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The burden of influenza among hospitalized febrile children in Ghana

Dissertation

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List of abbreviations

ALRI	—	Acute lower respiratory infection
APH	—	Agogo Presbyterian Hospital
BNITM	—	Bernhard-Nocht-Institute for Tropical Medicine
CI	—	Confidence interval
e. g.	—	Exempli grata
GISRS	—	Global Influenza Surveillance and Response System
H	—	Hemagglutinin
HIV	—	Human immunodeficiency virus
i.e.	—	Id est
ILI	—	Influenza-like illness
IQR	—	Interquartile range
N	—	Neuraminidase
OPD	—	Outpatient department
OR	—	Odds ratio
RNA	—	Ribonucleic acid
RR	—	Risk ratio
RSV	—	Respiratory syncytial virus
RT-PCR	—	Real-time polymerase chain reaction
SARI	—	Severe acute respiratory tract infection
SSA	—	Sub-Saharan Africa
WHO	—	World Health Organization

1 INTRODUCTION

Influenza is caused by ribonucleic acid (RNA) viruses of the family of *Orthomyxoviridae*, which are divided into four types, labelled A, B, C and D (1). The surface structure of influenza A expresses two glycoproteins: one hemagglutinin (H) and one neuraminidase (N). 18 H-subtypes (H1 – H18) and 11 N-subtypes (N1 – N11) are known so far (2). Depending on the combination of those surface proteins, influenza A viruses can be subdivided. Influenza A(H1N1)pdm09 and A(H3N2) are the current seasonal subtypes circulating in humans (1).

The transmission of influenza is extremely seasonal in character, mainly because of continuous evolvement of new influenza A virus strains, which can (partially) escape from pre-existing host immunity. Influenza viruses feature a broad genetic variability with two mechanisms of antigenic variations: "antigen drift" and "antigen shift", describing point mutations and recombinant reassortment of gene segments, when an animal host gets co-infected with two different viruses. Antigen shifts are responsible for pandemics with new influenza A virus strains (3). In history, the subtypes causing pandemics in humans were: H1N1 (1918), H2N2 (1957), H3N2 (1968), H1N1 (1977) and a new H1N1 subtype from swine origin (2009) (4).The name of the pandemic strain in 2009 was later officially modified to A(H1N1)pdm09 (5). Pandemic influenza does not follow any rules of seasonality.

Influenza A and B can cause a severe disease in humans, while influenza C probably causes, if any, only mild symptoms. Influenza D is not known to infect humans (1). The incubation period of influenza varies from 1 to 4 days and disease symptoms are characterized by a sudden onset of fever, severe malaise, non-productive cough, rhinitis, accompanied by head-, muscle- and joint-aches. Particularly children may present with gastrointestinal symptoms (4). Influenza patients usually recover within one week without any sequelae. However, individuals with risk factors (eg. young age or underlying diseases) are more prone to develop complications (1), such as secondary bacterial pneumonia, potentially leading to death. The contagiousness lasts from 1 day before up to 7 days after onset of symptoms. Infected children are more likely to spread the virus than adults; not only, because they maintain less personal hygiene and distance, but also, because they carry higher levels of virus loads in their respiratory tract (4).

According to the World Health Organization (WHO) targeted groups for influenza vaccination should be pregnant women, the elderly, children younger than 5 years of age, health-care workers and individuals with specific chronic medical conditions (6). These groups at risk of infection and influenza-related complications are prevalent in sub-Saharan Africa (SSA). Moreover, in Africa, the burden of influenza may be fueled by a high prevalence of co-morbidities with underlying diseases such as human immunodeficiency virus (HIV), tuberculosis and malnutrition (7). Nonetheless, although influenza vaccine is found to

be safe and effective (3, 8-10), virtually none is available and used in SSA (11).

The burden of influenza disease has been well understood in developed settings, while influenza is only now slowly being recognized as a major public health concern for developing countries. Worldwide, an estimated annual number of 90 million influenza cases with 870,000 influenza-associated hospitalizations and 20 million episodes of influenza-associated acute lower respiratory infection (ALRI) occur in children below 5 years of age. (12). The high burden among children in lower-income countries is illustrated by influenza-associated hospitalization rates three times higher in developing than in industrialized countries (10). Moreover, about 250,000 – 500,000 deaths worldwide have been ascribed to influenza with a more than 17-fold difference in case fatality ratios between developing and industrialized countries (12).

Consequently, influenza surveillance data from Africa generated since the A(H1N1)pdm09 pandemic in 2009 indicate a substantial disease burden with high mortality (13). However, local data on influenza frequency and transmission seasonality are still scarce for most African countries (14). In Ghana, influenza activity has only been studied among hospitalized children in urban settings (15, 16). However, these data are particularly important for health care professionals working in district hospitals with limited diagnostic facilities, where diagnoses are commonly made based on clinical signs and symptoms. Considering that influenza infections among children are not easily distinguishable from other non-specific febrile illnesses, such as malaria, it is crucial to have valid data on the relative contribution of influenza to febrile infections in hospitalized children. Providing data on influenza prevalence in a rural hospital in Ghana could add further evidence on the importance of this disease for health care professionals treating febrile children. Influenza diagnostics, such as rapid bedside tests, may eventually improve clinical management of patients, including the prevention of false treatment decisions.

2 AIMS OF THIS STUDY

The aims of the present study were to provide data on (i) the frequency and seasonal distribution of influenza A/B among hospitalized febrile children presenting to a district hospital in rural Ghana, (ii) to describe differential diagnoses to severe febrile infections, and (iii) to assess the symptoms, mortality and socioeconomic background of influenza-positive children.

3 MATERIALS AND METHODS

This cross-sectional study was conducted at the Agogo Presbyterian Hospital (APH), which is the referral hospital of the Asante Akim North district in the Ashanti Region of central Ghana (**Figure 1**). The APH sees about 6,000 patients per year (17) providing care for about 50 children on the paediatric ward at a time. The climate is tropical with a mean annual ambient temperature of 26°C and two rainy seasons; The major rainy season occurs from March to June, the second, minor one from September to October (17). The dry "harmattan" season prevails from December to February (18). Malaria transmission is holoendemic in this area with seasonal peaks (19).

Figure 1. Location of study hospital in Ghana. (20)

The population in Ashanti Ghana mainly consists of the native *Akan* people, mixed with migrants from mostly Northern Ghanaian tribes (21). The Asante Akim North district has approximately 140,000 inhabitants, from which roughly 40% are younger than 15 years of age (22).

Hospitalized children aged ≥1 month up to ≤14 years with a tympanic temperature of ≥38.0°C were included in the study on their day of admission. Recruitment took place between January 2014 and April 2015. Trained nurses and physicians conducted daily surveillance of inpatient admission lists, from which eligible children were enrolled. The study physician investigated respiratory (abnormal lung auscultation; blocked nose; breathing difficulties; chest indrawing; chest pain; coryza; cough; intercostal retractions; nasal flaring; oxygen saturation; sneezing; sore throat; stridor; wheezing), gastrointestinal (diarrhea, defined as lose stool >3x/ day; vomiting) and neurological (convulsions) signs and symptoms. Returning study children counted as new visits, if more than 30 days had passed since their last discharge.

Per WHO definition, severe acute respiratory tract infection (SARI) was diagnosed in all hospitalized patients with an acute respiratory infection with cough, a temperature of ≥38.0°C or a history of fever and an onset of symptoms within the last 10 days (23). In comparison, influenza-like illness (ILI) as defined by WHO is diagnosed, if the same conditions apply, except for hospitalization (23). Hospitalized children with ALRI are denoted by cough or breathing difficulties with an increased respiratory rate for age. Severe ARLI is diagnosed in case of additional chest indrawing (24). Malaria was diagnosed in all patients with a temperature of ≥38.0°C accompanied by asexual *Plasmodium falciparum* parasitaemia of ≥1 parasite per μl.

For influenza diagnostics, oropharyngeal swabs (Copan Flocked Nasopharyngeal Swabs number 553C) were taken from each study patient and immediately transferred in viral transport medium. The swabs were stored at -20.0°C until RNA extraction. RNA extraction was performed using the RTP® Pathogen Kit (Stratec biomedical, Birkenfeld, Germany) and screening of samples for influenza A and B by real-time polymerase chain reaction (RT-PCR) was done using the RealStar® Influenza RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) according to the manufacturer`s instructions (25). Further differentiation of positive influenza A samples for A(H1N1)pdm09 and A(H3N2) was done following a real-time RT-PCR protocol recommended by the WHO (26), using the Superscript ® III OneStep RT-PCR with Platinum Taq DNA Polymerase (Invitrogen, Karlsruhe, Germany) as described in Hogan B, Ammer L, Zimmermann M et al. (27).

For malaria diagnostics, Giemsa-stained thick and thin smears were performed for each patient. Two well-trained independent slide readers conducted malaria microscopy. In case of discording results, a third reading took place.

For each patient, a bacterial blood culture was performed on standard media from positive-flagged blood culture bottles (Oxoid, Basingstoke, UK). The inoculated bottles were kept at room temperature until incubation at 37.0°C. In case of positive results, gram staining enabled the identification of bacteria by microscopy. Environmental bacteria and bacteria belonging to the skin flora (e.g., coagulase negative Staphylococci, *Propionibacterium* spp., coryneform bacteria and *Bacillus* spp.) were considered as contaminants.

All statistical analyses were performed with Stata/ IC Software (Version 14.0; StataCorp LP, College Station, USA). Categorical variables were summarized as percentages and continuous variables as means with standard deviation (medians with interquartile ranges (IQRs)). All study children were described by sex and age. Influenza positivity and SARI rates were stratified by age group (<3 years, 3–5 years and >5 years) and month of the year. In order to quantify the association between a given exposure and outcome, odds ratios (OR) or risk ratios (RR) with their respective 95% confidence intervals (CI) were calculated.

The study's aims and purposes were explained to the parents or other related guardians on behalf of the study children. Informed consent was obtained by signature or thumbprint prior to study enrolment. All data was treated confidential. Ethical approval for this study was obtained from the Committee on Human Research, Publications and Ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, and the "Ethikkommission der Ärztekammer Hamburg", Germany. The cost for the children`s participation in the study was fully covered by the project budget. The patients were medically treated according to relevant hospital and national guidelines.

4 RESULTS

Between January 2014 and April 2015, 1,063 children with a median age of 2 years (IQR: 1–4 years) were enrolled. Overall, 274 (26%) children were diagnosed with SARI and 47 (4%) children were positive for influenza.

SARI was predominantly found in the age group <3 years (194/618; 31%), with a median age of 2 years (IQR: 1–3) (Table 1). In contrast to SARI cases, influenza patients had a median age of 3 years (IQR: 1–5) and were most frequently detected in the 3–5 years age group (18/292; 6%) with an OR of 2.0 (95% CI: 1.0–3.9) (**Table 1**). Among all recruitments, gender was slightly disparate with 591 male cases (55%) aged 0 to 14 years (median: 2 years, IQR: 1–4 years) compared to 483 female cases (45%) aged 0 to 13 years (median: 2 years, IQR: 1–4 years). Male cases also predominated among SARI patients (153/274; 56%), contrary to influenza patients, among whom female cases prevailed (27/47; 57%) (**Table 1**).

Table 1. Influenza and Severe Acute Respiratory Infection (SARI) cases stratified by sex and age.

			Influenza		**SARI**	
			n (%)	**OR (CI)**	**n (%)**	**OR (CI)**
Age group, years	<3	(n=618)	22 (4)	1 (N.A.) *	194 (31)	1 (N.A.) *
	3–5	(n=292)	18 (6)	2.0 (1.0–3.9)	58 (20)	0.6 (0.4–0.7)
	>5	(n=164)	7 (4)	1.5 (0.6–3.5)	22 (13)	0.3 (0.2–0.5)
Sex	Male	(n=591)	20 (3)	1 (N.A.) *	153 (26)	1 (N.A.) *
	Female	(n=483)	27 (6)	1.7 (0.3–3.1)	121 (25)	1 (0.8–1.3)

* N.A., not available (reference group)

Abbreviations: OR, odds ratio; CI, 95% confidence interval; SARI, severe acute respiratory infection; ALRI, acute lower respiratory infection

The influenza cases were composed of 26 (55%) cases for influenza B and 21 (45%) cases of influenza A (i.e. 15 (71%) A(H1N1)pdm09 and 6 (29%) A(H3N2) cases). Among SARI patients, 21 (8%) children had influenza, composed of 8 (38%) cases of influenza B and 13 cases (62%) of influenza A (i.e. 11 (85%) A(H1N1)pdm09 and 2 (15%) A(H3N2) cases), while 26 (3%; 26/789) of the non-SARI patients had influenza (OR=2.4; 95% CI: 1.3–4.4). A comparison of the (sub)type distribution reveals a higher proportion of influenza A cases among SARI patients (13/21; 62%) than among all study children (21/47; 45%) (**Table 2**). Other subtypes such as A(H1N1) or A(H5N1) could not be detected.

Table 2. Influenza subtype distribution among all study children and Severe Acute Respiratory Infection (SARI) cases.

		All study children (n=1063) n (%)	**SARI (n=274) n (%)**
(Sub) types	A(H1N1)pdm09	15 (32)	11 (52)
	A(H3N2)	6 (13)	2 (10)
	Influenza B	26 (55)	8 (38)

Abbreviation: SARI, severe acute respiratory infection

The median body temperature on admission was 39.0°C (IQR: 38.5°C–39.6°C). The temperature was not significantly different between influenza-positive and -negative children (OR: 1.1; 95% CI: 0.7–1.6). Among all study children, children presenting with cough had a higher likelihood of being positive with influenza (OR: 2.4; (95% CI: 1.3–4.1); Those, who presented cough, were 2.4 times more likely to have influenza than those without cough. Among all influenza-positive children, cough was the predominant respiratory symptom (45%; n=21), followed by coryza (19%; n=9) and chest indrawing (9%; n=4). The gastrointestinal symptom vomiting (38%, n=18) was identified as the second most common clinical manifestation overall among influenza-positive patients, after cough (**Table 3**). The oxygen saturation among all study children and among SARI cases ranged from 91%–100% with a median of 98% (IQR: 98%–98%). The median duration of hospitalization was 2 days (IQR: 2–4 days), while no death was recorded among the study children during their stay. 98% (n=1,042) of the study children were discharged with ongoing symptoms.

Table 3. Clinical symptoms among influenza-positive and -negative children.

Conditions	Characteristics	Influenza-positive children (n= 47) n (%)	Influenza-negative children (n= 1016) n (%)
Respiratory	Cough	21 (45)	253 (25)
	Coryza	9 (19)	96 (9)
	Chest indrawing	4 (9)	59 (6)
	Breathing difficulties	3 (6)	43 (4)
	Bronchial breath sounds	3 (6)	49 (5)
	Crackles	3 (6)	42 (4)
	Diminished breathing sounds	3 (6)	46 (5)
	Intercostal retractions	3 (6)	48 (5)
	Nasal flaring	2 (4)	70 (7)
	Blocked nose	1 (2)	34 (3)
	Chest pain	1 (2)	5 (<1)
	Pharyngitis	0	11 (1)
	Sneezing	0	3 (<1)
	Sore throat	0	3 (<1)
	Stridor	0	1 (<1)
	Wheezing	0	8 (<1)
Gastrointestinal	Vomiting	18 (38)	400 (39)
	Diarrhea *	3 (6)	204 (20)
Neurological	Convulsions	3 (6)	101 (10)

* (lose stool >3x/ day)

Influenza-positive cases were detected in each study month, except from September to December 2014. Positivity rates showed peaks in June 2014 and April 2015, which was the last study month. Increased frequency of influenza cases partially matched with the major annual rainy season (OR=2.9; 95% CI: 1.5–6.2), contrary to SARI prevalence, which seem to have no association with the rainy seasons (OR=1.2; 95% CI: 0.1–1.6). In 2014, the influenza cases were mainly caused by influenza B (71%; 24/34), followed by influenza A(H3N2) (18%; 6/34) and a small portion of influenza A(H1N1)pdm09 (9%; 3/34). In 2015, only two influenza (sub)types circulated: influenza A(H1N1)pdm09 (85%; 11/13) and influenza B (15%; 2/13) (**Figure 2**).

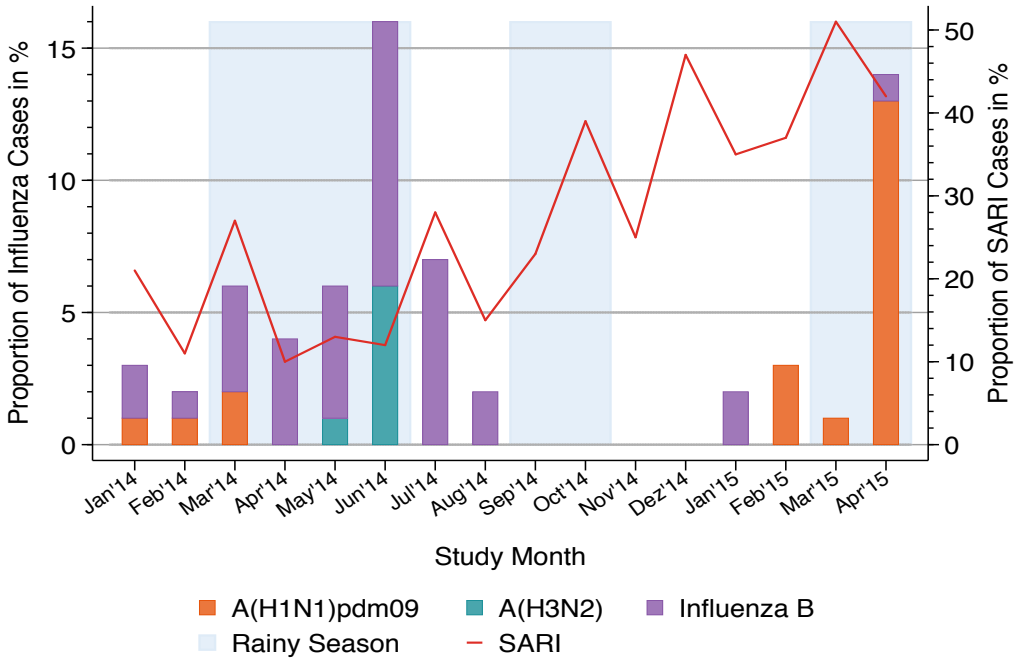

Figure 2. Influenza and Severe Acute Respiratory Infection (SARI) cases per study month.
Proportions of both influenza and SARI cases were calculated using the total number of recruited patients per study month. Influenza was further defined on subtype level: namely influenza B, influenza A(H1N1)pdm09 and influenza A(H3N2).

Study patients had 1 to 13 siblings (median: 3; IQR: 2–4) and 2–22 people lived together per household (median: 6; IQR: 4–7). Neither the number of siblings, nor the number of people eating together per household showed a statistically relevant relationship towards whether a case had influenza or not. None of the study patients were vaccinated with influenza vaccine.

Seven (15%) and two (4%) of the influenza-positive children were co-diagnosed with malaria (with a median parasitaemia of 12,231/µl; IQR: 8,606/µl – 35,599/µl) and with an invasive bacterial bloodstream infection (i.e. *Salmonella enterica*), respectively (**Figure 3**). Comparisons of malaria patients with and without influenza revealed a negative association between influenza and malaria (OR=0.1; 95% CI: 0.1–0.3).

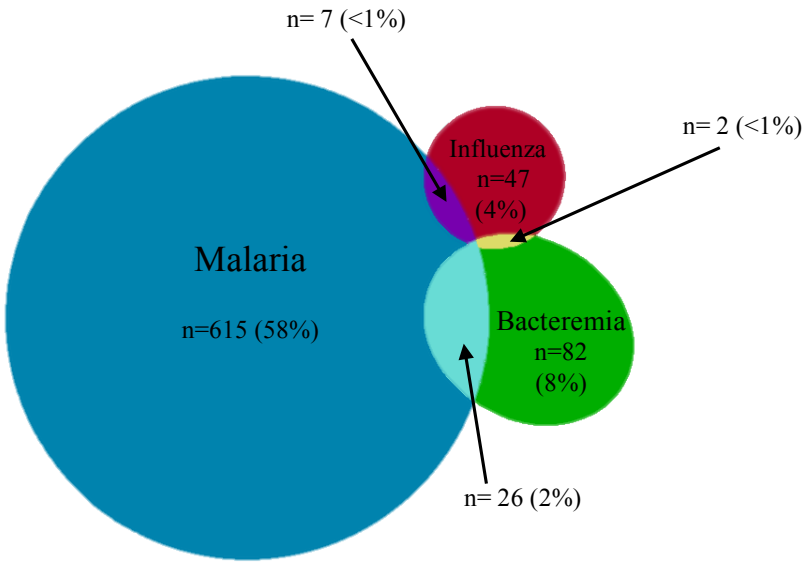

Figure 3. Overlap of diagnoses in study population (N=1,063).

5 DISCUSSION

Influenza and SARI contribute substantially to the burden of febrile illnesses in hospitalized children in rural Ghana. During the 16-month study period, 1,063 children were enrolled, of which 4% were positive with influenza and more than a quarter (26%) were diagnosed with SARI. In comparison to SARI, which predominated in children under 3 years of age, influenza was most frequently detected in children aged 3 to 5 years and during the major annual rainy season. 4% and 15% of all influenza-positive children had concomitant influenza/ bacterial bloodstream and influenza/ malaria infections, respectively.

In Africa, accurate determination of influenza activity is challenging. For this purpose, the SARI case definition is a commonly used surrogate in the absence of laboratory-based surveillance systems (15). However, influenza case definitions are highly age-dependent (28). Furthermore, a substantial proportion of influenza cases present with non-specific febrile illness without clear respiratory symptoms (15). One study in France found that 30% of all influenza-positive patients up to 11 months of age were lacking respiratory symptoms (29), while in Puerto Rico 48% of all influenza-positive hospitalized febrile children had no other respiratory symptoms than rhinitis. (30) In the present study, influenza was detected in 3% of non-SARI cases (**Table 2**). Particularly neonates and very young children often display fever only (10, 31). Even though neonates were not enrolled in the present study, 55% (26/ 47) of all influenza-positive patients were missing cough (**Table 2**). These high percentages of influenza-positive children, who did not meet the SARI definition, indicate that the utilization of the SARI definition alone will probably underestimate the true burden of influenza

infections and pinpoints the importance of broader screening criteria for influenza surveillance.

The observed younger median age of children with SARI compared to those with influenza can be explained by the high frequency of other respiratory tract pathogens circulating among very young children (10, 32). Globally, respiratory syncytial virus (RSV) is with 22% the most prevalent pathogen in young children with ALRI (12). In the outpatient department (OPD) of the study hospital, human parainfluenza viruses 1–4, entero- and adenoviruses predominated in children below 5 years of age, while influenza was the most frequently detected pathogen in children aged 5 to 12 years (17). A high proportion of influenza-positive ILI cases among school-aged children has been described before by Bonney et al. (3), who showed an increase of influenza-positive ILI cases with age (from 11% in infants to 31% in children aged 5 to 10 years, respectively). The more severe disease presentation among young children (10) explains the younger age of inpatients compared to outpatients with influenza.

Interestingly, the present study shows a slightly higher incidence of febrile admissions of boys than of girls (55%). Globally, this tendency could be attributed to earlier medical advice seeking behavior of parents for boys than for girls (24). Furthermore, smaller airway size in young boys compared to girls may increase the boy's risk of infection (33). However, in the present study, among influenza patients, the proportion of female patients overweighed (57%). It requires further research to determine to what extent sex differences affects the burden of influenza in Ghana.

Of all recruitments, SARI was diagnosed in roughly a quarter (26%), of which again 8% were influenza-positive. These findings are in concordance with surveillance data from 15 African countries, which detected influenza in 5%–26% of all SARI cases (34). Furthermore, results from Ghana showed influenza rates of 8%–9% among children with SARI (15, 35). Influenza B represents the predominant type with 55% of all influenza-positive children. The clinical manifestation of respective influenza (sub)types can vary greatly from patient to patient. However, the illnesses caused by influenza A subtypes and influenza B are generally comparable with the addition that A(H3N2) is associated with a greater disease severity and risk of complication compared to influenza B or A(H1N1)pdm09 (4). Individuals affected by strains associated with a milder course of disease may exhibit different health-seeking behavior (36). Nonetheless, the influenza virus (sub)type distribution pattern from this study is in line with data from the Global Influenza Surveillance and Response System (GISRS) by the WHO for Ghana in 2014 and 2015 (37), with the exception of missing A(H3N2) cases in 2015 in the present study. However, the study period ended in April 2015, hence robust analyses of the subtype distribution for 2015 were impossible.

The respiratory symptom cough is associated with influenza infection. Coryza and chest

indrawing were the next most common respiratory symptoms among influenza-positive children. More than a third of the influenza-positive children presented with the gastrointestinal symptom vomiting, endorsing the less typical manifestation of influenza in children than in adults (4). Interestingly, the findings of this study also underline a previously found negative correlation between the symptoms sore throat/ pharyngitis and influenza (28), as none of the influenza-positive children in this study was described with these symptoms (**Table 3**). Hypoxaemia is an important indicator of disease severity and a key predictor of ALRI mortality (38). In the present study, the peripheral measured oxygen saturation among all SARI patients was above 90%, which may support the finding that no influenza-related death took place in this cohort.

It has been assessed that 99% of deaths due to SARI (24) and 70% of all deaths charged to ILI in children below 5 years of age occur in developing countries (39). However, the mortality of the present study is in line with a 30-months surveillance, which also did not report any influenza-associated deaths in three Ghanaian tertiary hospitals (15). Low mortality due to SARI may be biased by poor access to hospital-based health care. Studies in Kenya (40, 41) and Gambia (42) reported a two- to ten-fold decrease in pneumonia-related admissions in areas farthest away from hospitals. Another study showed that fatality differs greatly between hospital-treated and non-hospital-treated severe ALRI (24). Hence, most deaths may still occur outside hospitals.

The increase of influenza cases during the main rainy season of the study area underpins other studies conducted in countries near the equator, including Tanzania (43) and Senegal (44, 45) suggesting a link between influenza transmission seasonality and climate factors, such as rainfall. However, opposed to results from the GISRS (37) and outpatient-based studies from Ghana (17, 35), no influenza cases were detected from September to December, given that this time stretch comprises the smaller second annual rainy season. In turn, from December to February, influenza cases occurred despite the dry season during this period (**Figure 2**). This underlines the less pronounced and less understood seasonality of influenza transmission in tropical areas than in temperate zones in the Eastern and Southern hemispheres, where outbreaks group in correlation with the local drier and cooler midwinter months (14, 46). After all, the study period fully covered only two rainy seasons in 2014. Influenza seasonality should therefore be interpreted with caution.

Socioeconomic factors such as increased household density and having siblings are risk factors for acute respiratory infection (47) and influenza-associated hospitalization (48), respectively. However, comparing data of the household size and the number of siblings of influenza-positive cases to negative ones, they seem not to play any mentionable role in explaining influenza infection in the present study. A community-wide transmission pathway may depend on contact with specific subpopulations, such as school-children (3).

In SSA, fever is a leading symptom in children presenting to outpatient departments in tertiary hospitals (49). It is also the main symptom of malaria, which temporally coincides with influenza peaking during the rainy seasons (7). Therefore, fever is easily mistaken as one of the symptoms of malaria. In this context, it is clinically relevant that in the present study, concomitant infections with influenza seem to be relatively rare; Influenza-positive study children were less likely to have malaria than those without influenza. This inverse association may be due to a hospital-induced selection bias, known as "Berkson's bias", where children with a severe infection have a reduced likelihood of another severe concomitant febrile illness (e.g. bloodstream infections) being the reason for their hospitalization (50). By point-of-care diagnostics, such as rapid tests on wards, influenza could be distinguished from other febrile infections. This would help to identify the causative agent for a substantial number of hospitalized SARI cases, which otherwise remain undiagnosed and underestimated. After all, improved diagnostics would reduce inadequate antimicrobial therapy risking the rise of antimicrobial resistance and failure or delay in treatment with poor clinical outcomes. However, rapid tests for influenza must be interpreted with caution, considering that a recently published meta-analysis described low sensitivities (51). Furthermore, in this study, almost one fifth (9/47; 19%) of all influenza-positive were concomitantly diagnosed with either an invasive bloodstream infection or with malaria. Taking this into account, in the light of a pragmatic therapeutic approach, additional antibiotic or antimalarial therapy may still be given for severely infected cases in settings with unreliable laboratory facilities.

This study has a few limitations. Oropharyngeal swabs were used for all patients. In some cases, better result may have been achieved with additional nasopharyngeal swabs to maximize the sensitivity for virus detection (52). The 16-months study period allowed only a rough estimation of seasonal patterns. Reliable analysis of seasonality, which is fundamental to prime public health care interventions, such as influenza vaccine implementation, require longer study periods of at least two years. Furthermore, the catchment population was narrowed down to hospitalized children belonging to a group of individuals, who had fairly good access to healthcare and were open to medical advice. Hence, as hospitalization rates are biased by access to care and cultural factors that affect health-care seeking behavior, the children attending the hospital may not have been representative for the overall population. Another limiting factor is the time delay from symptom onset to specimen collection, which might have caused underestimates of influenza prevalence in patients coming from very distant places. Finally, missing outcome data limited the ability to calculate reliable mortality rates.

6 CONCLUSIONS

SARI and influenza substantially contribute to febrile illnesses in hospitalized children in Ghana. Therefore, influenza should be recognized as a differential diagnosis in malaria-endemic regions. The study highlights increased influenza frequencies in children above 3 years of age during the first half of the year comprising the major annual rainy season. During this time, rapid tests might be most beneficial on paediatric wards. The fact that concomitant infections with influenza seem to be relatively rare in hospital settings supports the basic necessity for better diagnostics, such as with rapid tests, in order to better distinguish between influenza and other non-specific febrile illnesses. This would improve the clinical management of patients, including the prevention of false treatment decisions. However, further studies with longer study periods based on outpatient and inpatient surveillance with analysis of influenza burden by region and associated risk factors are needed. Future studies should also include the assessment of other respiratory pathogens. Such studies will trace novel influenza viruses and may guide public health care officials in Ghana to implement targeted interventions, such as influenza vaccination or health education campaigns.

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8 ABSTRACT

Introduction. Influenza surveillance data from Africa indicate a substantial disease burden with high mortality. However, from district hospitals with limited laboratory facilities local data are still scarce. This study aimed to assess the frequency and seasonality of influenza among hospitalized febrile children in a small district hospital in Africa, to identify the relative contribution of influenza and severe acute respiratory infection (SARI) to febrile infections.

Methods. Between January 2014 and April 2015 children ≥1 month and <15 years of age with a body temperature of ≥38.0°C admitted to a district hospital in rural Ghana were recruited. Oropharyngeal swabs were collected and screened for influenza A and B by RT-PCR. Malaria microscopy on Giemsa-stained thick and thin smears and a blood culture was performed for each patient.

Results. Of 1,063 enrolled children, 47 (4%) were positive for influenza and 274 (26%) were diagnosed with SARI. SARI was frequently diagnosed in children under 3 years of age and an association with the rainy seasons was not detectable (OR=1.2; 95% CI: 0.1–1.6). Influenza predominated in children aged 3 to 5 years and was more frequently detected during the major annual rainy season (OR=2.9; 95% CI: 1.5–6.2). Two (4%) and seven (15%) influenza-positive children were co-diagnosed with an invasive bloodstream infection or malaria, respectively.

Conclusions. Influenza contributes substantially to the burden of febrile illnesses in hospitalized children in rural Ghana being strongly dependent on age and the major annual rainy season during the first half of the year. During this period the use of rapid diagnostic tests may be beneficial to distinguish influenza from other non-specific febrile infections. The fact that concomitant infections with influenza seem to be relatively rare in hospital settings supports the basic necessity for improved diagnostics, in order to help prevent false treatment decisions.

Einleitung: Influenzadaten aus Afrika weisen auf eine erhebliche Krankheitslast mit hoher Sterblichkeit hin. Von Distriktkrankenhäusern mit fehlenden diagnostischen Kapazitäten gibt es jedoch bisher kaum Daten. Ziel dieser Studie war es, die Häufigkeit und Saisonalität von Influenza sowie die relativen Anteile von Influenza und schwerer akuter Atemwegserkrankung (SARI) an fieberhaften Erkrankungen bei hospitalisierten Kindern eines ländlichen Krankenhauses in Afrika zu untersuchen.

Methoden: Von Januar 2014 bis April 2015 wurden hospitalisierte Kinder im Alter von ≥1 Monat bis <15 Jahre mit einer Körpertemperatur von ≥38.0°C in einem ländlichen Distriktkrankenhaus in der Ashanti-Region Ghanas rekrutiert. Oropharyngeale Abstriche wurden entnommen und anhand RT-PCR auf Influenza A und B untersucht. Giemsa-gefärbte Dicke Tropfen und Blutausstriche sowie jeweils eine Blutkultur wurden für alle Patienten durchgeführt.

Ergebnisse: Von 1.063 rekrutierten Kindern hatten insgesamt 47 (4%) Influenza und 274 (26%) SARI. SARI wurde am häufigsten bei Kindern unter 3 Jahren diagnostiziert und zeigte keine nachweisbare Assoziation mit der Regenzeit (OR=1,2; 95% CI: 0,1–1,6). Influenza wurde häufiger bei Kindern zwischen 3 und 5 Jahren und während der Hauptregenzeit gefunden (OR=2,9; 95% CI: 1,5–6,2). Zwei (4%) und sieben (15%) der Influenza-positiven Kinder hatten zusätzlich eine systemische bakterielle Infektion beziehungsweise Malaria.

Schlussfolgerung: Influenza trägt erheblich zur Krankheitslast von fieberhaften Erkrankungen hospitalisierter Kindern im ländlichen Ghana bei, wobei die Häufigkeit stark vom Alter der Kinder abhängt und während der Hauptregenzeit in der ersten Hälfte des Jahres zunimmt. Während dieser Zeit könnten diagnostische Schnelltests helfen, Influenza von anderen unspezifischen fieberhaften Erkrankungen zu unterscheiden. Das relativ seltene Vorkommen von Koinfektionen weist darauf hin, dass eine bessere Diagnostik in stationärer Krankenhausumgebung zur Verringerung falscher therapeutischer Entscheidungen beitragen würde.

9 ERKLÄRUNG DES EIGENANTEILS

Hiermit wird bestätigt, dass Frau Luise Sophie Ammer im Rahmen der Publikation

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Des Weiteren waren folgende **Co-Autoren** beteilig:

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10 DANKSAGUNG

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11 CURRICULUM VITAE

Der Lebenslauf entfällt aus datenschutzrechtlichen Gründen in der elektronischen Version der Arbeit.

PUBLIKATIONEN

Benedikt Hogan, Daniel Eibach, Ralf Krumkamp, Nimako Sarpong, Denise Dekker, Benno Kreuels, Oumou Maiga-Ascofaré, Kennedy Gyau Boahen, Charity Wiafe Akenten, Yaw Adu-Sarkodie, Ellis Owusu-Dabo, the Fever Without Source (FWS) study group, Jürgen May: "**Malaria Coinfections in Febrile Pediatric Inpatients: A Hospital-Based Study From Ghana**" — *Clinical Infectious Diseases* Journal, 2018, Feb 2, https://doi.org/10.1093/cid/cix1120.

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12 EIDESSTATTLICHE ERKLÄRUNG

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe. Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe. Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Luise Sophie Ammer

13 ORGINALPUBLIKATION

Burden of influenza among hospitalized febrile children in Ghana

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ORIGINAL ARTICLE

WILEY

Burden of influenza among hospitalized febrile children in Ghana

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Background: Influenza surveillance data from Africa indicate a substantial disease burden with high mortality. However, local influenza data from district hospitals with limited laboratory facilities are still scarce.

Objectives: To identify the frequency and seasonal distribution of influenza among hospitalized febrile children in a rural hospital in Ghana and to describe differential diagnoses to other severe febrile infections.

Methods: Between January 2014 and April 2015, all children with a temperature of ≥38°C admitted to a district hospital in Ghana were screened for influenza A and B by RT-PCR and differentiated to subtypes A(H1N1)pdm09 and A(H3N2). Malaria microscopy and blood cultures were performed for each patient.

Results: A total of 1063 children with a median age of 2 years (IQR: 1-4 years) were recruited. Of those, 271 (21%) were classified as severe acute respiratory infection (SARI) and 47 (4%) were positive for influenza, namely 26 (55%) influenza B, 15 (32%) A(H1N1)pdm09, and 6 (13%) A(H3N2) cases. Influenza predominantly occurred in children aged 3-5 years and was more frequently detected in the major rainy season (OR = 2.9; 95% CI: 1.47-6.19) during the first half of the year. Two (4%) and seven (15%) influenza-positive children were co-diagnosed with an invasive bloodstream infection or malaria, respectively.

Conclusion: Influenza contributes substantially to the burden of hospitalized febrile children in Ghana being strongly dependent on age and corresponds with the major rainy season during the first half-year.

KEYWORDS

Africa, bacteremia, children, fever, influenza, malaria

Benedikt Hogan and Luise Ammer contributed equally to this publication.

†This work is part of the doctoral thesis by Luise Ammer.

1 | INTRODUCTION

Globally influenza has long been regarded as a major public health concern. Not only the elderly but also very young children have been identified as a vulnerable group for influenza infections. A recent meta-analysis estimated 90 million cases of influenza and 20 million episodes of influenza-associated acute lower respiratory infections (ALRI) annually worldwide in children below 5 years.[1] As a result, influenza is the second most common pathogen identified in children with ALRI after respiratory syncytial virus (RSV) and followed by parainfluenza virus.[2] About 870 000 annual hospitalizations in children below 5 years of age have been attributed to influenza with influenza-associated hospitalization rates being three times higher in developing than in industrialized countries.[3] It has been assumed that 99% of inpatient deaths from severe acute respiratory infections (SARI)[4] and 70% of all deaths attributable to influenza-like illnesses (ILI) among children below 5 years of age occurred in developing countries.[5]

The extent of influenza infections in Africa is now slowly being recognized due to strengthened national influenza surveillance systems across the continent.[6] Most countries have had inadequate data on the influenza disease burden until the influenza A(H1N1) pandemic in 2009. Since then, many countries implemented hospital-based influenza surveillance among SARI patients.[3,7]

In Ghana, ILI sentinel surveillance within outpatient facilities across all regions was established in 2007, which screened 2357 samples in 2014 (https://www.ghanahealthservice.org). The burden of influenza disease among hospitalized children in Ghana has so far only been studied in large tertiary hospitals.[8,9] However, data on the burden of influenza are particularly important for healthcare workers in small rural or district hospitals with no or limited laboratory facilities, considering that influenza infections are not easily distinguishable from other febrile infections such as malaria and may therefore lead to false treatment decisions.[10]

This study aims to identify the proportion and seasonal distribution of influenza infections among hospitalized febrile children in a rural district hospital in Ghana to inform healthcare workers on the contribution of influenza as a differential diagnosis to other severe febrile infections.

2 | METHODS

2.1 | Study site and sample collection

Study participants were recruited at the pediatric ward of the Agogo Presbyterian Hospital (APH), a district hospital with 250 beds, situated in the Asante Akim North municipality of the Ashanti Region in Ghana. The climate is tropical with two rainy seasons from March to June and from September to October.[11] The study area is located in a holoendemic malaria region with perennial malaria transmission.

Oropharyngeal swabs (Copan, Italy) were taken from all children aged between 1 month and 15 years with a tympanic temperature of ≥38°C between January 2014 and April 2015. Swabs were taken at admission and immediately transferred in viral transport medium and stored at −20° until RNA extraction. For each patient, two independent slide readers conducted malaria microscopy on Giemsa-stained thick and thin smears and a blood culture was performed on standard media (Oxoid, Basingstoke, UK). The following respiratory signs and symptoms were assessed by the study physician: abnormal lung auscultation, breathing difficulties, chest indrawing, chest pain, coryza, cough, intercostal retractions, nasal flaring, sore throat, and stridor. Repeated visits of study children were considered as new visits if they were at least 30 days apart. SARI was diagnosed according to the World Health Organization (WHO) case definition in a hospitalized patient with fever of ≥38°C or history of fever, with cough and an onset of illness within the last 10 days.[12]

2.2 | Sample processing and virus detection

RNA was extracted from oropharyngeal swabs with the RTP Pathogen Kit (Stratec biomedical, Birkenfeld, Germany) and eluted in 120 μL. Screening of samples for influenza A and B was conducted with the RealStar® Influenza RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) as described by the manufacturer with half the reaction volume. Positive influenza A samples were further differentiated for A(H1N1)pdm09 and A(H3N2) following the real-time RT-PCR protocol recommended by the WHO.[13] PCRs were performed in 25 μL using the Superscript® III OneStep RT-PCR with Platinum Taq DNA Polymerase (Invitrogen, Karlsruhe, Germany) containing 5.0 μL RNA template, 5.5 μL water, 12.5 μL 2× reaction buffer, 40.0 μmol/L of each forward (A(H3N2): 5′-AGCAAAGCCTACAGCAA-3′, A(H1N1) pdm09: 5′-GAGCTAAGAGAGCAATTGA-3′) and reverse (A(H3N2): 5′-GACCTAAGGGAGGCATAA-3′,A(H1N1)pdm09:5′-GTAGATGGAT GGTGAATG-3′) primer, 10.0 μmol/L probe (H3N2: 5′-Fam-CCGGCA CATCATAAGGGTAACA 3′-BHQ-1, A(H1N1)pdm09: 5′Fam -TTGCTG AGCTTTGGGTATGA -3′-BHQ-1), and 0.5 μL enzyme. Conditions for the reverse transcription PCR were 50°C for 30 minutes, followed by 2 minutes of initial denaturation at 95°C and 45 cycles at 95°C for 15 seconds and 55°C for 30 seconds.

2.3 | Epidemiological analysis

Categorical variables were described as frequencies and percentages and continuous variables as medians and their corresponding interquartile ranges (IQRs). All data analyses were performed with Stata 14 (StataCorp LP, College Station, TX, USA).

Children were grouped by age into the categories <3, 3-5, and >5 years. To quantify the association between a given exposure and outcome, odds ratios (OR) with their respective 95% confidence intervals (CI) were calculated.

2.4 | Ethical considerations

The Committee on Human Research, Publications and Ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana and the "Ethikkommission der

Ärztekammer Hamburg," Germany provided ethical approvals for this study. All participants were informed about the study's purpose and procedures. Written informed consent was obtained from the parents or the guardian on behalf of the study children prior to study enrollment.

3 | RESULTS

A total of 1063 hospital visits were made from 991 study children. Sex distribution was slightly unbalanced with 586 male cases (55%) aged 0-14 years (median: 2 years, IQR: 1-4 years) and 477 female cases (45%) aged 0-13 years (median: 2 years, IQR: 1-4 years). At recruitment, the children presented with a median temperature of 39.0°C (IQR: 38.5-39.6°C).

During the study period, 47 (4%) children were diagnosed with influenza, of which 21 (45%) tested positive for influenza A and 26 (55%) for influenza B. Influenza A cases were divided into the virus subtypes A(H1N1)pdm09 (n = 15; 71%) and A(H3N2) (n = 6; 29%).

In total, 274 (26%) children were diagnosed with SARI, which was predominantly found in the age group <3 years (194/618; 31%), with a median of 2 years (IQR: 1-3) (Table 1). Among SARI patients, 21 (8%) were positive for influenza while 26 (3%) of the non-SARI patients had influenza (OR = 2.4; 95% CI: 1.34-4.42). In contrast to SARI cases, influenza patients had a median age of 3 years (IQR: 1-5) and were most frequently detected in the 3-5 years age group (n = 18; 6%) with an odds ratio of 2.0 (95% CI 1.0-3.9) (Table 1).

All influenza-positive children were observed during the period between January and August 2014, with a peak in June, as well as January to April 2015 (last study month) with a peak in April (Figure 1). The observed influenza seasons partially overlap with the major annual rainy season (OR = 2.9; 95% CI: 1.47-6.19) of the study area (Figure 1). However, no influenza activity was detected in the months September to December 2014, including the short second rainy period in September and October. The SARI cases seem to have no association with the rainy season (OR = 1.2; 95% CI: 0.09-1.57) (Figure 1). Three different influenza subtypes circulated in the 2014 influenza season, with influenza B causing the majority (71%; 24/34) of cases, followed by A(H3N2) (18%; 6/34) and A(H1N1)pdm09 (9%; 3/34). In 2015, influenza was caused by the influenza strains A(H1N1)pdm09 (85%; 11/13) and influenza B (15%; 2/13).

Among influenza-positive children, cough was the most common respiratory symptom (45%; n = 21), followed by coryza (19%; n = 9) and chest indrawing (9%; n = 4). At least one respiratory symptom was observed among 45% (n = 21) of influenza patients. Influenza-positive and influenza-negative children showed no differences concerning tympanic temperature (OR: 1.1, CI: 0.7-1.63). The median duration of hospitalization was 2 days (IQR of 2-4 days) and none of the children died during their stay.

Among influenza cases, seven (15%) had concomitant malaria with a median parasitemia of 12 231/µL (IQR: 8 606/µL-35 599/µL). The detection of influenza was negatively associated with malaria disease (OR = 0.1; 95% CI: 0.05-0.25). Concomitant invasive bacterial bloodstream infections with *Salmonella enterica* were diagnosed in two influenza-positive children (4%).

Socioeconomic data showed that patients had 1-13 siblings (median: 3, IQR: 2-4) and 2-22 people living together per household (median: 6, IQR: 4-7). Neither the number of siblings nor the household size showed a statistically relevant relationship toward whether a case had influenza or not (data not displayed).

4 | DISCUSSION

Within our study group, SARI cases made up more than a quarter of all febrile pediatric hospital admissions. Of the SARI cases, 8% are diagnosed with influenza A/B, which is in line with a large African multicountry surveillance project, which identified influenza in 5%-26% of SARI cases.[6] From Ghana, similar SARI rates (8%-9%) have been reported.[8,14] Although the SARI case definition showed a good association with influenza in this study, it is noteworthy that influenza was still identified in 3% of non-SARI cases. It has been shown before that influenza case definitions are highly age-dependant and performances vary due to the unspecific clinical picture of influenza.[15] Hence, laboratory-based surveillance systems using a SARI definition will probably underestimate the true burden of influenza infections.

The median age of children with influenza was higher than the median age of SARI cases. This age distribution is probably due to the

TABLE 1 Influenza and SARI cases stratified by sex and age

		Influenza		SARI	
		n (%)	OR (CI)	n (%)	OR (CI)
Age group					
<3	(n = 618)	22 (4)	1 (N.A.)[a]	194 (31)	1 (N.A.)[a]
3-5	(n = 292)	18 (6)	2.0 (1.04-3.9)	58 (20)	0.6 (0.4-0.7)
>5	(n = 164)	7 (4)	1.5 (0.6-3.5)	22 (13)	0.3 (0.2-0.54)
Sex					
Male	(n = 591)	20 (3)	1 (N.A.) [a]	153 (26)	1 (N.A.)[a]
Female	(n = 483)	27 (6)	1.7 (0.3-3.08)	121 (25)	1 (0.76-1.33)

[a]Not available (reference group).
OR, odds ratio; CI, confidence interval; SARI, severe acute respiratory infection.

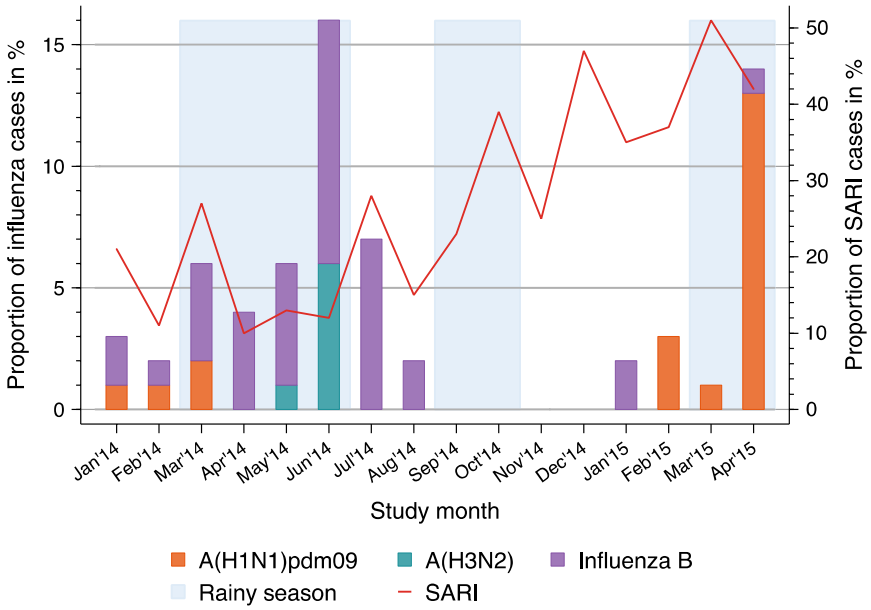

FIGURE 1 Influenza and Severe acute respiratory infection (SARI) cases per study month. Proportions of both influenza and SARI cases were calculated using the total number of recruited patients per study month. Influenza was further defined on subtype level: namely influenza B, influenza A(H1N1)pdm09, and influenza A(H3N2)

high frequency of other serious respiratory pathogens, notably respiratory syncytial virus (RSV), among very young children.[16]The outpatient department of the same study hospital reports human parainfluenza viruses 1-4, enteroviruses and adenoviruses as the predominant respiratory pathogens in children below 5 years of age, while influenza A/B was the most frequently detected respiratory virus in older children (5-12 years).[11] However, in the present study, samples were not tested for other respiratory viruses; therefore, this assumption could not be further investigated. The higher age of children with influenza at the outpatient department compared to hospitalized children in the present study can be explained by the more severe disease presentation among young children, which is illustrated by high death rates in this age group.[3] Influenza-related deaths were not reported during the present study, although high case fatality ratios, more than seventeen times as high as in industrialized countries, were estimated for developing countries.[1] This study's data are in line with findings from three Ghanaian tertiary hospitals, which also did not report any influenza-associated deaths during a 30 months surveillance period.[8] However, these findings may be biased by poor healthcare access, leading to an underestimation of the influenza disease burden including case fatalities.

Compared to data from the Global Influenza Surveillance and Response System (GISRS; http://www.who.int/influenza/gisrs_laboratory/en/), this study shows a similar pattern, in which influenza activity prevails end of a year/beginning of the next, until early/midsummer, which corresponds to the major rainy season. However, in contrast to outpatient-based studies from Ghana, no influenza viruses were detected from September to December, which comprises the smaller second annual rainy season.[11,14] Other studies confirmed that influenza seasonality in countries near the equator is roughly correlated to rainfall, but less pronounced than in southern African countries such as Zambia, South Africa, and Madagascar, where influenza corresponded with the drier, cooler winter months of June to August.[17,18] GISRS data for Ghana show a similar influenza subtype distribution for 2014 and 2015, despite the fact that subtype A(H3N2) was not detected in 2015 in this study. However, the study period ended in April 2015; therefore, data for 2015 should be interpreted with caution.

Clinically, influenza is difficult to distinguish from other tropical diseases, in particular malaria, which temporally coincides during the rainy seasons.[19] Interestingly, results of this study show that children hospitalized with influenza were less likely to have malaria than those without influenza. This effect is caused by hospital admission dynamics, meaning that children with a severe infection, such as influenza, have a reduced likelihood of another severe concomitant febrile illness (eg, malaria) being the reason for their hospital admission. This effect, also known as "Berkson's bias," can also be observed for concomitant influenza/bacterial bloodstream infections, which are very infrequent in this study.[20] The use of rapid diagnostic influenza tests in the study setting would help to distinguish influenza from other febrile infections and would identify the causative agent for the substantial number of hospitalized SARI cases, which otherwise remain undiagnosed. However, rapid diagnostic influenza tests have shown low sensitivities in a recently published meta-analysis and therefore must be interpreted with caution.[21]

This study has a few limitations. Oropharyngeal swabs were used for all patients, although the combination of nasopharyngeal and oropharyngeal swabs is reported to be more sensitive for virus detection.[22] The study was conducted throughout a 16-month period. Therefore, temporal patterns from this study can give a rough estimate, however, to time public health interventions, such as vaccinations, longer studies over a period of at least 2 years are required. Finally, this study aimed to assess the burden of influenza infections among hospitalized children. As hospitalization rates surely depend on the proximity to a healthcare facility and health-seeking patterns, these findings are not suitable to estimate influenza incidences in the general population. Another limiting factor of hospital-based surveillance is the time delay from symptom onset to specimen collection, which might underestimate influenza prevalence in patients living in very distant communities.

5 | CONCLUSION

SARI and influenza contribute substantially to the burden of hospitalized febrile children in Ghana. In comparison with SARI, which is most frequently found in children aged <3 years, influenza predominantly occurs in children aged 3-5 years and is associated with the major annual rainy season during the first half-year. During this time period, the use of rapid diagnostic tests may be considered on the pediatric ward, taking into account the test's low sensitivity. Distinguishing influenza from other non-specific febrile diseases, such as malaria and invasive bloodstream infections, could help to reduce the unnecessary application of antimicrobial and antimalarial drugs.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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