

Seroprevalence of Human Coronaviruses Among Patients Visiting Hospital-Based Sentinel Sites in Uganda

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Abstract

Background: Human coronaviruses are causative agents of respiratory infections with several subtypes being prevalent worldwide. They cause respiratory illnesses of varying severity and have been described to be continuously emerging but their prevalence is not well documented in Uganda. This study assessed the seroprevalence of antibodies against the previously known human coronaviruses prior 2019 in Uganda.

Methods: A total 377 serum samples collected from volunteers that showed influenza like illness in five hospital-based sentinel sites and archived were analyzed using a commercial Qualitative Human Coronavirus Antibody IgG ELISA kit. Although there is no single kit available that can detect the presence of all the circulating coronaviruses, this kit uses a nucleoprotein, aa 340-390 to coat the wells and since there is significant homology among the various human coronavirus strains with regards to the coded for proteins, there is significant cross reactivity beyond HCoV HKU-39849 2003. This gives the kit a qualitative ability to detect the presence of human coronavirus antibodies in a sample.

Results: The overall seroprevalence for all the sites was 87.53% with no significant difference in the seroprevalence between the Hospital based sentinel sites ($p=0.8$). Of the seropositive, the age group 1-5 years had the highest percentage (46.97), followed by 6-10 years (16.67) and then above 20 (16.36). The volunteers were divided into two broad categories, those below five years and those above five years, to calculate the odds ratio. The odds ratio of those seropositive with an age above 5 years with reference to those below 5 years was 0.62. This shows that those below 5 are more likely to be seropositive compared to those above 5 years. The seropositivity was generally high throughout the year with highest being recorded in March and the lowest in February and December.

Conclusions: The seroprevalence of Human coronaviruses is alarmingly high which calls for need to identify and characterize the circulating coronavirus strains so as to guide policy on the control strategies.

Background

Human coronaviruses are common causative agents of respiratory infections with several subtypes being prevalent in many parts of the world. Coronaviruses are a complex group of viruses of the subfamily *Coronavirinae* in the family *Coronaviridae* of the order *Nidovirales* (1, 2). This subfamily has four genera: Alphacoronaviruses, Betacoronaviruses, Gammacoronaviruses and Deltacoronaviruses, of which Alphacoronaviruses (HCoV-229E and HCoV-NL63) and Betacoronaviruses (HCoV-HKU1, SARS-CoV, HCoV-OC43 and MERS-CoV) infect humans (3, 4). They are enveloped with a linear, non-segmented, positive sense, single-stranded RNA genome ranging between 27kb to 32kb which shows that they are the largest among the RNA viruses (5, 6). Although they are phenotypically and genotypically diverse, they possess a common genomic organization with the replicase gene occupying two thirds of 5' end of the genome in which two overlapping large open reading frames, ORF1a and ORF1b are found (5, 7). The

ORF1b is the most highly conserved part of the genome encoding for conserved roles such as polymerase and helicase activities (8, 9). The remaining one third of the genome at the 3' end carries genes that encode for a set of structural proteins in the order 5'-spike (S) to envelope (E) to membrane (M) and lastly nucleocapsid (N) (5). The Nucleocapsid protein binds to the genome and elicits a humoral immune response because it contains several linear epitopes (10) while the spike protein which is the largest, forms oligomonomers on the virus surface.

Human coronaviruses were first discovered in the 1960s as causative agents of self-limited upper respiratory tract infections and until 2002, they were known to cause mild infections but this changed with the emergence of Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) (11, 12). To date there are seven known Human coronaviruses (HCoVs) that have been identified, these are HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV, MERS-CoV and the newest discovered, SARS-CoV-2 (4, 13). The first four HCoVs have been well known to be of worldwide distribution causing approximately 33.3% of human common cold infections (14). However in some cases, these can cause severe illness in the elderly, children and all immunocompromised persons and patients especially those with underlying infections medical conditions like diabetes, hypertension, tuberculosis and AIDs (15, 16). SARS-CoV suddenly emerged in Guangdong Province of China in 2002 causing severe pneumonia characterized by fever, headache and cough but later develops into life threatening respiratory failure and distress (17). The first cases to be diagnosed with SARS were food handlers and workers in wet live-animals' markets, but soon the disease spread to several countries in a short period infecting more than 8000 people with a mortality rate of 10–11% (18–20). SARS-CoV is believed to have been spilled over to human populations from civets that are intermediate host for SARS-CoV since it has been traced back to bats as their natural hosts (21). After ten years, in 2012, MERS-CoV appeared in Saudi Arabia causing severe human respiratory disease with clinical presentation similar to SARS-CoV but with a higher fatality rate of 35% (22, 23). MERS-CoV found its way into human population from bats via dromedary camels but unlike SARS-CoV, cases of MERS-CoV infections have been restricted to the Arabian Peninsula and cases outside this Peninsula like the outbreak that occurred in South Korea, the index patient had traveled to Saudi Arabia and United Arabs Emirates thus tracing the source of infection back to the Arabian Peninsula (24, 25). Currently the world is facing a pandemic caused by a novel human coronavirus that started from the Hubei Province in China and now has spread the whole world, this novel coronavirus that had been previous named 2019-nCoV is now known as SARS-CoV-2 due to its similarity to the symptoms induced by SARS-CoV(26–28). Initial studies of SARS-CoV-2, linked the infection to a wet wild animal market in Wuhan but genetic analysis have shown that it is similar to a coronavirus that had been isolated from bats but transmitted to human through a yet to be confirmed intermediate host (29). Nevertheless, studies have showed that human to human transmission through droplets and direct contact has been the most important mode of transmission to regions outside Hubei especially by asymptomatic carriers traveling from one area to another(30, 31).

Seroprevalence studies are important in understanding the prevalence of subclinical human coronavirus infections and the population's herd immunity against these viruses. The Seroprevalence of human coronaviruses varies greatly among studies because of the different antigens, methodologies used, age

and other demographic characteristics of the population studied (32). The Seroprevalence estimates for Human coronaviruses range from 5–30% of all respiratory infections with up to 21.6% of the general population having serum antibodies (33, 34). In a previous study to estimate the exposure level of individuals to the circulating coronaviruses in the U.S. Metropolitan population, established 91.3%, 59.2%, 91.8% and 90.8% seropositive for HCoV 229E, HKU1, NL63 and OC43 respectively which is similar to studies that showed 99%, 91%, 98% and 100% seropositive for the respective human coronaviruses (32, 35). These findings are consistent with the cross-sectional and longitudinal seroepidemiological studies that have showed that large proportions of children and adults have serum antibodies to these four non-SARS human coronavirus strains(7). The Seroprevalences of the zoonotic human coronaviruses (SARS, MERS and SARS-2) have been reported to be below 5% in humans especially among those who do not come into contact with the intermediate hosts for these viruses (36–39). Such results suggest that unknown asymptomatic and subclinical infections or unrecognized cases might exist in the general population that can underscore the role of human to human transmission.

Previous studies have also shown that Enzyme-linked immunosorbent assays are useful in diagnosis of HCoV infections with the biggest challenge being cross reactive antibodies that can give rise to false positives especially when the study aims at detecting a specific virus strain. Several studies have made it clear that cross reactivity on serological testing occurs especially when the target is the nucleocapsid proteins (40, 41).The nucleocapsid protein has been recognized as an important target in the development of human coronavirus diagnostics because it induces a good antibody response (42). However, this nucleocapsid protein has a highly conserved region that occurs in the N-terminal portion which induces cross reactivity antibodies among human coronaviruses (34, 43, 44). Whereas this cross reactivity is a setback, diagnostic kits that detect antibodies to the nucleoprotein can have a qualitative screening role during testing of samples. Compared to the nucleocapsid protein, the spike protein contains multiple conformational epitopes that are major inducers of neutralizing antibodies. These antibodies are more specific and more recommended in the confirmatory testing of a particular type of human coronavirus because the spike protein has the least sequence conservation among human coronavirus proteins(33, 45, 46).

Human coronavirus infections are commonly diagnosed by polymerase reaction using cDNA synthesized from RNA extracts from respiratory tract samples. However, for the establishment exposure rates among the population, seroepidemiological studies offer an important avenue for painting a picture on HCoV infections. Although an increasing threat of zoonosis and emerging pandemics caused by HCoVs is now more obvious than ever, there is little known about their seroprevalence in most developing countries especially Uganda. Here we report the seroprevalence of human coronavirus antibodies in hospital-based surveillance sentinel sites in Uganda before the global pandemic of SARS-CoV-2.

Methods

The study was conducted among the Makerere University Walter Reed project established hospital-based sentinel sites for surveillance activities on acute febrile illnesses. Samples were obtained from the five

hospital-based sites were established for surveillance which include Jinja (Eastern Uganda), Mulago (Central Uganda, Capital), Bwera (Western Uganda), Gulu (Northern Uganda) and Bombo Military hospital (Central Uganda) (Fig. 1).

Patients reporting to these sites aged 6 months or older presenting influenza like illness (ILI) were enrolled after written and signed consent for the adults while for minors, written and signed consent was obtained from their parents or legal guardians. All methods in this study were carried out in accordance with relevant guidelines and regulations. Blood samples were drawn from the volunteers and transported to the Emerging Infectious Diseases Laboratory. The serum samples were kept at -20°C . An ILI patient was defined as any individual having a fever of temperature 38°C and above, plus clinical signs including cough, sore throat, myalgia and headache according to the established guidelines by WHO with modifications in other studies(47). Demographic characteristics of the patients and other related illness and symptoms were recorded on a standardized form.

The archived serum samples were retrieved, thawed to room temperature and tested using a commercial Qualitative Human Coronavirus IgG ELISA kit Cat. No. MBS9301037 (renamed HCoV-HKU-IgG ELISA kit) from MyBiosecure. The kit uses a nucleoprotein, aa 340–390 to coat the wells and according to the manufacturer, since there is significant homology among the various human coronavirus strains with regards to the coded for proteins, there is significant cross reactivity beyond SARS-CoV HKU-39849 2003. This gives the kit a qualitative ability to detect the presence of human coronavirus antibodies in a sample. The assay was done according to manufacturer's recommendation. Briefly, the reagents and samples were brought to room temperature, the positive and negative control wells as well as the sample wells were set. 50 μl each of the positive control, negative control and undiluted samples were added to respective wells. 100 μl of HRP- conjugate reagent was added to all the wells, covered with an adhesive strip and then incubated for 60 minutes at 37°C . The plates were then washed four times after which 50 μl of Chromogen Solution A and 50 μl of Chromogen Solution B was added to each well successively, mixed gently and incubated for 15 minutes at 37°C when protected from light. 50 μl of stop solution was added to each well and then Optical Density read at 450 nm using an ELISA reader at five minutes after the addition of the stop solution. The cut off for positivity were calculated per assay as the average OD value for the negative control wells + 0.15.

Results

Demographic and clinical characteristics of enrolled volunteers

A total of 377 samples were randomly selected from 1485 samples that had been collected from the Sentinel sites between 2017 and 2018. The samples size of 377 was computed using the formula adapted from Veterinary Epidemiology by Michael Thrusfield at 95% confidence interval, prevalence of 50% (Xiuping et al., 2007) and precision of 5%. The demographic profile of the samples is summarized in

Table1. Majority of the samples (45%) were from volunteers of 1–5 years of age and were gender balanced (48.8% males, 51.2% females).

Table 1
Demographic characteristics of the samples

Demographic Characteristic	Numbers (%)
Gender	
Male	184 (48.8%)
Female	193 (51.2%)
Age	
< 1	28 (7.4%)
1–5	171(45.4%)
6–10	68 (18.0%)
11–15	27 (7.2%)
16–20	22 (5.8%)
> 20	61 (16.2%)
Activity	
Pre-school Children	160 (42.4%)
Schooling /Students	139 (36.9%)
Employed	30 (8.0%)
Unemployed	48 (12.7%)

Seropositivity Among The Samples

Of the 377 serum samples analyzed, 330 (87.53%) were seropositive while 47 (12.47%) were seronegative (Table 2). There was no significant no significant difference in the seroprevalence of Human Coronavirus antibodies in the different sentinel sites (95% CI, p-value = 0.8).

Table 2
Seroprevalence in different sites

Site	Seropositive Positive	Seronegative Negative	Seroprevalence (%)
Gulu (n = 76)	64	12	84.21
Mulago (n = 79)	64	15	81.01
Jinja (n = 68)	66	2	97.06
Bwera (n = 76)	70	6	92.16
Bombo (n = 78)	66	12	84.62
Over all (n = 377)	330	47	87.53

When we examined the seroprevalence according to age groups (Table 3), age group 1–5 years had the highest seroprevalence 90.6% with 47% of the seropositives falling in this age group. Further, 63% of all the seropositives were in the age bracket 1–10 years which also constituted 63% of all the samples. The percentage of the seropositive samples for each age was high with a mean of 86.63% and standard mean error 1.029.

Table 3
showing seroprevalence according to groups

Age group years (n)	Seropositive samples	% Seropositive within age group	% Seropositive in total samples
< 1(n = 28)	24	85.71	7.27
1–5 (n = 171)	155	90.64	46.97
6–10 (n = 68)	55	80.88	16.67
11–15 (n = 27)	23	85.19	6.97
16–20 (n = 22)	19	86.36	5.76
20< (n = 61)	54	88.52	16.36
Total (377)	330	87.53	

We categorized the results in two main groups (Table 4) to calculate the odds for seropositivity according to age of volunteers below five years and those above five years. The odds ratio of those seropositive with an age above 5 years with reference to those below 5 years was 0.62.

Table 4
showing the broad age categories

Age category	Seropositive samples	Odds Ratio
Below 5 (199)	179 (89.9%)	1
above 5 (178)	151(84.8%)	0.62

Seasonality Of The Seropositivity Among The Volunteers

The seropositivity was generally high throughout the year with highest being recorded in March and the lowest in February and December as shown in Fig. 2.

Discussions

Human coronaviruses are known to have a wide distribution and endemic to most countries in the world but usually limited information is available on their presence and circulation in Sub-Saharan countries like Uganda. Conducting serosurvey studies is important in establishing baseline knowledge of endemic viruses in a community especially examining the extent of exposure of the population to coronaviruses. We report on the level seroprevalence of human coronaviruses in hospital-based sentinel sites in Uganda at a time when country and the world at large is facing a pandemic caused by SARS-CoV2. This will provide a picture about the possible level of exposure and population-based immunity against human coronaviruses.

The study observed a high seroprevalence (87.5%) of human coronaviruses in volunteers that visited the hospital-based sentinel sites. This high seroprevalence was consistent with other studies elsewhere. A previous study showed that 91% of sera collected from individuals in Southern Iraq has antibodies against HCoV 229E and OC43 (48). Further, a study that compared the prevalence of antibodies to four human coronaviruses (229E, OC43, NL63 and HKU 1) in serum and lower nasal secretions showed an average seroprevalence of 97% in serum of adult patients (49). This is still consistent with other studies that have shown that human coronaviruses have a global distribution (7, 50). Although in our study we did not screen for specific strain of human coronavirus, the result shows a great exposure of the volunteers to human coronaviruses. The hospital-based sentinel sites from which the samples were collected are major referral health centers in the respective regions in the country, thus this result give an overall picture of the seroprevalence of human coronavirus antibodies in the country. A previous surveillance study in hospital-based sites in Uganda showed 1.5% PCR positivity for human coronaviruses which further proves that these viruses circulate within the population (51). The volunteers presented several symptoms like fever, headache, muscle pains, sore throat and cough that are associated with infections of human coronaviruses, it is probable that patients were infected with human coronaviruses although health centers rarely carry out diagnosis to prove the causes of the clinical signs presented beyond the commonly known infections (52–54).

The qualitative Human coronaviruses antibody IgG ELISA kit that was used had its wells coated with a nucleoprotein, aa 340–390 (SARS corona HKU-39849)(55). There is no commercially available kit which can universally detect antibodies against all circulating coronaviruses but given that here is significant homology among the various human coronavirus strains with regards to the coded for proteins, there is significant cross reactivity beyond SARS-CoV HKU-39849 2003. This gives the kit a qualitative ability to detect and give an overall picture of the seroprevalence of human corona antibodies in the volunteers. IgG antibodies are known to be detected 4 days after onset of disease and may persist in patients for a period of 36 months in individual after recovery (56). Since the kit was not quantitative in nature, we did not establish the antibody titer in the samples which could be correlated with the time of infection. Never the less, this suggests that this observed high seroprevalence among volunteers could be due to a recent or earlier exposure to the human coronaviruses. Without corresponding PCR or culture experiments for the tested samples, it was not possible to confirm the presence and the infectiveness of the virus in the volunteers. It is known that when viruses invade the body, immune responses are induced in form of host defensive mechanisms among which is the production of neutralizing antibodies that prevent the virus from invading new cells and initiate the killing of infected cells and these antibodies stay long enough after the virus has been cleared (57). With no deaths previously reported due to human coronavirus infection, it probable that the defense mechanisms of volunteers are strong enough to clear the viral infection.

In this study, seropositivity was highest among children between 0–5 years compared to other age group ranges. The samples were selected completely at random therefore the numbers per age group is not equal which limits our degree of comparison. However, it is true that the seroprevalence of human coronaviruses antibodies vary greatly depending on the age of the population (58, 59). The computed odds ratios show that children under five years are more likely to be seropositive compared to individuals above 5 years of age. This agrees with the study which suggested that primary exposure to and infection by HCoV takes place in childhood (7, 60) (61–63). The first 10 years of an individual usually sees them transit from purely parent protection and isolation to open exposure in school settings. It is probable that this exposure makes this age group more susceptible to contracting human coronaviruses resulting in the high seropositivity.

In terms of seasonality, the seroprevalence was generally constant throughout the year with slight variations observed. The highest seroprevalence was observed in March and the lowest in February and December. Although no data about climate was analyzed during this study, Uganda experiences little annual variation in temperature with the average coolest months between June and September, while the rainy seasons occur between March and June, then between September and November, notwithstanding that the Country experiences occasional light rains almost through the year especially for regions around Lake Victoria (64). These weather conditions seem to favor the circulation of human coronavirus which elsewhere have been documented to circulate more frequently during winter season (14). This is in line with the argument that the activity of human coronaviruses is sporadic throughout the year with no clear seasonality (65) unlike influenza whose seasonality in Uganda has been established (66). However, there

is need to continuously do serosurveillance over a longer period of time to establish and describe the seasonality of Human coronaviruses in Uganda.

This study had limitations. The small sample size that was analyzed, the sentinel sites are not evenly distributed in the country, the lack of measurement of climatic factor to relate with the observed seroprevalence.

Conclusion

This study reports the high seroprevalence of antibodies against Human coronaviruses. Human coronaviruses are important emerging pathogens and currently the world is facing a devastating pandemic caused by SARS-2, there is therefore need for continuous viral surveillance. There is need to determine whether these antibodies possessed due to previous exposure to coronaviruses can offer protection to these emerging viral strains.

Abbreviations

HCoV

Human Coronavirus

SARS

Severe Acute Respiratory Syndrome

MERS

Middle East Respiratory Syndrome

cDNA

complementary DNA

Declarations

Ethical approval and consent to participate:

This study was conducted under an approved wider surveillance study by Makerere University Walter Reed Project. The project was internally approved by Makerere University School of Public Health Institutional Review Board (IRB) and externally by Walter Reed Army Institute of Research. The adult Patients at each sentinel site were enrolled after informed consent. The minors (below 18 years) were enrolled after informed consent from parents and legal guardians

Consent for publication: Not applicable

Availability of data and materials: All data generated is available from the corresponding author on reasonable request

Competing interests

The Authors declare that they have no competing interests

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Authors' contribution

MNE did the bench work, drafted the manuscript and the included figures

DKB is the Principal Investigator of the project

RT reviewed the manuscript

FMW reviewed the manuscript

EW reviewed the manuscript

JK reviewed the manuscript

HN reviewed the manuscript

MM reviewed the manuscript

BE participated in sample collection

TT participated in sample collection

UQA participated in sample collection

All authors have read and approved the manuscript

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Figures

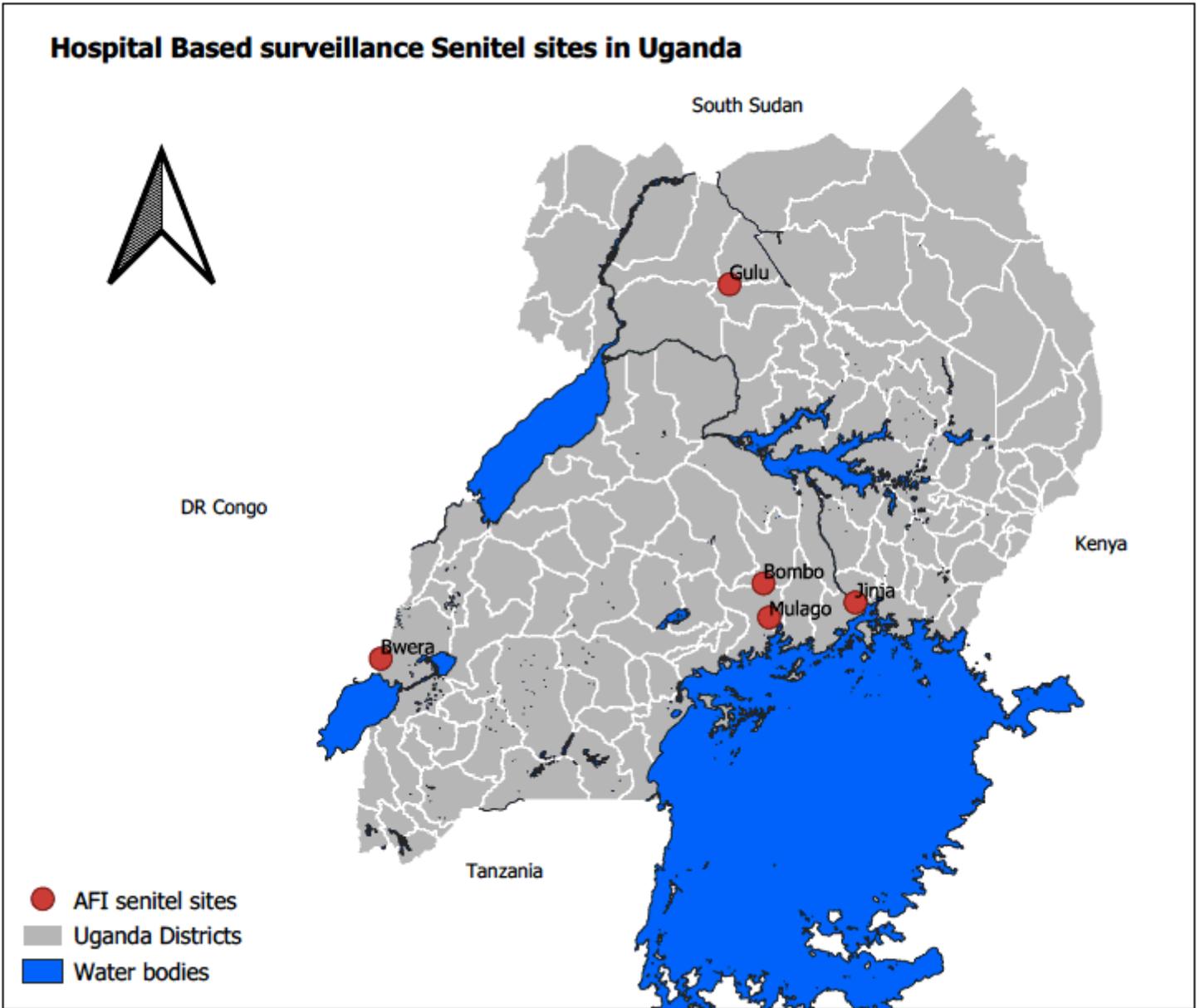


Figure 1

Map showing the Hospital based surveillance Sentinel sites in Uganda Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

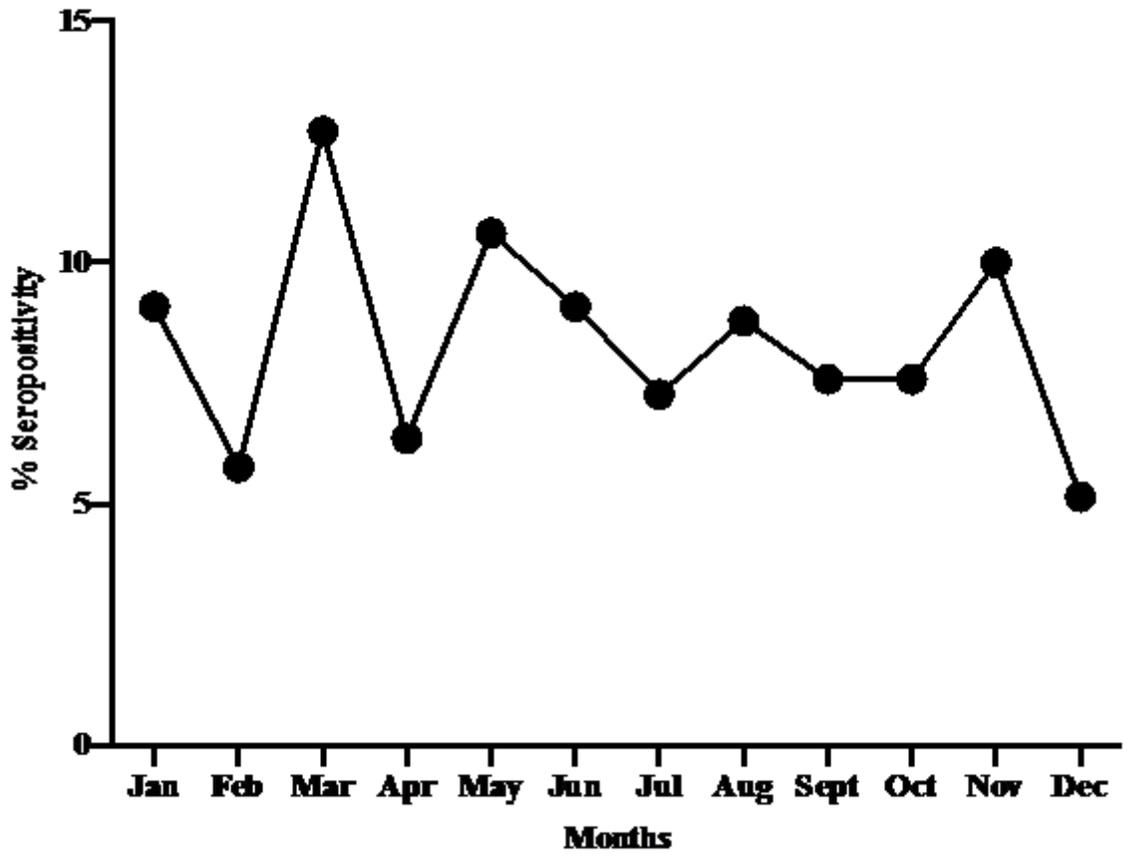


Figure 2

showing the variation of percentage seropositivity across the year