*Article*

# SARS-CoV-2 Detection in Fecal Samples in Sym-asymptotic Patients with Typical Findings of COVID-19 on Ag-RDT and SARS-CoV-2 RT-PCR Tests

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**Abstract:** Coronavirus is a disease caused by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which emerged as a global pandemic in 2019 from Wuhan, China. Since its emergence, it has caused immense suffering to human life, 6.27 million lives have been lost, movement curtailed and social dynamics disrupted. The golden standard for getting samples for SARS-CoV-2 detection is through oral- nasopharyngeal swab, this method of sample collection is invasive and uncomfortable, thus stigmatized the general population, and thereby impeded the progress of controlling the spread through mass testing. Being a contact disease, mechanisms to encourage mass testing is key to reduce the spread. This study thus developed a complimentary sample type to test for SARS-CoV-2, the use of human feces. Fecal samples were collected from 100 asym-symptomatic individuals suspected to be infected with COVID-19, virus RNA was then extracted and profiled through Real Time Polymerase Chain Reaction (RT-qPCR). The antigen rapid diagnostic test revealed high positivity rate of 44%, but the real time polymerase chain reaction results on nasopharyngeal and fecal samples revealed a significant variation, high number of the patients tested positive with stool samples compared to the nasopharyngeal swabs, with 43 and 37%, respectively. SARS-CoV-2 virus was detected in both symptomatic and asymptomatic individuals; however, the symptomatic registered a higher positivity of 25% compared to 20% among the asymptomatic patients. Vaccination only lowered the risk of infection, fully and partially vaccinated lowered the infection level to 10% compared to 20% among the unvaccinated. Finally, gender parity in relation to COVID19 was evaluated, more females (56%) compared to males were recruited in this study, out of which (20; 43.4%) were positive, and 26 (56.6%) were negative based on fecal RT-qPCR outcomes. Based on the outcome of this study, rapid diagnostic test (Ag-RDT) however cheap and or fast does not provide accurate information, moreover, the virus does not stay longer within the Oro-nasopharyngeal region, thus the invalid or negative results, thus use of feces should be adopted as a confirmatory test to ascertain the COVID19 status of an individual.

**Keywords:** COVID19; SARS-CoV-2 virus; Oro-nasopharyngeal; fecal; vaccination; asym-symptomatic; rapid diagnostic test

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## 1. Introduction

Coronavirus disease 2019 (COVID-19), which was first reported in Wuhan, China [1], is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is a pandemic disease that can manifest with fever, pneumonia and, in severe cases, with acute respiratory distress symptoms (ARDS) [2]. Gastrointestinal (GI) symptoms with vomiting and diarrhoea are often reported as other manifestations of the disease. A study done by Abbasinia et al [3] showed that diarrhoea was the main gastrointestinal symptom in 152 patients (15%) while abdominal pain (37 of 1,012; 3.7%) and vomiting (36 of 1,012; 3.6%) were the other GI symptoms occurring in these patients.

It is believed that SARS-CoV-2 is a zoonotic coronavirus, which jumped from an unknown host to humans [4], and mainly transmitted by respiratory droplets and fomes, and there is also evidence of fecal-oral transmission among humans [5]. The control of the virus has been challenging due to the asymptomatic and paucisymptomatic individuals who do not show any form of suffering but are able to transmit the virus to their contacts [6].

Real-time reverse transcriptase polymerase chain reaction (RT-PCR) on nasopharyngeal swab samples is considered the gold standard routine method for detection of SARS-CoV-2 [7]. However, SARS-CoV-2 RT-PCR tests giving negative results when nasopharyngeal swabs are used do pause a diagnostic challenge for clinicians in the management of patients, especially when these negative results do not confirm clinical manifestations. Moreover, due to high level of infection and traffic of suspected cases, compounded by the high cost of the gold standard RT-PCR, a less expensive and faster diagnostic test that detect the antigens specific for SARS-CV-2 infection have been designed and currently in use, commonly known as the antigen rapid diagnostic tests, or Ag-RDTs. If Ag-RDTs were accurate, could have a greater public health impact than the RT-qPCR due to: (i) requires minimal technical and laboratory expertize; (ii) may be performed locally in a decentralized locality with the associated logistic advantages; (iii) may facilitate timely decisions regarding quarantine and/or treatment regimens and epidemiological investigations of novel clusters [8].

Despite the revolution brought about by the use of RDTs, its greatest shortcoming is the high level of false positivity, which results into misdiagnosis. Furthermore, Ag-RDTs have been found to be less sensitive compared to RT-qPCR [8]. A recent Cochrane review [9] highlighted considerable study variability in sensitivity and specificity estimates; these also varied by Ag-RDT brand and viral load. For instance, a subgroup analysis by viral load defined by the cycle threshold (Ct) quantified a 53.8% absolute difference in sensitivity between samples with Ct ≤25 and >25 [10]. The World Health Organization (WHO), [11] recommends that SARS-CoV-2 Ag-RDTs should have a minimum of 80% sensitivity and 97% specificity. Furthermore, the European Centre for Disease Prevention and Control (ECDPC), 2020 recently proposed a more conservative threshold of ≥90% for the sensitivity parameter, especially in low-incidence settings.

Based on the dynamics of the various test methods, the use of complimentary method is critical in improving the accuracy level of diagnosis, thus the use of fecal sample to profile the SARS-CoV-2 RNA will support the diagnosis of COVID-19 in symptomatic and asymptomatic cases, in the presence of multiple negative RT-qPCR results, when conducted together with the SARS-CoV-2 antigen-rapid diagnosis tests. Moreover, recent studies have reported that the virus can persist for a long time in feces [1], and recommend the performance of SARS-CoV-2 RT-qPCR testing on fecal specimen as part of routine analyses for the detection of SARS-CoV-2, especially before the release of COVID-19 hospitalized patients. Based on this background, this study sought to investigate the effectiveness of use of fecal samples in the detection of SARS-CoV-2 virus compared to nasopharyngeal swab profiled through antigen rapid diagnostic test (Ag-RDT) and real time quantitative polymerase chain reaction (RT-qPCR).

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## 2. Materials and Methods

The Materials and Methods should be described with sufficient details to allow others to replicate and build on the published results. Please note that the publication of your manuscript implicates that you must make all materials, data, computer code, and protocols associated with the publication available to readers. Please disclose at the submission stage any restrictions on the availability of materials or information. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited.

Research manuscripts reporting large datasets that are deposited in a publicly available database should specify where the data have been deposited and provide the relevant accession numbers. If the accession numbers have not yet been obtained at the time of submission, please state that they will be provided during review. They must be provided prior to publication.

Interventionary studies involving animals or humans, and other studies that require ethical approval, must list the authority that provided approval and the corresponding ethical approval code.

### *2.1. Study Site*

The study was conducted in Kisumu and Siaya county referral hospitals located at 0.1017° S, 34.7556° E, and 0.0635° N, 34.2870° E respectively (Figure 1). The counties have a population of 2,148,757) accounting for approximately 4% of Kenya's population [12].

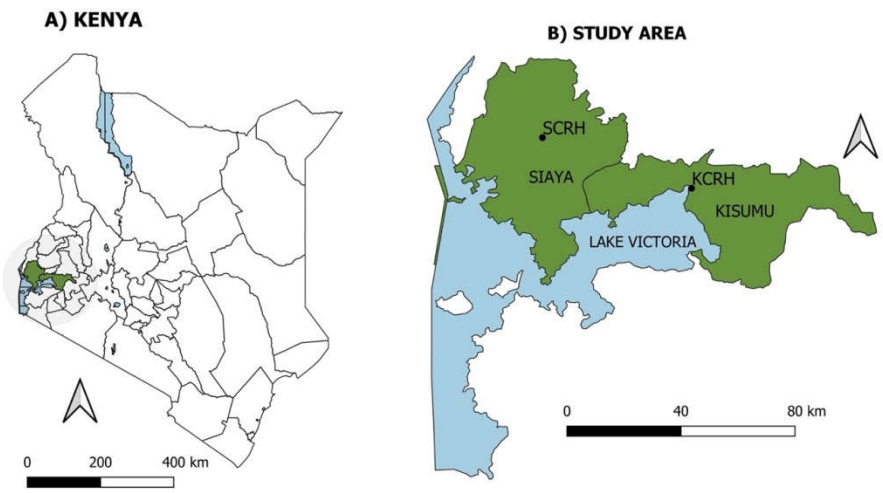


**Figure 1. (A). A map of Kenya illustrating the study sites (B).** Specific study sites in Siaya and Kisumu counties showing the exact sampling points. The map was drawn using QGIS Version 3.16 Hannover.

### *2.2. Sampling Design*

One hundred patients (50 from Siaya and 50 from Kisumu county referral hospitals) were purposively recruited and taken through informed consenting [13,14]. The cohorts were patients who voluntarily presented themselves for COVID-19 testing, including health workers and patients in isolation centers. Demographic details of the recruited participants such as name, age, gender, comorbidities and dwelling places were recorded.

### *2.3. Sample Collection and Storage*

Sample collection, handling and storage were done according to protocols described by the United States Centre for Disease Control, (2020 and 2021). Human samples were taken by health practitioners with the required PPEs following the guidelines of the

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Kenyan MOH, (2020). Nasopharyngeal specimens were collected using swabs with a synthetic tip, such as nylon or Dacron®, with a plastic shaft. The swab was gently inserted 2-3 cm into the nasal cavity and held parallel to the palate; then gently rubbed, rolled, and pulled out. The swabs were then placed into sterile tubes containing 2-3 ml of Gibco™ viral transport media in cooler boxes. The recruited individuals were requested to provide fecal samples in sterile plastic containers provided with spatulas, which were used to transfer the sample into well-labeled collection bottles containing Gibco™ virus transport media. The nasopharyngeal swab and fecal specimens were then transported to the Centre for Global Health Research, Kenya Medical Research Institute's (CGHR-KEMRI) biosafety level 3 laboratories in Kisian, Kisumu County. Samples were preserved in 10% formalin and PVA (polyvinyl-alcohol) at 1 v/v rate, and refrigerated at 4°C.

*2.4. Rapid Antigen Diagnostic Test*

The Panbio™ COVID-19 Ag Rapid Test Device with membrane strips was obtained from the county referral hospitals. Swab specimens and detection buffer were applied to the test cartridges. A dropper pipette (supplied) was used to aspirate the extracted specimen into the lateral-flow inlet and the results read after 15 minutes.

*2.5. SARS-CoV-2 RNA Profiling from Nasopharyngeal and Fecal Samples*

Individual nasopharyngeal swab samples were vortexed for 1 min within the transport medium, and 200 µl were used for SARS-Cov-2 RNA extraction. Fecal samples were pre-treated before RNA extraction in which 180 g was weighed in 1.5 ml Eppendorf tubes and 1 ml stool lysis buffer added, and vortexed for 1 min. The mixture was incubated at room temperature for 10 min and centrifuged for 3 minutes at 13000 rmp. About 200 µl of the supernatant was used for RNA extraction. The nasopharyngeal and fecal specimens were processed for viral RNA extraction using the QIAamp® Viral RNA extraction kit (United States), as per the manufacturer's instructions. Briefly, 140 µl of the specimen was treated with 560 µl of prepared buffer AVL containing carrier RNA (1µg/µl). After brief pulse vortexing and 10-minutes incubation at room temperature, the specimen was precipitated by adding 560 µl of pre-chilled ethanol. The treated specimen was then transferred to the spin column. Viral RNA was purified by consecutive treatment with 500 µl of buffer AW1 and AW2. Finally, it was eluted in 60µl buffer AVE

*2.6. RT-qPCR Reactions*

The extracted nucleic acid samples were tested for SARS-CoV-2 y RT-PCR using the 7500 fast real-time PCR system (PathoFinder, BV), in accordance with the manufacturer's instructions. The RT-qPCR reactions procedures were adapted from FastPlex® Triplex SARS-CoV-2 Detection Kit. The reactions procedures were as presented on Table 1 and were prepared using 200 µL sample input volume in a MicroAmp™ Optical 96-well reaction plate. The sealed plate containing the purified sample RNA, negative and positive controls (Table 2) were vortexed gently and centrifuged to collect the liquid at the bottom of the plate.

**Table 1.** The RT-PCR Master-mix reaction volumes.

| | Number of samples | 1 | 100 |
|---|---|---|---|
| Reaction mix | NC (ORF1ab/N) PCR reaction solution A- Specific primer probes, MgCl2, (NH4)2SO4, KCl, HCl | 8.5 µL | 850 µL |
| | NC (ORF1ab/N) PCR reaction solution B- Hot start Taq DNA polymerase, CMMLV enzyme, DNTPs. | 1.5 µL | 150 µL |

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**Table 2.** Reaction plate volumes that were used.

| Component | Volume per reaction | | |
|---|---|---|---|
| | RNA Sample reaction | Positive Control | Negative Control |
| Reaction Mix | 10 µL | 10 µL | 10 µL |
| Purified sample RNA (from RNA extraction) | 5.0 µL | — | — |
| Positive Control (diluted Taq Path™ COVID-19 Control, from step 3) | —` | 5.0µL | — |
| Purified Negative Control (from RNA extraction) | — | — | 5.0 µL |
| Total volume | 15 µL | 15 µL | 15 µL |

*2.7. Data Analysis*

The RT-PCR generated data and the Ag-RDT data were compared on SPSS, using one-way ANOVA. A receiver operating characteristic (ROC) curve was calculated for each test illustrating their diagnostic ability by plotting the true positive rate (sensitivity) against the false positive rate (1-specificity) at various threshold settings. For ROC analysis, PCR results were considered as reference to estimate sensitivity and specificity.

**3. Results.**

*3.1. Evaluation of faecal sample to detect the presence of SARS-CoV-2 virus*

Accurate detection of SARS-CoV-2 is critical and urgently needed globally in order to either eradicate or significantly control its spread. The high number of asymptomatic patients and yet highly infectious is a worry, and thereby impeding the process to reduce the level of SARS-CoV-2 infection rate. Out of 100 samples analysed, antigen rapid diagnostic test revealed high positivity rate of 44%, but the real time polymerase chain reaction results on nasopharyngeal and fecal samples revealed a significant variation, high number of the patients tested positive with stool samples compared to the nasopharyngeal swabs, with 43 and 37%, respectively (Figure 1). The results obtained are in agreement with the previous study conducted by [15] in which 15 positive COVID-19 patients with mild or no symptom were selected, and found that 73% were positive to fecal samples. Moreover, a study conducted by [16] found that 4.2%: 95% confidence interval 2.5–6.5% tested positive for SARS-CoV-2 virus on nasal/throat swabs and of these, 3/17 (18%: 95% CI 4–43%) had SARS-CoV-2 detected in stool from a sample size of 434 participants from 176 households. Furthermore, two of the participants exhibited faecal shedding of SARS-CoV-2, without showing any signs of gastrointestinal symptoms, after testing negative for SARS-CoV-2 in respiratory samples. The SARS-CoV-2 RNA profiling from the fecal samples is critical to controlling the infection; moreover, SARS-CoV-2 RNA lifespan is longer in the feces compared to the respiratory tract samples.

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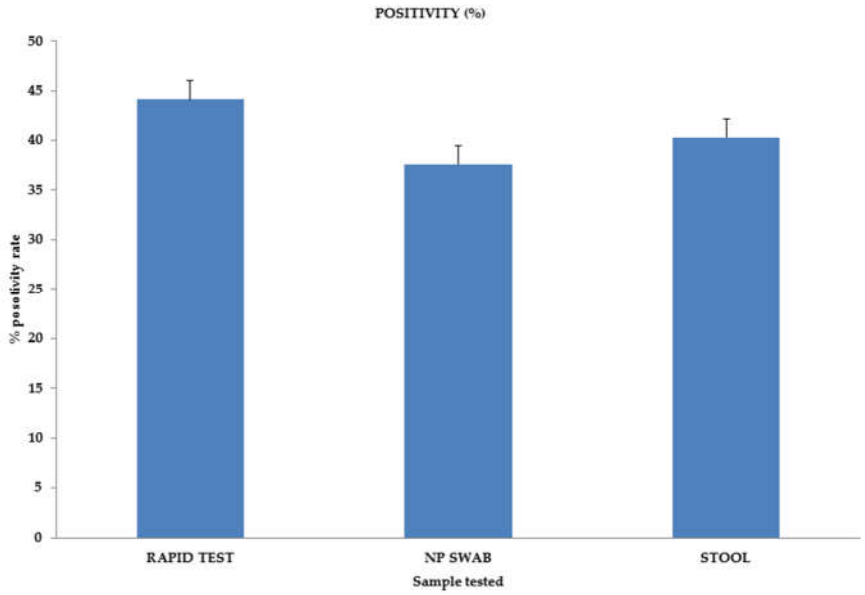


**Figure 1.** Evaluation of feacal samples to detect the presence of SARS-CoV-2 virus. Rapid test (antigen rapid diagnostic test) and NP (nasopharyngeal).

*3.2. Symptoms and level of infection (COVID-19 predictors)*

Since the outbreak of COVID-19, the affected individuals have been exhibiting varied signs and symptoms. These symptoms are classified into common, less frequent and rare symptoms. The common symptoms were like fever, chills, cough, fatigue, headache and gastrointestinal disorders such as vomiting and diarrhoea. The less frequent symptoms were shortness of breath, sore throat, difficulty in swallowing, conjunctivitis, exacerbation of chronic conditions, delirium, decreased or loss of appetite, and loss of smell and/or taste while the rare symptoms included skin manifestations, confusion, stuffy nose and eye manifestations (Abdelrahman and Bakheet, 2021; Article, 2020; Adhikari et al., 2020; Review, 2020). Furthermore, COVID-19 has presented a unique observation which has never been observed in the medical world, where an individual may harbour the pathogens, thus acting as an intermediate host and are able to infect people within their immediate environment. Moreover, it has been hypothesised that infected individuals who remain asymptomatic play an important role in the control of the spread of COVID-19 disease, but their relative number and effect have been uncertain [20]. In the entire sampled population, those who tested both positive and negative, the symptomatic individuals registered a higher percentage above 25% while the asymptomatic in either of the case registered below 20%. Hhowever, it is worth noting that, among the individuals who tested positive for SARS-CoV-2, approximately 4% were asymptomatic (Figure 2). The results are in agreement with previous studies in which the estimated proportion of the asymptomatic individuals stood at 17.9% out all the members who tested positive for COVID-19 [21].

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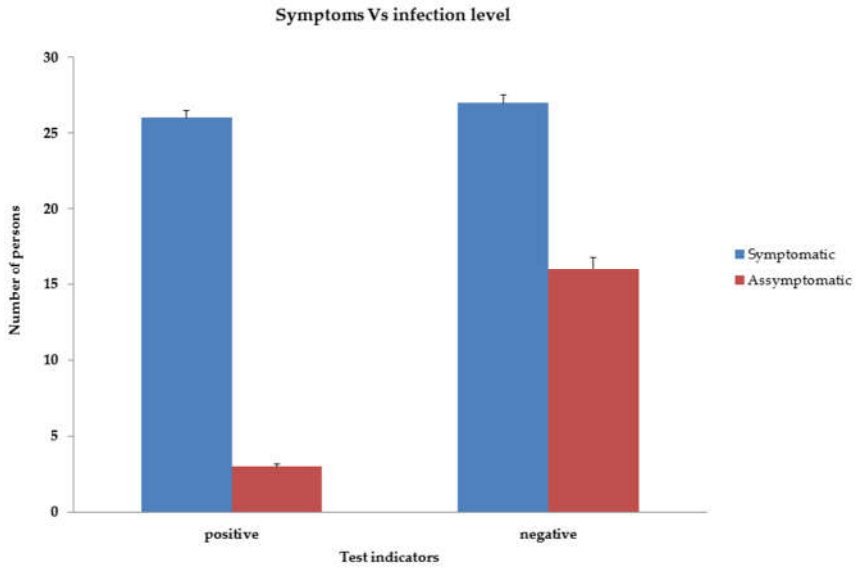


**Figure 2.** Symptomatic and Asymptomatic patients and SARS-CoV-2 virus detection.

*3.3. Age and COVID-19 infection*

The transmission dynamics of COVID-19 the world-over, led to more hospitalization of elderly and people with comorbidities [22] which warranted shielding strategies for at-risk individuals [23]. Although no age-specific threshold was observed for COVID-19 infection, severity risks due to isolated effect of age was observed to increase [24]. In this study, the age bracket of participants who volunteered to participate ranged between 10-73 years. The least number of positive cases (3%) of fecal samples was observed in the age brackets of 10-19 years which agrees with the findings of Davies, [25] that younger people have a lower propensity for COVID-19 infection. Indeed, a study conducted in the US by Monod *et al*., [26] revealed that adults aged 20-34 and 35-49 were the age groups that had sustained SARS-CoV-2 transmission. In this study, the highest infection rate was observed in the age bracket of 20-30 years, in which (10%) patients were positive. The same age bracket (20-30 years) reported the highest negative results (15%), followed by the age bracket of 10-19 years with 14% having negative results. The second highest number of positive fecal samples fell in two age brackets: 30-40 years and above 50 years, both of which had (7%) positive patients. and 40-50 years (Figure 3).

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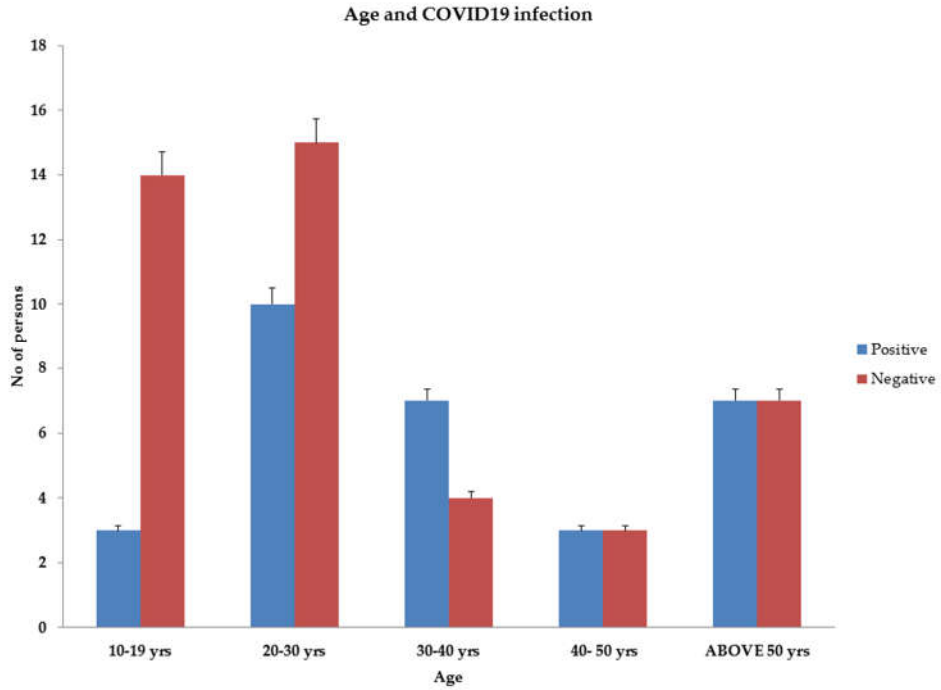


**Figure 3.** Age and SARS-CoV-2 infection level.

*3.4. Vaccination regime and SARS-CoV-2 infection level*

Since the emergence of COVID-19 over 6 million deaths have been recorded [27]. After the rapid development of anti-SARS-CoV-2 vaccines, 9.2 billion doses have been administered through national vaccination programmes [28]. Even though large population have been vaccinated, sporadic cases of COVID-19 are reported daily; therefore investigation was carried out to understand the infection level across the study cohort. The results revealed that infection was spread across the fully vaccinated, partially vaccinated and the unvaccinated group. however, the infection was observed to be highest among the none vaccinated with an infection level above 20%, while the vaccinated and the partially vaccinated, the infection level stood at less than 10%, though there were no significant difference between them (Figure 4). The findings of this study is in agreement with previous studies which showed that infections with the SARS-CoV-2 Delta variant affected both the vaccinated and the non-vaccinated persons [29].

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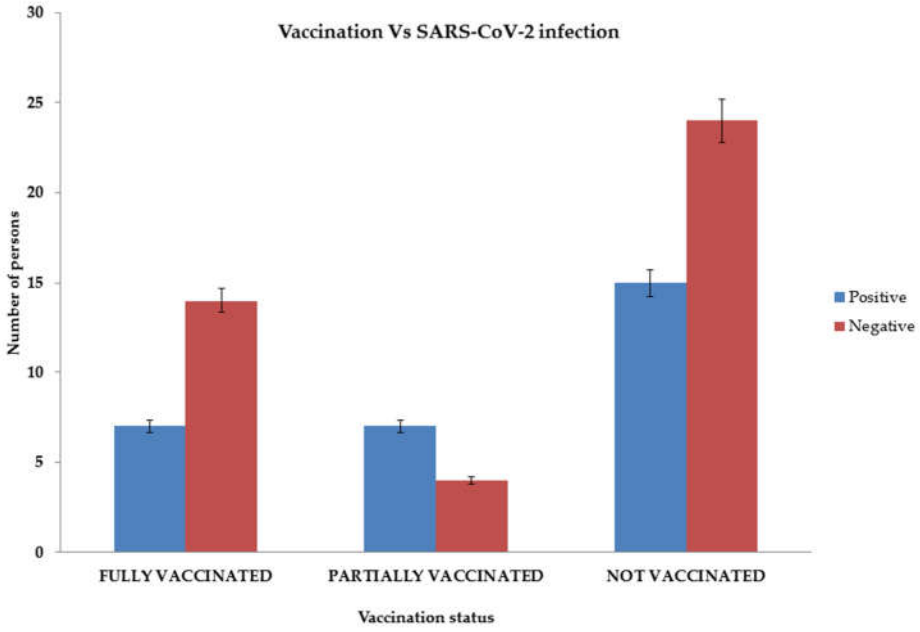


**Figure 4.** Vaccination status and SARS-CoV-2 infection.

*3.5. Gender and COVID-19*

A gendered dimension of COVID-19 infection was analysed to compare the ways in which males and females shaded the virus in feces matter, since gender norms influence health status, health seeking-behavior and associated barriers [30]. The overall effect of COVID-19 was disproportionately borne by women, children, elderly and the poor in Kenya [31]. In this study, more females (56%) compared to males were recruited in this study, out of which (20; 43.4%) were positive, and 26 (56.6%) were negative based on fecal RT-qPCR outcomes (Figure 4). Comparatively, fewer males (9; 34.6%) returned positive results, while (17; 65.4%) were negative. This outcome contrasts findings by Bwire, [32] that men are more vulnerable to COVID-19, due to lifestyle and genetic differences. However, the data in this study was obtained from voluntarily consenting participants, which resulted in more females turning out for the test.

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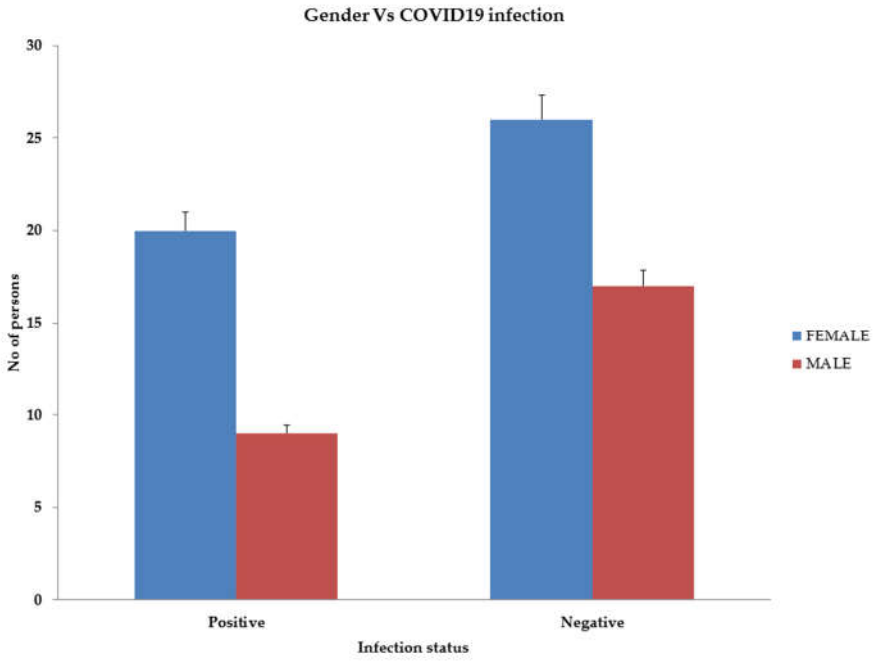


**Figure 5.** Gender and SARS-CoV-2 infection level.

## 4. Conclusions

COVID-19 caused by SARS-CoV-2 virus is an emergent and highly infectious disease that has spread across the globe to become a global pandemic. With the poor health care infrastructure and very low government expenditure on the health care systems, worst predication was made for the African continent. Due to the global prediction of massive deaths in Africa, the low- and middle-income countries were concerned thus it became a health scare, resulting into some of the knee jack reactions, such as lockdown and curfews instituted by various governments. What remains to be a mystery is the deaths were relatively low in Africa, and whether the containment measures yielded fruit is hard to tell. But one can point to courteousness and adoption of mass testing could have led to this reduced level of infection among the African populace. Even though majority were willing to present themselves for testing, the invasive and uncomfortable nature of the swabbing method created a hindrance, thus the use of feces created some relief. Moreover, those who tested negative presented positive results with fecal analysis, thus the adoption of fecal matter as a clinical sample for profiling for the SARS-CoV-2 RNA increased the accuracy and efficiency in the SARS-CoV-2 virus diagnosis.

**Author Contributions:** Conceptualization, ROM, BO, SSA and BE.; methodology, ROM, BO,AO,JNK, and BE.; software, ROM, JNK, MAO, SM, FO, AO, FO, DA, FOO, JOM, RN and FA.; validation, ROM, BO, JNK and SSA.; formal analysis, ROM, JNK,SSA,BO and DA.; investigation, ROM, EM, SM, FO, AO, CO, RN, EO, SI and JNK.; resources, BE.; data curation, ROM, BO, AO, SSA, PW, JNK, MAO, EM, SM, FO, DA, JOM, FOO and FA.; writing—original draft preparation, ROM.; writing—review and editing, ROM, JNK, BO, AO, and SSA; visualization, ROM, JNK, SSA, and BO.; supervision, BE.; project administration, BE.; funding acquisition, ROM and BE. All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** Data supporting the findings are included in figures and tables within the manuscript

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**Conflicts of Interest:** The authors declare no conflict of interest.

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