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Research Article

Astrovirus and *Norovirus* Infections among Asymptomatic Edible Straw-Colored Fruit Bats (*Eidolon helvum Kerr*) in Southwest Nigeria

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ABSTRACT

Introduction: Several zoonotic infections such as haemorrhagic fever and respiratory diseases have been described to emanate from direct contact, consumption and trading in wild animals. Nevertheless, the propensity of the fruit bats as reservoirs of enteric virus infections have been less described in Africa, where the meat is a sought delicacy. This study was designed to detect the presence of some enteric viruses of gastroenteritis from *Eidolon helvum* bat species in Nigeria, to determine the prevalence and risks of transmission of the viruses via consumption of the meat.

Methods: From January 2018 to January 2019, we initiated surveillance in roosting colonies of *Eidolon helvum* to detect enteric *Astrovirus* and *Norovirus*. By convenience sampling, fecal samples were collected from freshly trapped fruit bats in Akure and Epinmi Akoko, Nigeria. The virus antigen was detected from the fresh stool samples using the CerTest 4th generation quadruple Enzyme Immunochromatographic assay. The generated data were analysed using descriptive statistics and Chi square at $\alpha_{0.05}$.

Result: Fifteen *Astrovirus* positive samples (15/140:10.7%) were obtained from Akure while two positive samples (2/40:5%) were obtained from Epinmi Akoko, giving a total prevalence of 9.4% (17/180) from the two locations. Similarly, six *Norovirus* positive samples (6/140:4.3) were obtained from Akure while four positive samples (4/40:10) were obtained from Epinmi Akoko, giving a total prevalence of 5.6% (10/180). A statistically significant difference was found between *Astrovirus* and *Norovirus* infection at Akure ($\chi^2 = 4.17$; df = 1; p = .41). No statistically significant association was found in the rate of virus detection from bats in the two locations ($\chi^2 = 1.96$; df = 1; p = .16).
Conclusion: The results indicated that bats are reservoirs of *Astrovirus* and Noroviruses and can be a potential source for the outbreak of gastroenteritis and diarrhea of unknown etiology in the study area. The prevalence and serological evidences can be used to infer periods of increased virus circulation and gastroenteritis that coincides with periodic hunting of fruit bats for consumption. The collective data can be used to identify high risk periods for gastroenteritis outbreaks between December and April, to inform strategies for prevention and control of the associated viral diarrhea. The systematic surveillance for viruses in local and global bat populations can help identify potentially zoonotic or pandemic pathogens prior to their emergence in humans and/or domestic animals.

Keywords: *Eidolon helvum*; *Astrovirus*; *Norovirus*; Gastroenteritis; Ondo state

ABBREVIATIONS

FECA: Federal College of Agriculture; Akure; COVID-19: Coronavirus Disease 2019; HAstV: Human Astrovirus; SaVs: Sapoviruses

INTRODUCTION

Bats are mammals of the order Chiroptera (Latin for "handwing"), making them the only mammal that are naturally capable of true and sustained flight. Bats are natural reservoirs of a large number of zoonotic pathogens and many emerging viruses such as lyssavirus, Hendra virus, Nipah virus, Ebola virus, and Marburg virus that causes severe human diseases [1-3]. Most of the known bat viruses have been discovered in apparently healthy bats. When bats are experimentally infected with henipavirus or the rabies virus, the bats shed the virus but do not produce any clinical syndrome like those observed in other animals and humans [4]. Bats are the second largest order of mammals (after the rodents) with about 1,240 species worldwide [5]. About 70% of bat species are insectivores, others are fruit eaters while other species such as the vampire bats feed on blood. Environmental changes and deforestation have severely influenced many ecosystems, intensifying the contact between wildlife and humans, thereby exposing man to a huge number of potentially pathogenic viruses of animal origin [6]. The straw-colored fruit bat, *Eidolon helvum* Kerr, is a frugivorous animal in the order Megachiroptera [7]. In West Africa, there are over 120 species of fruits and nectar eating bats, feeding on a wide range of trees in the forest landscape [8]. They feed on leaves, flowers, and large proportion on fruits of different families of tropical forest plant species. *E. helvum* is known to feed on both cultivated and wild fruits including, *Musa sapientum* (Musaceae), *Carica papaya* (Caricaceae), *Mangifera indica* (Anacardiaceae), *Kigelia aethiopica* (Bignoniaceae), *Terminalia* spp.(Combretaceae) [9,10]. In Nigeria, *E. helvum,* feeds almost exclusively at night, visiting only trees that have food resources, whereas trees visited during the day are only for roosting [7]. They may assist in the pollination of the flowers of some trees [11].

More than 70 viruses have been isolated from bats [12]. They can be transmitted within the large populations of bats between the individuals of one species and as they roost in the same habitat, intra-specific transmission occurs frequently. However, other bats may overcome the infection and seem to store the virus in their body. Several of these "bat"-viruses are known to be transmitted to other mammals including humans. Recently, Severe Acute Respiratory Syndrome and novel groups of Astroviruses in apparently healthy insectivorous bats belonging to the genera *Miniopterus* and *Myotis* have been reported [13]. In bats, astroviruses were mostly found in apparently healthy animals and since 2008, a growing number of bat species have been found to carry Astroviruses with a noticeable prevalence and diversity [14]. Human *Astrovirus* and *Norovirus* have been identified as the causative agent of severe diarrhea in children and immunocompromised patients [15]. Knowledge about the tissue tropism of Astroviruses has been expanded to some neurotropic strains that have recently been shown to be responsible for encephalitis in humans and livestock [16].

Astroviruses belong to the *Astroviridae* family which comprises two genera: *Mamastrovirus*, including 19 species, designated *Mamastrovirus 1–19*; and *Avastrovirus* including three species, formerly assigned as turkey, chicken and duck astrovirus. This virus family comprises a diverse group of small, non-enveloped positive sense single-stranded RNA viruses with a characteristic star-like appearance [17]. Altogether 19 species of mamastroviruses, have been identified with a wide geographic distribution in a great number of domestic animals, in wildlife including bats, as well as in humans [18]. Most of the infections caused by astroviruses are assumed to be asymptomatic but, depending on the affected species, the age and immunological status of the affected host, the infection can be associated with diarrhea, hepatitis, nephritis or, more recently, encephalitis [19]. Intense hunting of large-bodied fruit bats for food is reported across West Africa. Nigeria allows hunting of about 90 species of her bats which is one-third of Africa's bat diversity, and

some of these are of near threatened or vulnerable conservation status in which the straw-colored fruit bat is arguably the most hunted fruit bat in Africa [17,20,21].

New viruses including rotaviruses, orthoreoviruses, and astroviruses have been described in different bat species around the world [22-24]. Following the first report on BtAstVs in 2008 [14], only a few additional studies revealed bats as reservoirs of AstVs in Hungary and China [24-27].

Norovirus prototype strain was first identified as the cause of a gastroenteritis outbreak in Norwalk, Ohio, in 1968, hence the common name, "Norwalk-like virus" [28]. *Norovirus* is a small, non-enveloped, positive sense, single stranded RNA virus belonging to the family *Caliciviridae*, in the genus *Norovirus* and species Norwalk virus. Noroviruses are approximately 38-40 nm in diameter, classified into at least ten genogroups (GI-GX) that were further divided into more than 40 genotypes which are responsible for acute gastroenteritis and encephalitis in humans, livestock, pets, wild animals, marine mammals and bats [29-32]. Some previous studies have reported the detection of *Norovirus* from intestinal tissue of insectivorous bat species, namely *Rhinolophus pusillus, Rhinolophus sinicus* and *Rhinolophus affnis* in china. However, less report exist on bigger frugivorous bat species [33,34].

Information on the ecology and evolution of bat viruses are still scarce, as they were under-studied, underappreciated and under severe threat, particularly in Nigeria. Bats are frequently considered the reservoir host for a broad variety of newly emerging viruses, especially in the tropics, although their general role in the epidemiology and spillover of zoonotic viral diseases is still not fully understood. Therefore, more extensive surveillance of different bat species from different geographic areas is needed. Hence, the objective of this study was to detect the presence of *Astrovirus* and *Norovirus* from *Eidolon helvum* bat species in Nigeria, to determine the prevalence and risks of transmission of the viruses via direct contact and consumption of the meat and meat products.

MATERIALS AND METHODS

Study area

This study was conducted in Akure, Akure South Local Government and Epinmi in Akoko South East Local Government Areas in Ondo state, Nigeria. Akure is a city in south-western Nigeria, and is the largest city and capital of Ondo State. The city had a population of 484,798 as at the 2006 population census and an estimate of 570, 500 in 2011 with a density of 490/km^2 (1,300/sq mi), 350m (1,150ft) Elevation, 991km^2 (383 sq mi) and coordinates 7^{0}15'0"N 5^{0}11'42"E. Ondo State is bounded by Ekiti and Osun state

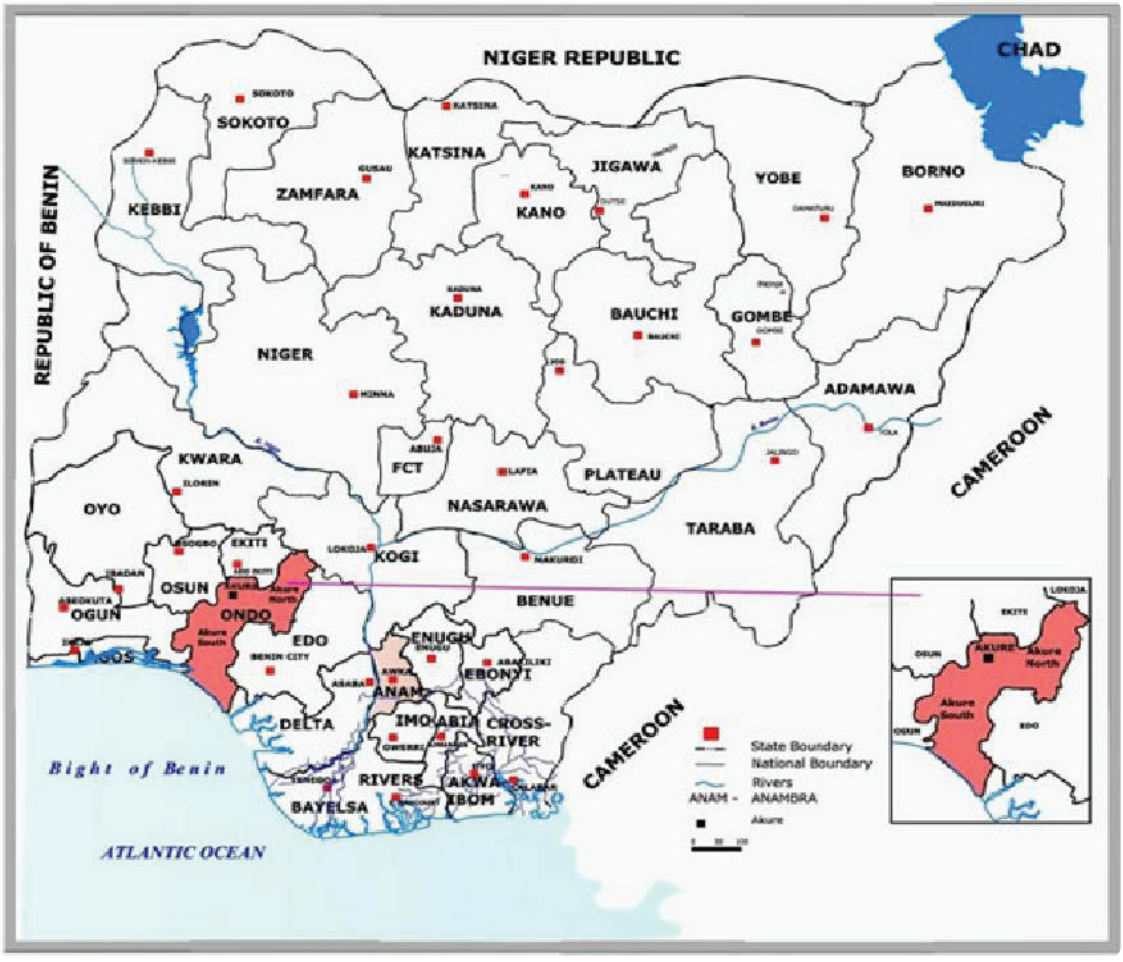

Figure 1: Map of Nigeria showing the study area (Ondo state).

to the North, Ogun state to the West, Edo to the East and the Bight of Benin Atlantic Ocean to the South (Figure 1). The study sites were the Federal College of Agriculture, Akure, Ondo State, Nigeria, located along Ikale Street off Ijapo Estate road Akure as well as Arborheal roosting forest in Epinmi Akoko, Nigeria.

Sample collection and preparation

Between January 2018 and January 2019, convenience sampling was adopted in the collection of fecal samples from freshly trapped fruit bats roosting at several colonies in the Federal College of Agriculture, Akure (FECA) and a foraging colony at Epinmi Akoko, Nigeria. Bats were captured or trapped by harp traps at swarming sites and in their natural foraging habitats. All captured bats were identified for species by an experienced chiropterologist according to [35,36]. The animals were freed from traps immediately and put into sterile, disposable, perforated paper bags individually and were left hanging for a maximum of 30 min to defecate according to a previous method [24]. Fecal samples were aseptically collected from the bags into 200 µL of phosphate-buffered saline in sample bottles, tightly screw-covered and labeled accordingly (Figure 2). Samples were tested immediately or transported in cold chain and stored refrigerated prior to testing at the Microbiology Department of Adekunle Ajasin University, Akungba Akoko, Nigeria. After sample collection, live bats were tagged and released into the wild at the netting site. Although invasive bat sampling is not prohibited in Nigeria, we nevertheless observed noninvasive procedures on the live bats during collection of fecal samples, and all examined bats were handled according to previous guidelines, as bat species has been enlisted as endangered species [37,38].

Screening for astrovirus and norovirus using lateral flow enzyme immunoasssay

According to the manufacturer's instructions and minor modifications to previous method [39], the fecal samples from the bats were tested for the presence of Astroviruses and Noroviruses using CerTest® Biotec Rota+Adeno+Astro+Noro one step combo card. The tests samples and controls were maintained at room temperature (15-30° C) prior to testing. The pouches were not opened until the performance of the assay. The cap of the sample collection tube was taken out. About 200 µL of the centrifuged supernatant (extract) was dispensed into the sample extraction buffer/diluent. The tube was gently agitated in order to assure good sample dispersion. The mixture was left for 2 minutes so as to allow proper extraction of viral particle after which the cap of the specimen extraction tube was snapped open and 4 drops of the aliquot sample were dispensed into each of the sample port lettered A, B, C and D of the CerTest Rota+Adeno+Astro+Noro combo card. The results were then read after10 minutes of incubation. Appearance of red band and green band at the test and control portions of the result window indicated positive test, that is, when reaction was indicated by two lines (1 test, 1 control lines) in the cassette. Absence of *Astrovirus* or *Norovirus* antigen that is, negative result was indicated by one upper green band (1 control line) while invalid result was indicated when there was no green line or both the control line and the test line were absent. The results were then recorded accordingly. This device has an internal quality control comprising the test and control lines embedded in the cassettes which were invisible before application of sample. The control band appears only when the test procedures and reagents were performed properly.

RESULTS

A total of 180 fecal samples from a common bat species were collected and tested for the presence of *Astrovirus* and *Norovirus* from January 2018 to January 2019. At Akure, *Astrovirus* was detected in the months of January, February, March, November and December 2018 as well as January 2019 while Noroviruses were detected in February, March, October, November, December 2018 and January 2019. Representative positive samples in the viral assay are shown in figures 3 and 4.

Figure 2: Collection of fresh fecal sample from a bat

Figure 3: Representative viral assay of the fecal specimen of bat sample AK1 showing positive result for *Astrovirus*.

Figure 4: Representative viral assay of the fecal specimen of bat sample AK13 showing positive results for *Astrovirus* and *Norovirus*.

The highest numbers of samples (16) were collected in the month of January 2019, followed by fifteen in January, February, March, Oct., November and Dec. 2018. A total of 17 bats were found positive for at least one virus, with two samples containing a mixed infection. Fifteen *Astrovirus* positive samples (15/140:10.7%) were obtained from Akure while two positive samples (2/40:5%) were obtained from Epinmi Akoko, giving a total prevalence of 9.4% (17/180) from the two locations (Table 1). Similarly, six *Norovirus* positive samples (6/140:4.3) were obtained from Akure while four positive samples (4/40:10) were obtained from Epinmi-Akoko, giving a total prevalence of 5.6% (10/180). A statistically significant difference was found between *Astrovirus* and *Norovirus* infection at Akure ($\chi^2 = 4.17$; df = 1; $p = 0.41$) while there was no statistically significant association between *Astrovirus* and *Norovirus* infection at Epinmi ($\chi^2 = .72$; df = 1; $p = 0.39$). Similarly, no statistically significant association was found in the rate of virus detection from bats in the two locations ($\chi^2 = 1.96$; df = 1 $p = .16$).

DISCUSSION

The increasing relevance of bat-transmitted viruses in public health cannot be overemphasized as new viruses have continued to emerge in the last decades causing worldwide epidemics. Owing to the paucity of data regarding the presence of bat-transmitted viruses among bat species in Nigeria and sequel to complaints of abdominal pain, fever, headache, diarrhea anorexia and confusion consequent upon the consumption of bat delicacies in the study area, we embarked on this research to detect any enteric viruses in the locally consumed *Eidelon helvum* fruitbats, from roosting colonies in Akure and Epinmi Akoko, Ondo state, Nigeria. From the two locations, monthly rate of detection showed that the highest rate of detection for *Norovirus* occurred in the month of October, November and December 2018, while the highest rate of detection for *Astrovirus* was obtained in the month of December 2018. No virus was detected in the months of April to September 2018 in bat samples from the two locations. It was observed that low fecal samples were obtained between April and October 2018 owing to low bat population as previously reported [40]. This observation coincides with a period of low *E. helvum* population from the roost, and when the trees habitat may have time to recover from the foraging activities of the bats who feeds on the flowers and nectars of many indigenous trees, such as *Azadiractha indica, Deloniix regia, Solanum erianthum, Ficus exasperate* and other plant species in the study sites.

From our result, the high risk periods for probable disease is between December and April which coincides with the dry peak season reported for hunting and consumption of bats as they provide an important food source during the "lean" agricultural season thereby substantiating the previous report in Ghana [21].

The detection of high rate of *Astrovirus* infections among asymptomatic bat populations in this study corroborates the earlier reports of [14] who detected astroviruses from several bat species including *Micropteropus magnater, Micropteropus pusillus, Micropteropus schreibersii, Micropteropus chinensis, Micropteropus ricketti,* and *Pteropus abramus*. Our findings indicate that bats can shed several viruses and yet remain asymptomatic as none of the captured samples showed any evidence of disease.

In Europe, only the *M. myotis*, *M. daubentonii*, *M. bechsteinii*, and *P. auritus* species have been addressed before as reservoirs of AstV. However, evidence from literature suggests that this is the first report of Astroviruses and Norovirus in *Eidolon helvum* from Nigeria thereby substantiating a recent report of high rate of Astroviruses from asymptomatic bats in Wuhan China where the current COVID-19 pandemic emanated [41]. Bats are known to harbor highly pathogenic viruses of humans, yet they do not develop severe clinical symptoms upon several viral infections. The reasons are still not fully understood and little is known about the immune system of

Table 1: Overall distribution and rate of *Astrovirus* and *Norovirus* detection by month and locations of bats in Ondo state Nigeria.

2018-2019	Akure			Epinmi			Total		
	No. of samples Tested	No. (%) Positive for Astro	No. (%) Positive for Noro	No. of samples Tested	No. (%) Positive for Astro	No. (%) Positive for Noro	No. of samples Tested	No. (%) Positive for Astro	No. (%) Positive for Noro
Jan 2018	12 (8.6)	5 (41.7)	00	3	00	00	15	5(33.3)	00
Feb	12 (8.6)	2 (16.7)	1(8.3)	3	00	00	15	2(13.3)	1(6.7)
Mar	12 (8.6)	2 (16.7)	1(8.3)	3	00	00	15	2(13.3)	1(6.7)
Apr	8 (5.7)	00	00	3	00	00	11	00	00
May	8 (5.7)	00	00	3	00	00	11	00	00
June	10 (7.1)	00	00	3	00	00	13	00	00
Jul	8 (5.7)	00	00	3	00	00	11	00	00
Aug	12 (8.6)	00	00	3	00	00	15	00	00
Sep	10 (7.1)	00	00	3	00	00	13	00	00
Oct	12 (8.6)	00	1(8.3)	3	00	1(33.3)	15	00	2(13.3)
Nov	12 (8.6)	2 (16.7)	1(8.3)	3	00	1(33.3)	15	2(13.3)	2(13.3)
Dec	12 (8.6)	2 (16.7)	1(8.3)	3	1(33.3)	1(33.3)	15	3(20)	2(13.3)
Jan 2019	12 (8.6)	2 (16.7)	1(8.3)	4	1(25)	1(33.3)	16	3(18.8)	2(13.3)
TOTAL	**140**	**15 (10.7)**	**6(4.3)**	**40**	**2(5)**	**4(10)**	**180**	**17(9.4)**	**10(5.6)**
***p*-value**	**$\chi^2 = 4.17$; $p = .41$; df = 1**			**$\chi^2 = 0.72$; $p = .39$; df =1**			**$\chi^2 = 1.96$; $p = .16$; df = 1**		

Abbreviations: No.: Number; Astro: *Astrovirus*; Noro: *Norovirus*

bats and its interaction with pathogens. However, in one of previous studies, this resistance was ascribed to the synthesis of an antiviral immune pathway called the STING-interferon pathway elicited by bats to maintain just enough defense against illness without triggering a heightened immune reaction [42]. The risk of transmission of these viruses to human is high as viruses generally, especially enteric viruses in bats may be transmitted to humans directly through bites or via exposure to saliva, fecal droplets, infected tissues, through consumption of contaminated flesh as delicacies, as well as indirectly through contact with infected intermediate hosts, such as swine [1]. As obtained in Nigeria and other African countries, the current overhunting of the straw-colored fruit bat (*E. helvum*) for bush meat culinary is very high and is classified internationally as near-threatened bat species, even as they contaminate the environment with fecal droppings, urine and exfoliated plants [43-45].

Notably, one of the important properties of astroviruses include the ability of the *Astrovirus* capsid to act as an enterotoxin, disrupting the gut epithelial barrier, thereby eliciting diarrhea upon consumption. Interestingly, a typical HAstV infection is characterized by mild, acute watery diarrhea that lasts one to four days and, in some cases, it is associated with vomiting, fever, anorexia, and abdominal pains that may resolve spontaneously without medical attention [46]. However, recent discovery of a great genetic diversity among animal and human astroviruses emphasizes the potential of these viruses, particularly some neurotropic strains to cross species barriers and elicit more severe disease such as encephalitis in humans and livestock. In immunocompromised individuals, the symptoms can be prolonged, leading to wasting, and occasional systemic spread, resulting in encephalitis and meningitis [47,48]. Similarly, little is known about norovirus infections in most non-human hosts, but the close genetic relatedness between some animal and human noroviruses coupled with lack of understanding of the sources of newly emergent human norovirus genotypes and variants has led to the hypothesis that norovirus may not be host restricted but capable of jumping the species barrier [32]. *Norovirus* is infectious at very low doses with less than 100 virus particles sufficient to cause disease and transmits rapidly by the feco-oral route via contaminated food and water, causing a severe, sporadic or epidemic diarrhea and vomiting in all human age groups, especially during the winter [49].

Therefore, deciphering where emergent astroviruses, noroviruses and other enteric viruses originate and whether they arise from a certain reservoir or reservoirs is important, as is the need to determine whether there are strains that have yet to be identified circulating in humans [50]. Contrary to previoud reports of frequent detection of *Norovirus* in smaller insectivorous bat species, particularly in Asia, [33,34,51] this current study emphasizes the presence of Norovirus in frugivorous *Eidolon helvum* bats in Nigeria. In addition, other newly found viruses in *Eidolon helvum* include SaVs belonging to the Sapovirus genus, in the family Caliciviridae. They have been associated with gastroenteritis in humans and in pigs but not in other animals. In addition, some strains from pigs, chimpanzees and rodents show close sequence identity with human SaVs thereby suggesting the possibility of interspecies transmissions [52].

Virus 'spillovers' from bats to humans occur either directly, through contact with infected bats or indirectly through intermediate hosts such as domestic animals or wildlife that have been contaminated by blood, saliva, urine or feces of bats. Noroviruses are resistant to heat, freezing, extreme pH and disinfection. Outbreaks of most zoonotic diseases have increased in recent decades consequent upon bushmeat consumption as well as human encroachment into natural habitats by deforestation and agricultural intensification. These factors may thus help in the transmission and amplification of gastro-enteric infections in susceptible host. In some regions of the world, a variety of bat dishes, minced bat meat, and even hot pot with the whole bat cooked in a pot of soup are available in restaurants in southern China. Dried bat droppings are used as traditional Chinese medicine, for the treatment of diseases such as night blindness. Bat dung that is mined in caves as guano can be used as organic fertilizers. All these uses of bats and their derived products have created countless opportunities of human-bat interaction, and hence, have increased the chance of virus transmission [53]. These submissions give credence to our report of detection of *Astrovirus* and *Norovirus* in *Eidolon helvum* bat species that may act as reservoirs for outbreak of viral gastroenteristis in the susceptible human populations, as bat species continued to be exploited for bush meat and medicine [54].

CONCLUSION

The detection of Astroviruses and Noroviruses in bats samples in this investigation portends the inherent danger in consumption of improperly processed meat from this widely sought "bushmeat". Contamination of bat derived delicacies by these viruses may be responsible for gastroenteritis of previously unknown etiologies in the study area. The systematic surveillance for viruses in local and global bat populations can help identify potentially zoonotic or pandemic pathogens prior to their emergence in humans and/or domestic animals. These measures are valuable to safeguard health.

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