

# Human coronaviruses in persons with acute respiratory infections in Ghana

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## Abstract

**Background:** Acute respiratory infections (ARI) remain a leading cause of morbidity, mortality, and economic loss globally. Until recently, human coronaviruses (HCoV) have been mainly associated with mild respiratory tract infections. The 2003 outbreak of severe acute respiratory syndrome coronavirus (SARS-CoV) and cases of Middle East respiratory syndrome coronavirus (MERS-CoV), since 2012, illustrate the potential of coronaviruses to cause severe disease.

**Objective:** This study investigated the presence of human coronaviruses in acute respiratory illness in Ghana.

**Methods:** As part of routine influenza surveillance, nasopharyngeal and oropharyngeal (NP/OP) swabs obtained from 200 patients (100 hospital inpatients and 100 outpatients) with Influenza-like-illness from sentinel health facilities in Ghana from January 2013 to March 2014, were screened for the presence of HCoV at the National Influenza Centre using real-time reverse transcriptase polymerase chain reaction assays.

**Results:** Human CoVs were detected in 7 (3.5%) out of 200 cases investigated: HCoV HKU1 in 3 patient, HCoV 229E in 2 patients, HCoV OC43 in 1 patient, and HCoV NL63 in 1 patient. No co-infection with HCoV types was detected. Out of 7 patients with HCoV infections, 6 were aged 5 yr. or greater. Also, HCoV were detected more frequently in outpatients (5/100) than in hospitalized patients (2/100) with acute respiratory tract infections, though statistically insignificant ( $p > 0.005$ ). None of the respiratory specimens tested were positive for MERS-CoV, indicating the absence of MERS-CoV infection in Ghana between January 2013 and March 2014.

**Conclusion:** This work provides an important reference point for coronavirus infections in humans in Ghana noting the current concern on the 2019 novel coronavirus.

**Keywords:** Acute respiratory infections, Ghana, human coronavirus, real-time reverse transcriptase PCR

## INTRODUCTION

Viruses including human coronaviruses (HCoV) have been shown to represent a considerable proportion of the pathogens associated with acute respiratory infections (ARI), a major global public health problem that causes around 4 million deaths annually [1-4]. The HCoV have also been reported to contribute significantly to ARIs with a wide spectrum of clinical presentation in upper and lower respiratory tract illnesses [3-10]. Coronaviruses (CoVs) are enveloped viruses with a positive-sense, single-stranded RNA genome between 26–32 kilobases. They belong to the family *Coronaviridae* and the sub-family

*Coronavirinae* that infect a wide host range, including mammalian and avian species [11-13]. Currently, these viruses are classified into 4 genera designated Alpha coronavirus (former groups 1a and 1b), Beta coronavirus (groups 2a to 2d), Gamma coronavirus (group 3), and Deltacoronavirus [14]. There are currently 6 HCoV that cause respiratory infections including 2 alpha CoVs (HCoV-229E and HCoV-NL63) and 4 beta CoVs [HCoV-OC43, severe acute respiratory syndrome coronavirus (SARS-CoV), HCoV-HKU1 and Middle East respiratory syndrome coronavirus (MERS-CoV)] [15-19]. The SARS-CoV and MERS-CoV are well known for their association with severe respiratory illness and high fatality [17,20-24]. Whereas the other HCoV have been mainly associated with mild upper respiratory tract infections [25-29]. However, in young children, the elderly and immunocompromised patients, these viruses are known to cause severe respiratory tract disease that required

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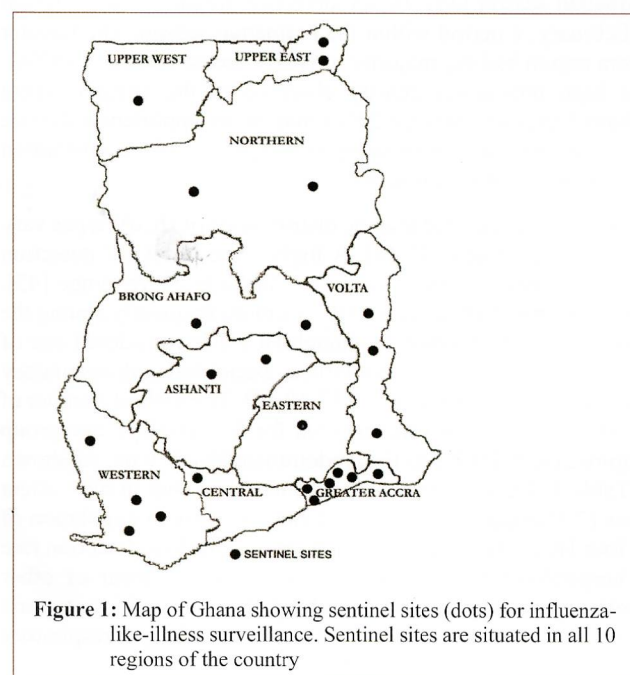
hospitalization [9,10,18,30]. The outbreak of the SARS-CoV in 2003 [17,19] and recently, MERS-CoV in 2012 [20,24], illustrates the epidemic potential of coronaviruses to threaten global health. The lack of sufficient data from developing countries on the burden of disease due to respiratory viral infections such HCoV has been identified by the Battle against Respiratory Viruses (BRaVe) initiative although HCoVs excluding SARS and MERS-CoVs are reported to be globally distributed [31-33].

## MATERIALS AND METHODS

### Study design

Ghana lies in the coastal belt of West Africa, bordered by Togo on the East, Cote D'Ivoire on the West, Burkina Faso on the North and the Atlantic Ocean on the south. At the time of the study the country was divided into 10 administrative regions: Greater Accra, Ashanti, Brong-Ahafo, Central, Eastern, Northern, Upper East, Upper West, Volta and Western regions. Ghana now has 16 administrative regions. Influenza-like illness (ILI) is defined as an acute respiratory infection with the following symptoms onset within the last 10 days: fever of  $\geq 37.5^{\circ}\text{C}$  (Axillary) and any respiratory symptom including cough, sore throat, rhinorrhoea, chest pain, difficulty in breathing, wheezing.

The National Influenza Centre (NIC) which is based in the Virology Department of the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon is responsible for the laboratory monitoring of influenza viruses in Influenza-like-illness (ILI) cases. The NIC receives respiratory specimens from ILI cases seen at influenza sentinel health facilities. These patients are either out-patients (ambulatory) or hospitalised patients with ILI. The sentinel sites have been established at various health facilities situated in all 10 regions of the country (Figure 1) and are required to submit to the NIC each week, the first five oropharyngeal (OP) and/or nasopharyngeal (NP) specimens from cases that meet the ILI case definition. An



anticipated frequency of 16% was adapted [29] to calculate the sample size of 200 at 95% confidence level. This was done with the OpenEpi34 (Version 3) using a 5% confidence limit as percentage of 100 and a Design effect (DEFF) set at 1.0. During the period of January 2013 to March 2014, specimens from outpatients with ILI that had been processed by the NIC and found to be negative for influenza type A and B viruses were stratified according to age groups ( $\leq 1$  yr., 2-4 yr., 5-14 yr., 15-49 yr., 50-64 yr., and  $\geq 65$  yr.), adapted from recommendations for Global Epidemiological Surveillance Standards for Influenza [29]. 100 specimens were then selected from these by a random sampling technique using Microsoft Excel version 2013. These were in representative proportion to the respective age group numbers for the period of interest. These 100 samples together with 100 specimens from hospitalized ARI patients for the same period (January 2013 to March 2014) were analysed.

### Laboratory processes

Viral RNA was extracted using the QIAamp® Viral RNA Mini Kit according to manufacturer's recommendations (Qiagen, Hilden, Germany). Viral RNA was then eluted in 60  $\mu\text{L}$  of RNase/DNase-free elution buffer. Specific real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assays for the detection of four HCoVs namely HCoV-229E, OC43, NL63 and HKU1 were then conducted according to standardized protocols as described in rRT-PCR assays for non-influenza respiratory viruses by the Centres for Disease Control and Prevention (CDC) in Atlanta, USA (rRT-PCR Assays for Non-Influenza Respiratory Viruses Centres for Disease Control and Prevention ver.003a.2010) [29]. The AgPath-IDTM One Step RT-PCR Kit (Life Technologies, USA) was used. Primers and probes based on previously published sequences [7,33] were synthesized by a commercial supplier (Eurofins MWG Operon, USA). Samples were tested in 25  $\mu\text{L}$  reaction volumes containing 5  $\mu\text{L}$  of RNA, 12.5  $\mu\text{L}$  of 2 x RT-PCR Buffer (Ag-Path-IDTM), 1  $\mu\text{L}$  of reverse transcriptase/Taq mix (25 x RT-PCR Enzyme Mix, Ag-Path-IDTM), 0.5  $\mu\text{L}$  of each type specific primer and probe set. Amplification was performed on the Applied Biosystems® 7500 Fast Real-Time PCR instrument (Life Technologies, USA). Cycling conditions consisted of a reverse transcriptase step of  $45^{\circ}\text{C}$  for 10 min, followed by a Taq polymerase activation step of  $95^{\circ}\text{C}$  for 10 min and then 45 cycles of  $95^{\circ}\text{C}$  for 15 sec (denaturing) and  $55^{\circ}\text{C}$  for 1 min (annealing and extension step). Data was collected during the final annealing and extension step ( $55^{\circ}\text{C}$  for 1 min). Additionally, an rRT-PCR screening assay for the detection of the upstream E gene (upE assay) for the presence of MERS-CoV was applied [35]. Positive controls for HCoV-229E, OC43, NL63 and HKU1 were obtained from the International Reagent Resource at CDC, while positive controls for the MERS-CoV UpE gene were obtained from the Institute of Virology, University of Bonn Medical Centre, Germany. All the PCR runs used nuclease free water (Life Technologies, USA) as negative control.

## RESULTS

### Demographic and clinical characteristics

The respiratory specimens tested were obtained from patients aged 1 mo to 95 yr. (Median = 13 yr.), of which 113 (56%) were males with an average age of 19 yr. and 87 (44%) were females

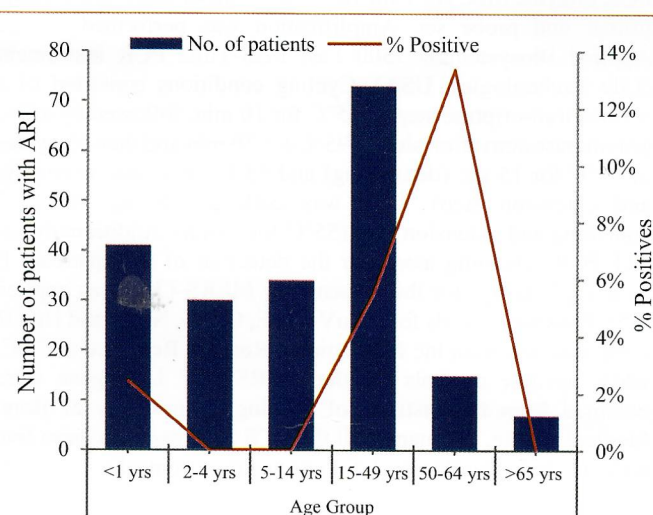


with an average age of 23 yr. (Table 1). There were more samples (36.5%) from patients in the 15-49 yr. age group than any other (Figure 2). Specimens were from 9 out of 10 regions in Ghana. Headache was the least reported symptom (6%, 12 out of 200). Four samples were from patients with pre-existing medical conditions of asthma (1) and chronic heart disease (3). Majority (76.5%) of samples were collected within 7 days of illness onset.

**Table 1:** Demographic characteristics of archived ARI samples screened for HCoV

Variable	X'tics	Cases n = 200 (%)	HCoV Pos n = 7 (%)
Case Syndrome	ILI	100 (50)	5 (71.4)
	SARI	100 (50)	2 (28.6)
Region	Northern	Northern	24 (12)
		Upper East	1 (0.5)
		Ashanti	1 (0.5)
		Brong Ahafo	8 (4)
	Southern	Volta	12 (6)
		Eastern	6 (3)
		Central	2 (1)
		Greater Accra	128 (64)
		Western	18 (9)
Zones	Southern	166 (83)	5 (71.4)
	Northern	34 (17)	2 (28.6)
Sex	Female	87 (43.5)	4 (57.1)
	Male	113 (56.5)	3 (42.9)
Age Group	0-1 yr	41 (21)	1 (14.3)
	2-4 yrs	30 (15)	0 (0)
	5-14 yrs	34 (17)	0 (0)
	15-49 yrs	73 (37)	4 (57.1)
	50-64 yrs	15 (8)	2 (28.6)
	≥65 yrs	7 (4)	0 (0)

\*X'tics, characteristics; HCoV Pos, human coronavirus positive; ILI, Influenza-like-illness; SARI, severe acute respiratory tract infection.



**Figure 2:** Graph showing occurrence of HCoV infections in the different age groups of ARIs investigated. Detection rate (% positive) was compared amongst age groups.

\*HCoV, human coronavirus positive; ARIs, acute respiratory infections.

## Detection of HCoV by rRT-PCR

Four types of HCoVs were detected in 7 patients and comprised HKU1 (3), 229E (2), OC43 (1), and NL63 (1). No co-infection of HCoV types was detected. Amongst these 7 positive cases, HCoVs were detected more frequently (6) in specimens collected within the first 7 days of onset of illness as compared with specimen obtained after 7 days (1). There was more detection of HCoVs in patients greater than 5 yr. (6) than in children less than 5 yr. (1). Also, HCoVs were detected more frequently with all 4 types present, in outpatient cases (5) than in hospitalized patients (2) (Table 2). All the HCoV positive cases were patients diagnosed with upper respiratory tract infection. Cough and sore throat were the most common (5/7) symptoms in HCoV positive patients. There were no pre-existing medical conditions in the 7 positive cases (Table 2). The median age of HCoV-infected patients was 35 yr. (range, 5 mos to 57 yr.). There was no detection of MERS-CoV in respiratory specimens from hospitalized patients.

## DISCUSSION

The prevalence of HCoV infections in respiratory specimens reported in literature range from 2% to 16%, probably due to differences in study areas and study populations [31,33,36-45]. In this study, HCoV was detected in 3.5% (7/200) of ARI cases. Our study reports the prevalence in specimens collected from 9/10 regions of Ghana which is lower than the 12.4% reported previously in 3 rural communities [45].

Association of HCoV infections with seasonality have been published in several studies, though there are variations in regional and annual circulation of different HCoV types [5,31,32,39,41,46], with reports of HCoV winter seasonality in temperate regions. In this study, although there is not enough data to describe seasonality of HCoV in relation to the wet and dry season climate of Ghana, we observed a trend of HCoVs circulation similar to a previous report for Ghana [45]. Consistent with high proportions of HCoVs identified in the harmattan season [45]. HCoV detection frequency was highest in February, a period within the harmattan season. The Greater Accra region had the majority of HCoV cases, 4 out of 7 (57%). The high population density observed in the Greater Accra (Ghana Statistical Service 2010) may be an important influence to this occurrence as respiratory infections are easily transmitted in populated communities [47].

Studies have suggested that the distributions of HCoV types vary between age groups [42,48]. A higher rate of HCoV detection has been reported among adults in the 50 to 59 yr. range [43]. Similarly, this study detected HCoVs more frequently among the adult age groups (Figure 2), though HCoV is considered one of the most common respiratory viruses associated with respiratory tract infections in children [26,37,40,49]. The highest number of HCoV positive cases was recorded for the 50-64 yr. age group (Figure 2) with HKU1 as the predominant HCoV type. As shown in Table 1, HCoVs were more frequently present in out-patient cases (2.5%) than in hospitalized cases (1%) with circulation of all four HCoV types in out-patient cases. The low detection rate in hospitalized cases may suggest the involvement of other respiratory pathogens; and this observation is similar to reports from Brazil [43,44]. A study in France reported upper respiratory



**Table 2.** Details of acute respiratory infection cases that were positive for human coronavirus

ID	Region	Age (yr.)	Sex	Symptoms aside fever	Sample type	Sample Date	HCoV Type
ILI-014	Greater Accra	51	F	Cough	OP	13-Mar-2013	229E
ILI-042	Western	15	F	Cough, Sore throat	OP	11-Jun-2013	NL63
ILI-060	Upper East	22	F	Breathing difficulty	OP	18-Oct-2013	HKU1
ILI-088	Greater Accra	35	M	Sore throat	OP	14-Feb-2014	HKU1
ILI-090	Greater Accra	45	M	Cough, Sore throat, Head ache	OP	20-Feb-2014	OC43
SARI-019	Greater Accra	5	M	Cough, Coryza	OP & NP	19-Nov-2013	229E
SARI-084	Northern	57	F	Cough, Sore throat, Headache, Breathing difficulty	OP & NP	17-Feb-2014	HKU1

\*F, female; M, males; OP, oropharyngeal; NP, nasopharyngeal; HCoV, human corona virus

tract infections (URTI) as the most common clinical presentation during HCoV infections [42]. Human CoVs have been suggested to play significant role in upper respiratory tract infections among adults and older children in rural areas of Ghana [45]. All HCoVs were detected in patients with URTI confirming the role of HCoV in URTI. Clinical signs reported in HCoV associated ARI included fever, cough, sore throat and headache, which is consistent with other studies [27-29,50]. Negative findings for MERS-CoV in these hospitalized patients with ARI is indicative that the virus was not in circulation in Ghana in March 2014, consistent with a previous report [45].

The World Health Organization (WHO) had recommended retrospective testing of respiratory specimens from patients with unexplained respiratory disease ("Guidelines for Investigation of Cases of Human Infection with Middle East Respiratory Syndrome Coronavirus" July, 2013). There is serological evidence suggesting the circulation of MERS-CoV or MERS-like CoV in dromedary camels before March 2012 in Nigeria and other Eastern and Northern African countries [51-53]. However, there are still no reports of MER-CoV in humans in West Africa which is concordant with findings of this study in Ghana. Our study had some limitations. The selection of samples from patients with influenza-like illness that were negative for influenza virus may have underestimated the prevalence of coronaviruses and excluded the detection of co-infections with influenza viruses. Only a snapshot was achieved rather than a full profile of HCoV which requires a systematic collection of samples over a long period of time. Despite the above limitations, this study has shown that HCoVs are associated with ARI in Ghana. Results from this study provide some insight into human coronavirus infections in the Ghanaian population. Lack of evidence for the presence MERS-CoV in hospitalized patients with ARI indicates that the virus was not circulating in Ghana as at March 2014. Findings from this study show the need for enhanced and continuous surveillance for MERS-CoV and other HCoVs in Ghana [54,55,56]. This is the first study that reviews the prevalence of coronavirus at a national level.

## Conclusion

In view of the brewing coronavirus global pandemic, the results of this study assume national and regional significance in that it provides a baseline reference to allow risk stratification and

evaluation of any potential arrival of the novel coronavirus infections within the borders of Ghana and the West African sub region. Currently, all countries are required by the International Health Regulations (2005) to implement policies for active surveillance, early detection, isolation and case management, contact tracing and prevention of spread of 2019-nCoV. Countries are also required to share quickly their data with the WHO to ensure public safety. The number of countries affected and the type of global attention it has received suggest that this novel coronavirus could rapidly become a worldwide pandemic [56].

## DECLARATIONS

### Ethical considerations

Ethical approval for the study (Protocol identification number MS-Et/M.9-P3.1/2013-2014) was given by the Ethical and Protocol Review Committee, University of Ghana College of Health Sciences, Accra, Ghana.

### Funding

None

### Competing Interests

No potential conflict of interest was reported by the authors.

### Author contributions

PKP developed and conducted study, JAAM supervised the study, MN coordinated the study, MA, KB, IA, EYB, KB, BS, KK, SAO assisted with conduct of study and paper writing, BSA supported paper writing and WKA developed and supervised the study.

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### Availability of data

Data is available upon request to the corresponding author.



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