RESEARCH ARTICLE

Surveillance of endemic human coronaviruses (HCoV-NL63, OC43 and 229E) associated with pneumonia in Kilifi, Kenya [version 1; peer review: 2 approved with reservations]

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Abstract

Introduction: Human coronaviruses (HCoVs) circulate endemically in human populations, often with seasonal variation. We describe the long-term patterns of paediatric disease associated with three of these viruses, HCoV-NL63, OC43 and 229E, in coastal Kenya.
Methods: Continuous surveillance of pneumonia admissions was conducted at the Kilifi county hospital (KCH) located in the northern coastal region of Kenya. Children aged <5 years admitted to KCH with clinically defined syndromic severe or very severe pneumonia were recruited. Respiratory samples were taken and tested for 15 virus targets, using real-time polymerase chain reaction. Unadjusted odds ratios were used to estimate the association between demographic and clinical characteristics and HCoV positivity.
Results: From 2007 to 2019, we observed 11,445 pneumonia admissions, of which 314 (3.9%) tested positive for at least one HCoV type. There were 129 (41.1%) OC43, 99 (31.5%) 229E, 74 (23.6%) NL63 positive cases and 12 (3.8%) cases of HCoV to HCoV coinfection. Among HCoV positive cases, 47% (n=147) were coinfected with other respiratory virus pathogens. The majority of HCoV cases were among children aged <1 year (66%, n=208), though there was no age-dependence in the proportion testing positive. HCoV-OC43 was predominant of the three HCoV types throughout the surveillance period. Evidence for seasonality was not identified.
Conclusions: Overall, 4% of paediatric pneumonia admissions were associated with three endemic HCoVs, with a high proportion of cases co-occurring with another respiratory virus, with no clear seasonal pattern, and with the age-distribution of cases following that of pneumonia admissions (i.e. highest in infants). These observations suggest, at most, a small severe disease contribution of endemic HCoVs in this tropical setting and offer insight into the potential future burden and epidemiological characteristics of SARS-CoV-2.

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Keywords
Human coronavirus, NL63, OC43, 229E

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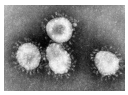

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Introduction

To date, seven human coronaviruses (HCoVs) have been identified, of which four (NL63, HKU1, OC43 and 229E) are known to be endemic among humans[1–4]. Endemic HCoV types are detected in a small but non-negligible proportion of respiratory tract infections; mild cases occur across a wide age-range and severe disease is predominant in young children and the elderly[5–8]. A further three HCoVs have emerged in recent years and caused epidemics: SARS-CoV the agent of severe acute respiratory syndrome in China[9], MERS-CoV the cause of Middle East respiratory syndrome in the Middle-East[10] and most recently SARS-CoV-2, the aetiological agent of the current pandemic of coronavirus disease 2019 (COVID-19)[11]. To date, there are limited preventive options against HCoV infections and no effective anti-viral treatment[12]. Understanding the epidemiology of HCoVs can play a critical role in prediction, prevention and control of HCoV infection. In addition, data on endemic HCoVs may inform expectations for SARS-CoV-2 if it becomes endemic. Our study aims to describe the circulation patterns of HCoVs (OC43, 229E, and NL63) over time using data from a long-term surveillance programme in a rural coastal setting in Kenya, 3° south of the equator.

Methods

Study setting

A prospective study was established in 2007 for the long-term continuous respiratory virus surveillance among pneumonia admissions to Kilifi County Hospital (KCH)[6] in order to develop improved epidemiological understanding, estimate disease burden and provide suitable baseline data for future vaccine studies. KCH is the referral hospital within the Kilifi Health and Demographic Surveillance System (KHDSS), in the northern coastal region of Kenya[13]. The location experiences two rainy seasons approximately from April to July and October to December, with median maximum temperature of 33°C (IQR: 31–36), median minimum temperature of 23°C (IQR: 22–24), and median relative humidity of 78% (IQR: 71–87) (unpublished weather station data). Children aged 1 day to 59 months admitted to KCH with clinical symptoms of severe or very severe pneumonia were recruited. Written informed consent was sought from parents/guardians of the children prior to sample collection. In this paper we define severe pneumonia as history of cough or difficulty breathing and chest indrawing while very severe pneumonia is defined as history of cough or difficult breathing and at least one of inability to feed, prostration, unconsciousness, or oxygen saturation of <90% by fingertip pulse oximetry. We use the term pneumonia to refer to all cases of clinically severe or very severe pneumonia[14]. The variables extracted from the hospital surveillance database include; Demographic characteristics (sex, KHDSS residency status, age), presence/absence of clinical features (history of cough, difficulty breathing, cyanosis, nasal flaring, chest indrawing, crackles, wheeze, inability to drink, vomits everything, fever defined as axillary temperature ≥37.5°C , oxygen saturation levels, conscious level: agitated, lethargic, prostration or unconscious, pneumonia status: severe or very severe), laboratory test results for RSV (A and B), rhinovirus, HCoVs (NL63, OC43, 229E), influenza (A, B and C), parainfluenza virus (1–4), adenovirus, and human metapneumovirus, and hospitalisation outcomes (admission to the high dependency unit, discharge outcomes; alive or dead)

Laboratory methods

Specimens collected between January 2007 and December 2019 were processed and screened for three HCoVs (OC43, NL63 and 229E) and at least 12 other respiratory viral pathogens using real-time polymerase chain reaction (RT-PCR). Sample testing was initially performed in 2007 using the LightCycler Fast Start DNA MasterPLUS HybProbe kit (Roche)[6], then multiplex RT-PCR using Qiagen Quantifast multiplex RT-PCR kit (Qiagen, United Kingdom) in triplex sets on an ABI 7500 system, from January 2007 until the present day[15,16]; additionally, a proportion of samples were tested using a 33-pathogen multiplex quantitative PCR (FTD Resp-33, Fast Track Diagnostics, Sliema, Malta) as part of the multi-country PERCH study[17], between August 2011 and December 2013. A variety of collection methods was used: nasopharyngeal flocked swab, nasal wash or combined nasopharyngeal swab and oropharyngeal swab.

Data analysis

Data analysis was done using STATA version 13.0 (Stata Corp, College Station Texas, USA). Summary statistics (counts, proportions, measures of central tendency and variation) are presented for continuous and categorical data as appropriate. We estimated unadjusted odds ratios to measure the association between demographic and clinical characteristics of the study participants and testing positive for HCoV. Three Poisson regression models, one for each HCoV type, were used to investigate the presence of seasonality. In the models a trend variable was included and residuals plotted against month. Identification of a strong pattern by visual inspection of the residual plots would suggest presence of seasonality. The chi-square test of proportional trends was used to test for a linear trend in the proportions of samples tested or not tested for HCoV over time. To check for an association between categorical variables the chi-square test of association or Fisher's exact test was used as appropriate. The analysis code is provided as *Extended data*[18].

Ethical approval

This study was approved by the Kenya Medical Research Institute Scientific Ethics Review Unit (Approval number: KEMRI/SERU/CGMR-C/027/3178).

Results

Characteristics of patients infected with HCoVs

During the 13 years of surveillance, there were 49,409 paediatric admissions of children aged 0–59 months at KCH. A total of 11,445 (23.2%) admissions were due to severe (n= 7808, 68%) and very severe (n=3637, 32%) pneumonia. Out of the eligible cases, 69.5% (n=7957) were tested for the three HCoVs while the remainder were not tested due to refusal of consent (13.8%), discharge (13%) or death (3.7%) prior to sample collection. Cases untested did not differ from those tested in age distribution or sex ratio, but were more likely to be very severe (40.0% versus 28.3%, Fishers exact P-value <0.001).

Of the 7957 samples tested, 5312 (66.7%) were aged <1 year, 1454 (18.3%) were aged 12–23 months, 620 (7.8%) were aged 24–35 months and 571 (7.2%) were in the 36–59 months age band. The proportion of tested individuals with elevated axillary temperature (≥37.5°C), cough and difficulty breathing was 58.3%, 83.2%, 92.6%, respectively. A total of 314 (3.9%) tested positive for at least one of the three HCoV targets. Among the HCoV positives, 129 (41.1%) had OC43, 99 (31.5%) had 229E, 74 (23.6%) had NL63 and 12 (3.8%) had coinfections between the three HCoV types. Among all the samples tested, the overall prevalence of NL63 was 1% (n=80), 1.7% for OC43 (n=137) and 1.4% for 229E (n=109).

The characteristics of the patients positive for any and for each HCoV type or infection combination are described in Table 1. HCoV positive cases were predominantly children aged <1 year (66.2%) and those aged 12–23 months (18.2%). The burden of infection with at least one HCoV, among all pneumonia admissions, was highest in infants and decreased with increasing age (2.6% for those under 1 year and 0.7% for 12–23 months). The same pattern was seen for each individual HCoV type (not shown). However, the proportion of samples testing positive (3.9%) for HCoV did not vary with age group (Fisher's exact p-value = 0.753). Among all HCoV positive participants, mean age was 11 months (median 7 months), there

Table 1. Demographic and clinical characteristics of study children (aged under 5 years) admitted with severe or very severe pneumonia to Kilifi County Hospital, Kilifi, Kenya 2007–2019, by HCoV type (n=314).

Variable		NL63 (n=74)	OC43 (n=129)	229E (n=99)	HCoVs coinfections (n=12)	Total (n=314)
		n (%)	n (%)	n (%)	n (%)	n (%)
Age (months)	Mean (SD)	11.9 (11.2)	11.0 (11.9)	12.1 (12.8)	6.9 (7.4)	11.4 (11.9)
	Median (IQR)	8 (3-20)	7 (2-15)	8 (2-19)	5.5 (2-8.5)	7 (2-16)
Age categories	0–11 months	49 (66.2)	87 (67.4)	61 (61.6)	12 (91.7)	208 (66.2)
	12–23 months	11 (14.9)	24 (18.6)	22 (22.2)	0 (0.00)	57 (18.2)
	24–35 months	11 (14.9)	8 (6.2)	9 (9.1)	1 (8.3)	29 (9.2)
	36–59 months	3 (4.0)	10 (7.8)	7 (7.1)	0 (0.00)	20 (6.4)
Sex	Female	22 (29.7)	60 (46.5)	36 (36.4)	6 (50.0)	124 (39.5)
	Male	52 (70.2)	69 (53.5)	63 (63.6)	6 (50.0)	190 (60.5)
Pneumonia status	Severe	49 (66.2)	101 (78.3)	76 (76.8)	10 (83.3)	236 (75.2)
	Very severe	25 (33.8)	28 (21.7)	23 (23.2)	2 (16.7)	78 (24.8)
Clinical presentation	Cough	55 (74.3)	112 (86.8)	85 (85.9)	12 (100.00)	264 (84.1)
	Breathing difficulty	67 (90.5)	118 (91.5)	94 (95.0)	10 (83.3)	289 (92.1)
	Fever*	43 (58.1)	75 (58.1)	54 (54.6)	10 (83.3)	182 (58.0)
	Prostrate/unconscious	17 (23.0)	18 (14.0)	14 (14.1)	1 (8.3)	50 (15.9)
	Chest Indrawing	65 (87.8)	122 (94.6)	89 (89.9)	12 (100.00)	288 (91.7)
	Wheeze	7 (9.5)	16 (12.5)	18 (18.2)	0 (0.0)	41 (13.1)
	Crackle	27 (36.5)	44 (34.1)	40 (40.4)	3 (25.0)	114 (36.3)
	Nasal flaring	40 (54.1)	66 (51.2)	54 (54.6)	6 (50.0)	166 (52.9)
	Shock*	12 (16.2)	10 (7.8)	6 (6.1)	0 (0.00)	28 (8.9)
	Hypoxemia (O_2 <90%)	12 (16.2)	14 (10.9)	13 (13.1)	1 (8.3)	40 (12.7)
	Cyanosis	1 (1.4)	1 (0.8)	1 (1.0)	0 (0.00)	3 (1.0)
	Inability to drink/feed	5 (6.8)	11 (8.5)	7 (7.1)	0 (0.00)	23 (7.3)
	Vomits everything	7 (9.5)	6 (4.7)	0 (0.00)	0 (0.00)	13 (4.14)
Duration of hospital stay	Mean (SD)	6.3 (6.8)	5.7 (6.1)	5.2 (5.5)	3.8 (2.3)	5.6 (6.0)
	Median (IQR)	4 (3-6)	3 (2-7)	3 (2-6)	6.5 (3-10)	4 (2-6)
Outcomes	HDU*	27 (36.5)	31 (24.0)	22 (22.2)	2 (16.7)	82 (26.1)
	Died	10 (13.5)	10 (7.8)	7 (7.1)	0 (0.00)	27 (8.6)

* Fever is defined as axillary temperature ≥37.5°C, shock is defined as capillary refill of >3 seconds, temperature gradient or weak pulse volume. HDU indicates study participants who were critically ill and were transferred from tde general ward to tde high dependency unit.

** tde high proportions of cough, breatding difficulty, chest indrawing, hypoxemia, prostrate/unconscious and inability to drink/feed among HCoV positive cases should be interpreted witd caution because these clinical signs form part of our study's eligibility criteria.

were fewer females than males and fewer with very severe compared to severe pneumonia. At least half of the HCoV positives presented with fever (58%) and nasal flaring (53%).

Clinical outcomes of HCoV-infected patients

Over a quarter of those positive for at least one HCoV were admitted to the high dependency unit and of those positive for OC43, 13.5% (n=10) died while 7.8% (n=10) and 7.1% (n=7) died of those positive for NL63 and 229E, respectively. A large proportion of these deaths were observed among those with underlying co-morbidities (Figure 1). None of the HCoVs were statistically significantly associated with any of the specific clinical signs or outcomes investigated (p-values>0.05) except death among NL63 cases (Table 2); however, we had limited power to detect associations given the small number of HCoV positive cases.

Co-infection with other respiratory viruses

About 47% (n=147) of the 314 HCoV cases were co-infected with other viral respiratory pathogens; respiratory syncytial virus (RSV) and human rhinovirus (HRV) jointly accounted for >50% of all HCoV coinfections with other pathogens. A similar coinfection pattern was observed for each HCoV tested (Figure 2). Throughout the surveillance period, there were three cases (one of NL63 and two of OC43) aged <1 year that were readmitted and tested positive for the same HCoV as the first admission. The NL63 readmission occurred 10 days after discharge from the first admission while the OC43 readmissions were at 3 and 21 days after discharge from the first admission. The NL63 case had a discharge diagnosis of neonatal sepsis for the first admission and gastroenteritis plus lower respiratory tract infection (LRTI) for the readmission. One of the OC43 cases had a discharge diagnosis of LRTI for both admissions while the other had immunosuppression plus malnutrition in the first and immunosuppression plus septicaemia for the second admission.

Temporal patterns of different HCoVs

NL63 and OC43 were observed fairly consistently throughout the surveillance period while fewer cases of HCoV-229E were observed from the middle of 2011 and it disappeared after 2016 (Figure 3). The highest numbers of cases were observed in the periods April to June for NL63, June to September for OC43 and January to March for 229E. Pooling data for all

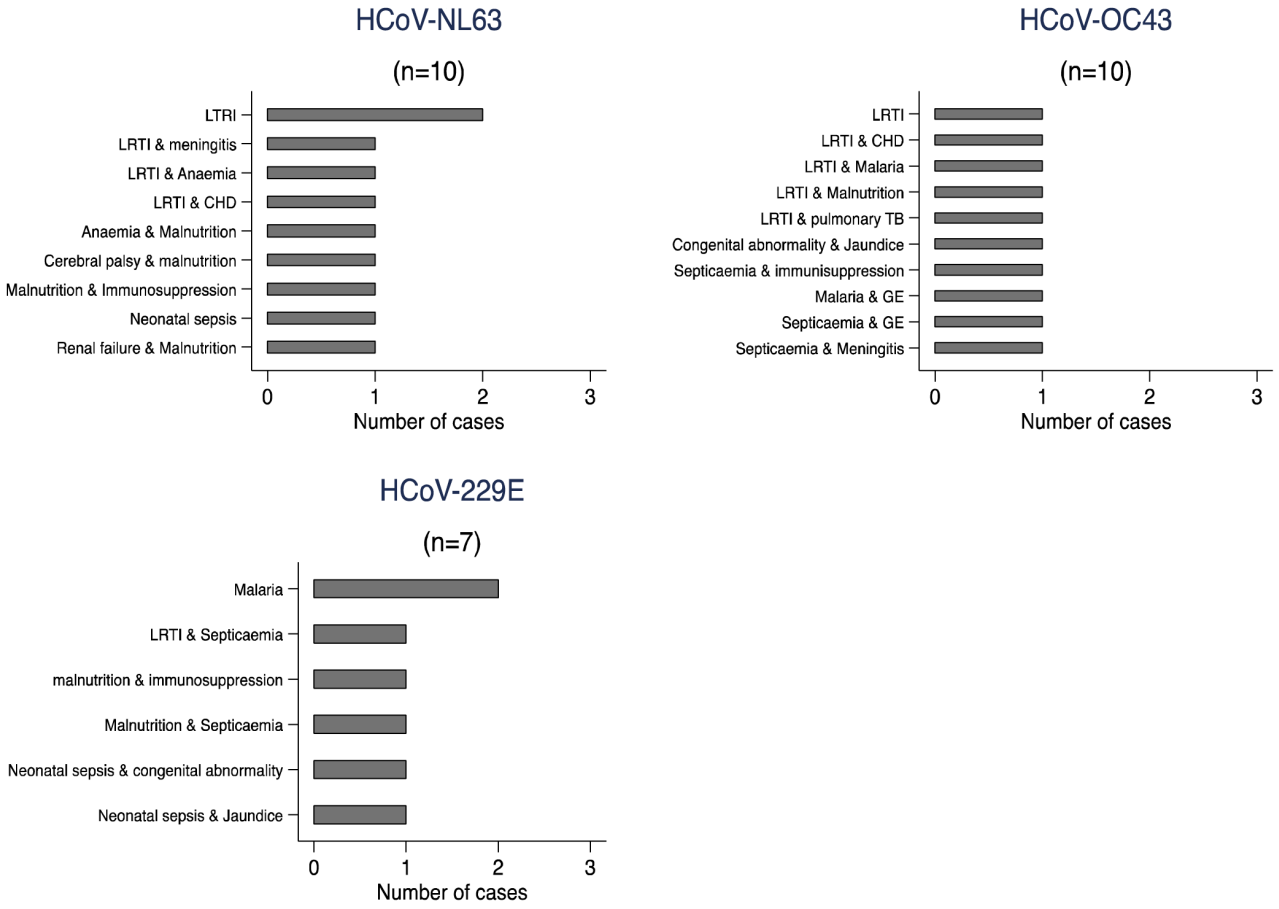

Figure 1. Frequency distribution of discharge diagnosis for mortality cases admitted to Kilifi County Hospital, Kilifi, Kenya 2007–2019, by HCoV type. LRTI=lower respiratory tract infection, CHD=congenital heart disease, TB=Tuberculosis, GE=Gastroenteritis.

Table 2. Unadjusted Odds Ratios (ORs) for demographic and clinical characteristics and the hospitalisation outcomes for children (under 5 years) admitted with severe or very severe pneumonia to Kilifi County Hospital, Kilifi, Kenya 2007-2019 by the 3 HCoV types.

		NL63		OC43		229E	
		OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	0-11 months	Ref		Ref		Ref	
	12-23 months	0.74 (0.39-1.42)	0.369	0.92 (0.59-1.45)	0.723	1.15 (0.71-1.86)	0.569
	24-35 months	1.92 (1.02-3.61)	0.043	0.72 (0.35-1.48)	0.371	1.23 (0.63-2.39)	0.547
	36-59 months	0.51 (0.16-1.65)	0.263	0.98 (0.51-1.89)	0.949	0.93 (0.43-2.03)	0.854
Sex	Female	Ref		Ref		Ref	
	Male	1.62 (1.01-2.60)	0.046	0.81 (0.58-1.14)	0.221	1.27 (0.86-1.88)	0.233
Cough	No	Ref		Ref		Ref	
	Yes	0.64 (0.38-1.08)	0.098	1.43 (0.86-2.39)	0.168	1.37 (0.78-2.42)	0.268
Breathing difficulty	No	Ref		Ref		Ref	
	Yes	0.63 (0.31-1.27)	0.196	0.83 (0.46-1.52)	0.544	1.38 (0.61-3.17)	0.440
Fever*	No	Ref		Ref		Ref	
	Yes	1.13 (0.72-1.78)	0.587	1.04 (0.73-1.46)	0.839	0.94 (0.65-1.38)	0.766
Prostration/ unconsciousness	No	Ref		Ref		Ref	
	Yes	1.44 (0.83-2.46)	0.189	0.85 (0.52-1.39)	0.517	0.84 (0.49-1.46)	0.543
Chest indrawing	No	Ref		Ref		Ref	
	Yes	0.68 (0.33-1.37)	0.279	1.62 (0.75-3.48)	0.217	0.86 (0.44-1.65)	0.64
Inability to feed	No	Ref		Ref		Ref	
	Yes	0.54 (0.22-1.35)	0.189	0.71 (0.38-1.32)	0.283	0.62 (0.29-1.34)	0.226
Hypoxemia (O_2<90%)	No	Ref		Ref		Ref	
	Yes	0.92 (0.51-1.67)	0.780	0.58 (0.34-0.99)	0.046	0.64 (0.34-1.14)	0.131
Pneumonia status	Severe	Ref		Ref		Ref	
	Very severe	1.22 (0.76-1.95)	0.406	0.71 (0.47-1.06)	0.094	0.71 (0.45-1.12)	0.143
Hospital stay	<= 4 days	Ref		Ref		Ref	
	> 4 days	1.06 (0.68-1.65)	0.807	0.85 (0.60-1.21)	0.373	0.72 (0.49-1.08)	0.110
Death	No	Ref		Ref		Ref	
	Yes	1.98 (1.02-3.87)	0.045	1.07 (0.56-2.05)	0.842	0.93 (0.43-2.03)	0.872

*Fever is defined as auxiliary temperature ≥37.5°C.

HCoVs, there were more cases in the colder months (May to September) than the hotter months (October to April) (Figure 4), as for OC43, but NL63 was more common in the first half of the year, and 229E in the second half of the year. However, time series models did not indicate a seasonal pattern for any of the HCoVs (Figure 5) over the years. The proportion of samples tested for HCoV did not change over time among those with severe pneumonia $\chi^2_{(1)} = 3.11$; p-value = 0.078) but changed among those with very severe pneumonia $\chi^2_{(1)} = 149.11$; p-value < 0.001).

De-identified raw data for this study are available as *Underlying data*[18].

Discussion

We have described the circulation patterns of endemic HCoVs (NL63, OC43 and 229E) in a long-term surveillance study of childhood pneumonia hospitalisations in coastal Kenya. We observed a small proportion of pneumonia admissions positive for one or more HCoVs (3.9%). While 65% of HCoV infections occurred in children in their first year of life (either cumulatively for all HCoVs or for each individual HCoV type), this reflected the age-distribution of pneumonia admissions to the ward. Hence, contrary to other reports[19], this suggests age is not a risk factor for coronavirus associated pneumonia hospital admission. Our reported prevalence is equivalent to that from a long-term hospital surveillance of seasonal coronaviruses

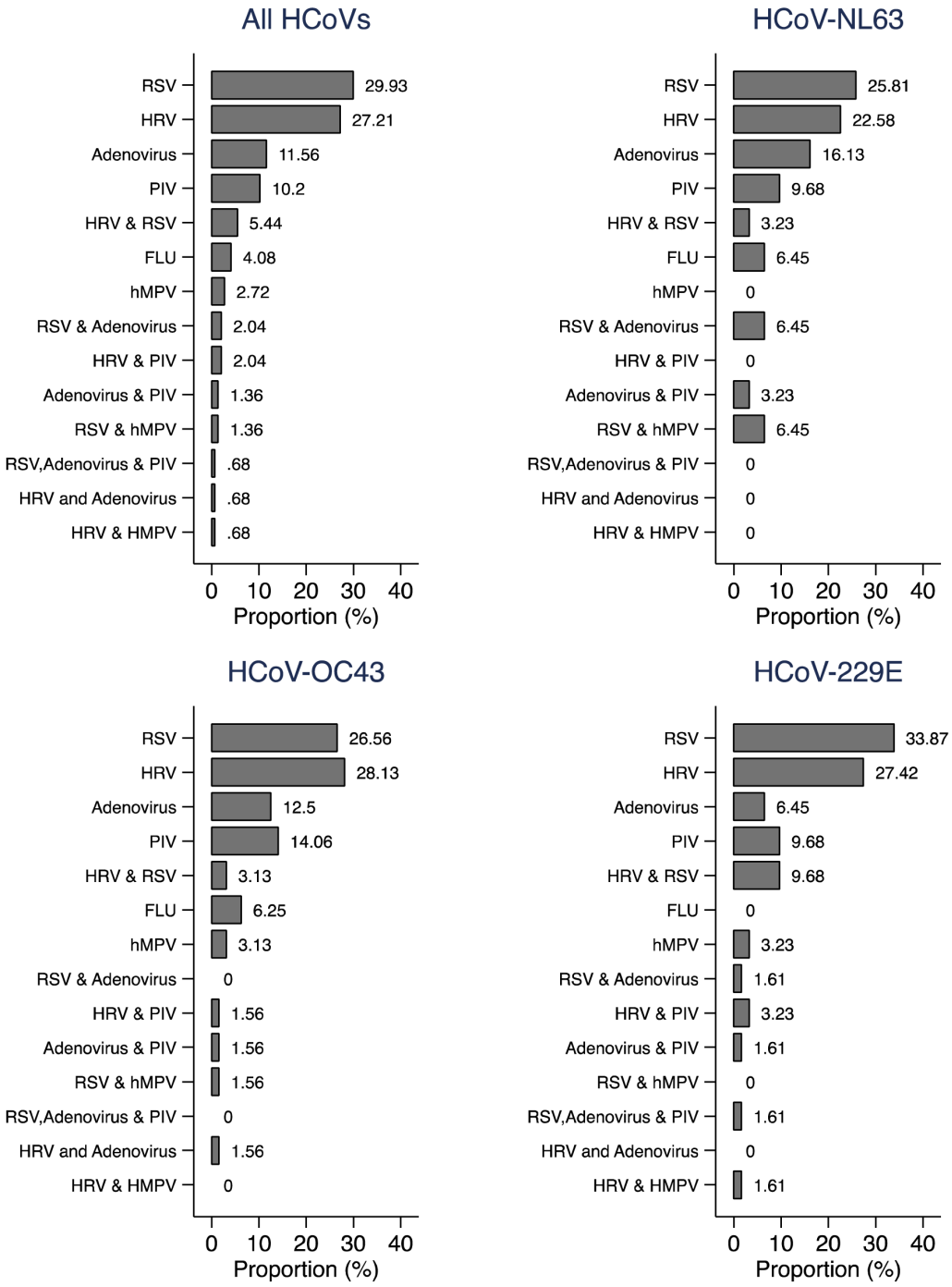

Figure 2. Percentage distribution of coinfections between HCoVs and other viral pathogens for pneumonia cases admitted to Kilifi County Hospital, Kilifi, Kenya 2007–2019. RSV=respiratory syncytial virus (A and B), HRV=human rhinovirus, PIV=parainfluenza, FLU=influenza (A, B and C), hMPV= human metapneumovirus.

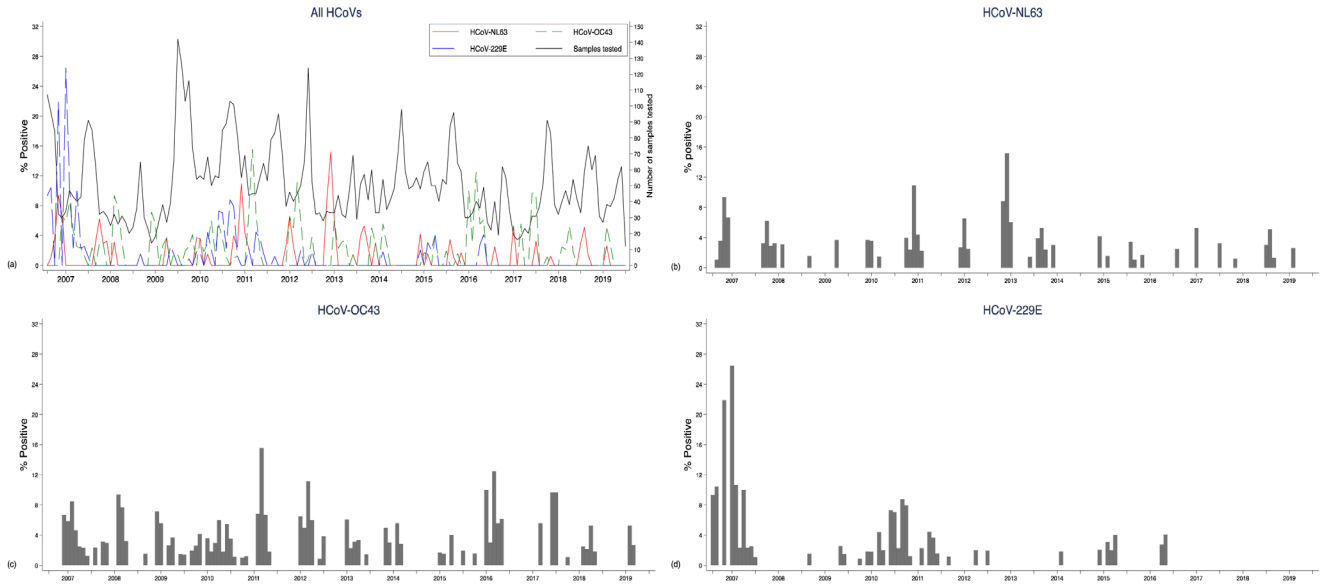

Figure 3. Monthly prevalence (%) pneumonia admissions at Kilifi County Hospital, Kilifi, Kenya 2007–2019 by HCoV type. The panel shows proportions for all HCoVs (**a**), NL63 (**b**), OC43 (**c**) and 229E (**d**).

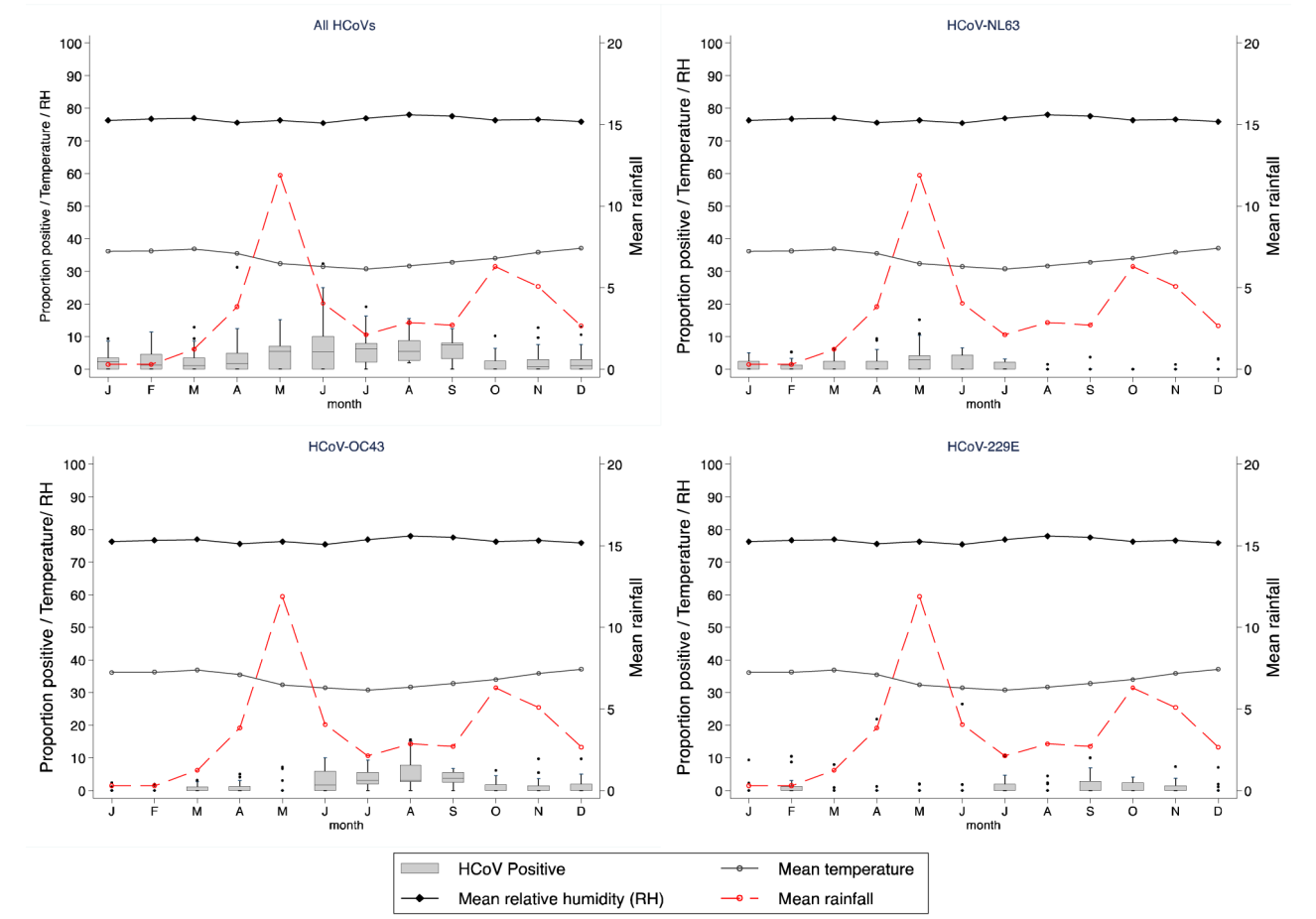

Figure 4. Proportion of monthly positive cases observed at Kilifi County Hospital, Kilifi, Kenya by HCoV type over a period of 13 years (2007–2019). The primary y-axis denotes the proportion of samples positive for HCoV and the average monthly maximum temperature in °C while the secondary y-axis denotes the average monthly rainfall in millimetres.

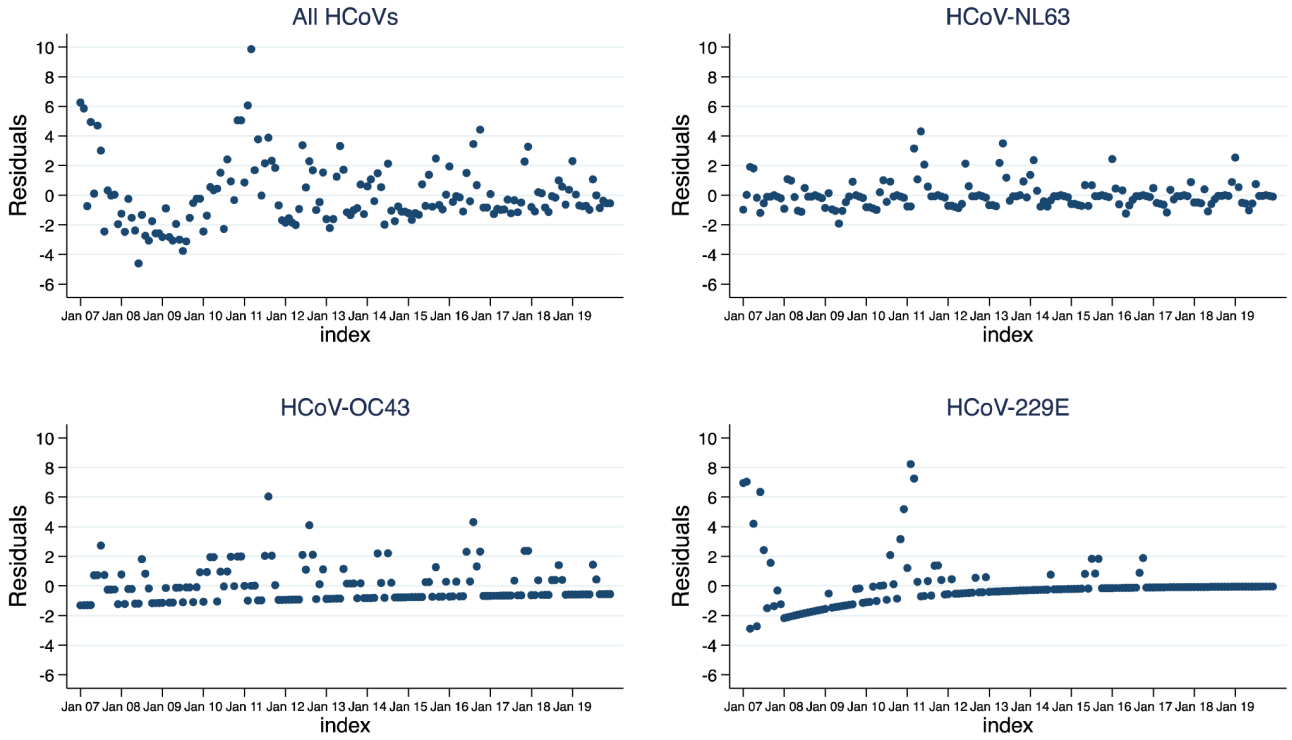

Figure 5. Plot of time series model residuals against month separated by HCoV type using pneumonia surveillance data at Kilifi County Hospital, Kilifi, Kenya 2007–2019. See *Methods* for statistical details.

in Scotland (4%)[8] but lower compared to a multi-centre study across Kenya for the same three HCoVs (26/417 6.2% versus 314/7643 3.9%; $\chi^2_{(1)} = 5.33$, p-value=0.021)[7]. This latter study included locations with a wide range of climate conditions that might influence prevalence; however, the study was not big enough to stratify by location.

We did not observe seasonal variation of HCoVs compared to some other respiratory viral pathogens like RSV, as previously reported from our site[14]. In addition, neither peak months for pneumonia admissions nor the long rain periods (April to July) in Kilifi translated to HCoV peaks. This is in contrast to data from temperate settings where seasonality of HCoVs has been reported[8,20–22], with increased occurrence during the colder winter months. The HCoVs we have studied are known to continuously circulate among humans[1], although in Kilifi we observed low numbers for all types. Of interest is that pneumonia associated with 229E admissions faded out in the later years of the surveillance. We attempted to investigate if this was due to primers or probe mismatches. For all the three tested endemic CoVs we did not observe significant mismatches on the primer/probe pairs against data available from GenBank database although this investigation suffered a limitation of few sequences available globally in recent years (2015–2019) and none from East Africa. With the highest numbers and consistent presence compared to NL63 and 229E, our results suggest that OC43 is the predominant HCoV type in the coastal region of Kenya.

The present study did not have a control group by which to assess an aetiological association between the HCoVs and pneumonia. In the PERCH multi-country case-control study[17] HCoVs contributed less than 1% of the etiological fraction. In our study the contribution to disease is not known (except for the relatively small set of samples from 2011–13 that were part of the PERCH study), but it is of note that around 50% co-occurred with another respiratory virus (most commonly with RSV), the risk was not age-dependent, there was no clear association between any of the viruses with the any of the specific clinical signs or outcomes investigated and in 26% of deaths with a HCoV detected there was a likely alternative diagnosis to pneumonia. While we have been able to sequence the virus from a proportion of the positive specimens[23,24], we cannot assume 100% specificity, and even a modest level of false positivity could account for many of the positive diagnoses and argues for caution in interpreting the prevalence estimates. Of relevance also is that few (~1%) of the 314 children positive for at least one HCoV were subsequently HCoV-positive readmissions. While this is a crude analysis which ignores censoring at the start and end of the surveillance, and alternative hospitals where patients may have been admitted, it might be an indicator of low probability of severe reinfection.

Over the surveillance period, we have changed our sample collection and testing methods. This is a limitation; we did not conduct a sensitivity analysis to compare the different PCR methods, and the addition of an OP swab increases the number

of viruses found by NP alone (by 14% for HCoVs)[15]. Some of the observed patterns may have been influenced by these changes. It should be noted that fever, neither history or elevated measured temperature at admission, was not an inclusion criterion for eligibility, which might have influenced the prevalence of HCoV. However, interestingly, only 58% of the HCoV positive cases had axillary temperature of >=37.5°C. A further limitation is that only a fraction (70%) of pneumonia cases was tested for HCoV. We have previously shown that those untested tend to be more severely ill and less likely to be virus positive[14]. The proportion tested has not substantially changed over time among individuals with severe pneumonia but changed among those with very severe pneumonia. Similarly, there was a significant age difference for those tested and those untested for HCoV across time.

In conclusion, in this tropical setting we find little evidence of a substantial aetiological contribution of these endemic HCoVs and no clear seasonal variations. As the pandemic of COVID-19 takes its course, it is of interest to speculate whether the SARS-CoV-2 virus will become endemic and continuously co-circulate in the human population with the existing HCoVs[7,25]. The epidemiology of endemic HCoVs can be used to inform our expectations of SARS-CoV-2 in childhood, its potential severity and inter-species interactions and competition.

Data availability

Underlying data

Harvard Dataverse: Replication Data for: Surveillance of endemic human coronaviruses (HCoV-NL63, OC43 and 229E) associated with pneumonia in Kilifi, Kenya. https://doi.org/10.7910/DVN/ZQ1DJY[18].

This project contains the following underlying data:

- KCH_paed_ARI_surv_pneumo (CSV). (De-identified underlying data for each patient in the study.)
- KCH_paed_ARI_surv_pneumo-1 (SAV). (De-identified underlying data for each patient in the study.)
- GPOtieno_HCOV_Codebook (PDF). (Data dictionary and codebook.)

The data have been de-identified, and hence lack personally identifiable information. To request access to additional variables from this dataset go to 'Data Governance' on http://kemri-wellcome.org/about-us/#ChildVerticalTab15 and submit an 'External Request' to the Data Governance Committee (dgc@kemri-wellcome.org).

Extended data

Harvard Dataverse: Replication Data for: Surveillance of endemic human coronaviruses (HCoV-NL63, OC43 and 229E) associated with pneumonia in Kilifi, Kenya. https://doi.org/10.7910/DVN/ZQ1DJY[18].

This project contains the following extended data:

- 1_descriptive_analysis (DO). (Scripts used to generate information in the tables, data on frequencies, HCOVs virus distributions and proportions.)
- 2_graph_outputs (DO). (Code used to generate charts in the paper.)
- 3_ORs (DO). (Code to fit univariable logistic regression models for each HCoV type.)
- 4_seasonality (DO). (scripts for analyses of HCoV type seasonality.)

Acknowledgments

We thank the study participants and their parents/ guardians, clinical and research team members of the Virus Epidemiology and Control group (http://virec-group.org/) who were involved at various stages of this study including sample collection and processing. This paper has been approved for publication by the director of the Kenya Medical Research Institute.

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Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 21 July 2020

https://doi.org/10.21956/wellcomeopenres.17592.r39427

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This study describes patterns of endemic human coronavirus detections over a thirteen year period among paediatric cases of pneumonia in coastal Kenya.

The authors should be commended for the impressive collation of clinical features, discharge diagnosis, and hospitalisation outcomes alongside multiplex PCR tests which is rarely available for a study spanning this length of time.

The manuscript is well written and clearly presented throughout. I do however have some concerns requiring clarification.

1. The authors mention in Methods that a variety of specimen types were collected. Could the authors clarify whether this is at the individual patient level? If relevant, I suggest authors describe how duplication of samples from individual patients was handled in the analyses (since this can affect probabilty of virus detection and violates statistical assumptions of independent data points).

2. Suggest authors add to Methods a brief outline of the changes in testing procedure over time (as mentioned in the Discussion) to aid interpretation of detection trends, as different specimens are likely associated with different probabilities of virus detection.

3. It is unclear why the laboratory screen omitted HCoV-HKU1. Suggest the authors include a comment on the anticipated influence on the overall detection of HCoV.

4. It is unclear whether exclusion of untested discharged patients (presumably milder cases) have led to an under-estimation of HCoV detection proportions. Suggest authors add a statement to describe any anticipated influence of this exclusion.

5. The % of pneumonia cases with HCoV detected is clearly low. However, it would be helpful if the authors could discuss this result in the context of other common respiratory viruses detected among cases of pneumonia in this setting, such as influenza and RSV.

6. Discussion page 6: "...suggests age is not a risk factor for coronavirus associated pneumonia hospital admission". The role of age as a risk factor for pneumonia cannot be determined here without a non-pneumonia control group. Suggest authors reword this statement and consider whether the lack of variation in age-specific proportions may potentially reflect (i) no variation in age-specific community incidence of infection in children under five, together with lack of variation in severity (likelihood of hospitalisation) across ages, or (ii) variation in age-specific community incidence by age but with a disproportionate probability of hospitalisation.

7. Discussion page 9: "...and in 26% of deaths with a HCoV detected there was a likely alternative diagnosis to pneumonia". This seems to suggest the role of HCoV on overall hospital burden may be under-estimated by pneumonia cases. Although not an aim of the study, I suggest the authors consider adding a brief comment regarding the generalisability of their findings to other clinical groups to place the results into a wider context.

8. Discussion page 9: Can the authors comment on the possibility that readmissions reflect prolonged virus shedding, rather than indicating reinfection? Longest duration between admissions of 21 days possibly bordering an anticipated peak detection within first few weeks (e.g. for SARS-CoV-2 [1]). Suggest authors consider adding a brief statement to include this possibility.

9. Discussion page 10: " fever... was not an inclusion criterion for eligibility, which might have influenced the prevalence of HCoV". Suggest authors make clear the anticipated direction of effect on the prevalence - presumably underestimation?

10. Discussion page 10: "...there was a significant age difference for those tested and those untested for HCoV across time." This statement seems to be at odds with Results page 3 "..cases untested did not differ from those tested in age distribution.." Suggest the authors reword as appropriate.

11. Conclusions page 10: "...little evidence of a substantial aetiological contribution...": The aetiological role of HCoV cannot be established without a control group (as the authors do mention on page 9) i.e. it is not known whether the prevalence of HCoV is lower among cases of ARI without pneumonia. Suggest authors rephrase to put the low HCoV detections within the context of relative contribution of pathogens/other causes to disease aetiology i.e. most pneumonia cases are attributable to other causes.

12. Although presumably outside the scope of this study to analyse, I suggest the authors consider commenting briefly on any potential role of coinfecting respiratory bacteria on HCoV-associated pneumonia in this population; bacterial coinfection may influence the chance of pneumonia/testing for viruses.

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

Yes

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

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

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

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

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Temporal and spatial dynamics of infectious diseases; epidemiology of viral respiratory infections

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

Reviewer Report 06 July 2020

https://doi.org/10.21956/wellcomeopenres.17592.r39270

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David P. Moore
[1] Medical Research Council, Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand, Johannesburg, South Africa
[2] Department of Paediatrics and Child Health, Chris Hani Baragwanath Academic Hospital, University of the Witwatersrand, Johannesburg, South Africa

Thank you for the opportunity of reviewing this well-written manuscript by the accomplished research team at Kilifi. They describe the prevalence of endemic human coronavirus (HCoV) pneumonia hospitalisations in children <5 years of age in Kilifi over a 13-year surveillance period.

Major comments:

1. Why was HCoV-HKU1 not included in the surveillance of endemic human coronaviruses in this study? Lack of testing for HCoV-HKU1 should be mentioned as a limitation of the study.

- PERCH samples were included in the analysis, which would have included HCoV-HCoV-HKU1: mention should be made that, as HKU1 was only tested for in the 24-month PERCH surveillance period, it was considered to be under-represented over the study time period and was therefore not included in the analysis.

2. Mention is made of a variety of specimen types that were tested for respiratory viruses in the study. Is there evidence to suggest that any one type gives better yields than others? This should be elaborated on briefly in the discussion section, potentially as a limitation of the study.

3. The authors mention that an alternative attribution could be made in 26% of cases that died with a positive detection of HCoV in their study (page 9, left hand column, third sentence); however, this in not explored at all in the Results Section. This should be presented in the Results section, so as to contextualise the assertion made in the Discussion.

Minor comments:

1. Suggest change the title to reflect that paediatric pneumonia was the focus of attention in this study. Suggest “Surveillance of endemic human coronaviruses (HCoV-NL63, OC43 and 229E) associated with childhood pneumonia in Kilifi, Kenya”.

2. Correct the redundant double comma in affiliation 2 at the top of the title page of the manuscript.

3. In the results section of the Abstract, qualify that 3.9% of the pneumonia admissions tested positive for at least one of the three endemic HCoVs surveyed in the study.

4. It is unclear what is meant by “no age-dependence in the proportion testing positive” for HCoV in the abstract. Suggest omit this ambiguous phrase. Rather state “… n=208), and there was no clear seasonal pattern of HCoV infection. HCoV-OC43 was predominant…”

5. Suggest reword the Abstract conclusions as “… co-occurring with another respiratory virus, no clear seasonal pattern, and with the age-distribution…”

6. Suggest omit the speculation as to the impact of SARS-CoV-2 at the end of the last sentence of the Abstract.

7. At the end of the Introduction, suggest reword as “Our study aims to describe the circulation patterns of three of the endemic HCoVs (OC43, 229E, and NL63) over time…”

8. Suggest omit the definite article in the first sentence of the Methods section, i.e. “… in 2007 for long-term continuous respiratory virus surveillance among…”

9. Consider amending slight punctuation errors in page 3, including:
   - Left column, six lines from the bottom: “… ≥37.5°C, oxygen…”
   - Right column, second line: “… alive or dead).”

10. There are two mentions of which viruses were tested for on page 3: suggest concatenate into one mention (probably best placed in the “Laboratory methods” paragraph). Reword at the bottom of page 3 on the right hand column to “… or very severe), laboratory test results, and hospitalisation outcomes…”

11. The link to code in *Extended data* is not currently active in the manuscript: please correct this.

12. At the bottom of page 3, suggest reword as “Untested cases did not differ… more likely to have very severe pneumonia (40.0% versus 28.3%; X^2=152.5, P<0.001).”
    - Why resort to the use of Fisher’s exact P-value in the analysis of tested and untested cases (the Chi-square P-value is every bit as “significant”)?
13. Children are not “samples”: suggest reword the sentence at the top of page 4 as “Of the 7957 children tested, 5312 (66.7%) were aged <1 year, 1454…”

14. Suggest clarify the meaning of the second sentence on page 4 as follows: “The proportion of tested individuals with fever (axillary temperature ≥37.5°C), cough, and difficulty breathing was 58.3%, 83.2% and 92.6%, respectively.”

15. On page 4, suggest reword the third last line on the left hand column as “… testing positive for HCoV (3.9%) did not vary with age group…”

16. In Table 1, suggest present Age (months) as median and interquartile range only.

17. In Table 1, rather use the term “Difficulty breathing” which is more widely used than “Breathing difficulty”, also use “Prostration” rather than “Prostrate/unconscious”.

18. There are typos in the footnote to Table 1: “tde” should be “the”; “breatding difficulty” should be “difficulty breathing”; “witd” should be “with”.

19. Suggest reword the first sentence under the heading “Clinical outcomes of HCoV-infected patients” on page 5 as: “… positive for at least one of the three endemic HCoVs investigated for in this study were admitted to…”

20. In Figure 1, the HCoV-OC43 figure y-axis requires correction of spelling of the word “immunosuppression”; the HCoV-229E figure requires change of the “Malnutrition & immunosuppression” label on the y-axis to have an initial capital “M”.

21. Suggest reword the first sentence under the heading “Temporal patterns of different HCoVs” on page 5 as: “… middle of 2011, and were not detected subsequent to 2016 (Figure 3).”

22. In Table 2, suggest add in an analysis for all HCoVs combined.

23. Suggest reword the first sentence of the Discussion as “… patterns of three endemic HCoVs (NL63, OC43 and 229E) in a… positive for one or more of the HCoVs that were investigated for (3.9%).”

24. Check the calculation of the comparison between Sipulwa et al and the current study, in the second line on the right hand column on page 9: I get X^2=3.92, p=0.048.

25. In the second sentence of the second paragraph on the right column on page 9, suggest reword to “… nor the long rainy periods (April to July) in Kilifi were associated with HCoV peaks.”

26. Suggest reword the fifth sentence in the right hand column on page 9 as “Of interest is that pneumonia associated with 229E was not detected in the later years of surveillance.”

27. Please correct the spelling or “axillary” in the footnote to Table 2.

28. The authors do not consider differences in participant recruitment as being a potential explanation as to why 229E-associated pneumonia was not detected subsequent to 2016: were there systematic differences in participant recruitment strategies that might have impacted on detection of this virus?

29. Suggest reword the third sentence on the right hand column on page 10 as “It should be noted that fever, either on history or as measured at the time of admission, was not an inclusion criterion…”

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

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

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

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

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

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Paediatric pneumonia aetiology; paediatric infectious diseases.

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