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Absence of molecular evidence of filovirus circulating in bats and rodents in Laikipia North sub-County, Kenya: a cross sectional study

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Abstract

In the recent decade, pathogenic zoonotic viruses have emerged in different geographical locations almost annually. These changes have led to new complex interactions between humans, animals and the environment, creating unique opportunities for pathogens unique opportunities to pass between hosts. Most emerging pathogens are RNA viruses such as filovirus. Numerous factors such as anthropogenic activities, changes in local ecosystem and climate change have contributed to this spillover. While Kenya has not reported any filovirus outbreaks in humans, a filovirus (i.e Bombali Ebola virus) have been detected in Kenyan bats, which have been implicated as reservoir hosts. The goal of this study was to detect and molecularly characterize known and novel filovirus circulating in bats and rodents in Laikipia North sub-County, Laikipia County, Kenya. In May 2018, a total of 477 samples (blood, oral and rectal swabs) were collected from 159 bats and 159 rodents in Laikipia North sub-County, Kenya. Ribonucleic acid was extracted from all samples and screened using consensus polymerase chain reaction targeting the long-gene of filovirus. All samples were negative. These results suggest that circulation of filovirus was uncommon during the month of May, 2018 in rodents and bats from Laikipia North sub-County. Considering our findings, future sampling should be conducted both longitudinally and with significantly larger sample sizes for a more in depth assessment of the prevalence of filoviruses in bats within the region studied.

Introduction

Emerging and re-emerging viruses are of great concern not only on the African continent, but all over the world with a majority of the outbreaks occurring in the tropics including East Africa, Central Africa, West Africa [1]. These viruses cause outbreaks at unexpected times with severe consequences. In addition, there is a global movement of people across national borders which makes possible the transfer of infection to regions that were previously not known to harbor these viruses [2]. In this decade, examples of worldwide reported outbreaks of emerging and re-emerging viruses include severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Ebola virus (EBOV), Middle East respiratory syndrome coronavirus (MERS-CoV) and influenza viruses. All of these viruses have emerged or re-emerged from animals [3]. Some of the most devastating viral disease outbreaks reported have been caused by viruses in the family *Filoviridae*[4]. Filovirus are amongst the highly pathogenic emerging and re-emerging viruses in humans, with a mortality rate of up to 90% in both humans and non-human primates [5]. So far, only three genera of this family (*Marburgvirus, Ebolavirus and Cuevavirus*) have been described [4]. Members of this viral family are known to cause periodic outbreaks with severe hemorrhagic disease in both humans and non-human primates (NHPs) and respiratory disease in pigs [6]. The frequency of filovirus (Ebolavirus) outbreaks has increased over the last decade [7].

The first documented human filovirus outbreak was with *Marburg virus* in 1967, (Germany and Yugoslavia), from African green monkeys (*Chlorocebus aethiops*) imported from Uganda. Subsequently, in 1976 the first EBOV case in humans was reported in Nzara, South Sudan [8]. Thereafter, over 40 outbreaks of Ebolavirus and 10 outbreaks of Marburg virus have been reported in Africa and imported cases in the Netherlands, Yugoslavia, United States of America, Italy and United Kingdom [7,9]. To date, the most devastating EBOV outbreak occurred in West Africa in 2014-2016, with a case fatality rate as high as 62% [10]. In previous filovirus outbreaks, wildlife has been suspected to be the main source of filovirus emergence. The potential mammalian natural reservoir hosts of filoviruses include bats, duikers, rodents and NHPs [11]. Evidence shows that bats are the putative hosts and play a major role in transmission of filoviruses. Ebolavirus antibodies have been detected in several bats species in Africa (Guinea, Democratic Republic of

Congo-DRC, Sierra Leone, Ghana, Liberia, South Africa, Uganda, Ivory Coast, Gabon), Asia (Philippines, Singapore, Bangladesh, India, China) and Europe (Spain) [8,12-14] and even isolated and sequenced from samples from Sierra Leone, China, DRC and Gabon [12,13,15]. Ebolavirus has also been serologically detected in several rodent species (*Arvicanthis spp., Mastomys spp., Mastomys spp., Mus spp. and Praomys spp.*) from the Central African Republic [16]. In fact, experimental studies have demonstrated the ability of rodents to adapt to filoviruses, meaning they can be infected [17]. Infected bats with filovirus pose as a risk of illness to humans.

Bats may represent a source of filovirus spillover in many African countries to both humans and other mammals. This may occur during hunting and preparation of bush meat or through direct exposure to fruits contaminated by infected saliva, feces or urine [18]. Kenya harbors a rich diversity of wildlife, lending it to becoming one of the top tourist destinations in Africa. Tourism accounts for a major percentage of Kenya´s gross development product (GDP), contributing approximately 9.7% of GDP in 2017 [19]. However, wildlife plays an important role in transmission of zoonotic diseases at the animal-human interface, which is extensive within Kenya. At the animal human interface in Laikipia North sub-County, it is not well understood what viral exchange occurs. Even though Kenya has not reported any filovirus outbreaks, filovirus antibodies have been detected in humans from the Central African Republic and DRC where no outbreak has been reported [20,21]. In addition, filoviruses have been detected serologically and have been isolated from bats and rodents, respectively. Moreover, in 2017, Kenya was listed as one of the countries at risk of imported filovirus (Ebolaviruses) despite the fact that it has only reported one outbreak of Marburg virus in 1980 [22]. We investigated the presence of known and novel filovirus RNA in bats and rodents from Laikipia North sub-County, Kenya.

Methods

Study design: this was a cross sectional study conducted in May, 2018 in Laikipia North sub-County, Laikipia, Kenya. The aim of this study was to detect the presence of known and novel filoviruses circulating in bats and rodents in Laikipia North, county Kenya.

Study area: Laikipia is a county in Kenya that lies between latitudes 0° 18" and 0° 51" North and longitude 36° 11" and 37° 24" East. Laikipia county is ranked as the second in wildlife population with a wide range of animals (both domestic and wild animals) that freely transverse the county [23,24]. Laikipia North has a human population size of 36,184 who interact with wild animals, thus increasing the risk of virus jump from animals to humans [25].

Sample collection: we employed convenience sampling technique, where we sampled the animals to saturation. To avoid bias, all the animals trapped were sampled. We sampled during the wet season, when insects and animals are found in large numbers. Insectivorous bats majorly feed on insects. In addition, during feeding, animals come in close contact, thus increasing the chances of filovirus infections. Blood clots, oral swabs and rectal swabs were collected from different species of bats and rodents from Ol Jogi and Ilmotiok villages from Laikipia North sub-County, Kenya (Figure 1). Depending on species of small mammals, different capture, bleeding techniques and sites were used. The animals were sampled in duplicate, collecting oral swabs, rectal swabs and blood. Rodents were captured using Sherman traps, while bats were captured using mist nets and harp nets. The animals were anesthetized using 0.4ml of isoflurane in cotton balls. Depending on the rodents' species blood was collected from the retro-orbital, lateral tail vein, jugular vein or ventral tail vein and in bats blood clots were collected from propetagial (cephalic) vein, the uropetagial (saphenous) vein, or the brachial

vein [26,27]. The samples were stored in 500µl of virus transport media (VTM) while the other in 500µl of TRIzol™ media (LOT. No. 175806, Ambion® Life Technologies™). The samples were immediately placed in dry shippers containing liquid nitrogen, then shipped to the Institute of Primate Research Karen, where they were transferred to -80°C freezer.

RNA extraction and nested RT-PCR: RNA was extracted from each sample using DIRECT-Zol RNA-MiniPrep kit (Cat. #. R2052, Zymo Research). Following RNA extraction, the quality of RNA was confirmed using mitochondrial cytochrome-b RT-PCR [28]. Reverse transcription was performed using Superscript III first strand kit (Cat. # 18080-051, Invitrogen) for cDNA synthesis. Manufacturer´s instructions on use were followed. This was followed by a nested PCR using Platinum TM Taq DNA Polymerase kit (Cat. # 10966-026, Invitrogen). The amplification was carried out using PREDICT 11 filovirus protocol with modified primers from Zhai *et al.,* 2007, from the conserved L-Gene of filovirus (Unpublished filovirus primers from University of California; Davis RT-PCR protocol [29]. The primers were modified to increase their sequence degeneracy in order to improve filovirus detection and discovery. The primary and secondary amplification conditions were 40 cycles, initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 1 minute, annealing at 52°C for 1 minute, extension at 72°C for 1 minute, final extension at 7°C for 7 minutes. The positive control used in this study was a plasmid derived from reston filovirus (Filoreston, FiloR) provided by USAID PREDICT 11 project. For negative control we used PCR water that was used during the RT-PCR.

Ethical approval: this study was carried out in strict accordance with the recommendations in the Guide for Care and Use of Laboratory Animals in Kenya. Ethical approvals were granted by the Institute of Primate Research Ethical Review Committee, Ref. No: IERC/08/16. Research Permit was granted by National Council of Science and Technology (NACOSTI) permit number: NACOSTI/P/19/48092/2643.

Results

Samples were collected during the wet season between 4th-11th May, 2018. The molecular prevalence of filovirus circulating in Laikipia North sub-County, was assessed in 477 samples from 159 bats and rodent species, each animal giving 3 samples (Table 1). A total of 100 bats (n=80 *Chaerephon pumilus* and n=20 *Scotophilus dinganii*) from the families Molossidae and Vespertilionidae were sampled. Each bat gave 3 samples i.e blood, oral swabs and rectal swabs. A total of 59 rodent species, 6 species from family *Muridae* and 1 species from family *Nesomyidae*, were sampled, each rodent giving 3 samples (blood, oral swabs and rectal swabs). All the blood samples tested negative for filovirus L-gene by RT-PCR. In addition, all the oral swabs and rectal swabs from the two animal taxa tested negative.

Discussion

In the year 2017, Kenya was listed as one of the countries at risk of imported filovirus (*Ebola viruses*) despite the fact that it has never had outbreaks since 1980 (*Marburg virus*). Filovirus continue to emerge or re-emerge, with the most recent cases reported in April 2020 [30]. Filovirus ecology, reservoir hosts and spillover event to humans are still not completely understood. The suspected reservoir hosts (bats) are elusive and may be a source of infection to humans. Fruit bats have been linked through different index cases as potential sources of Marburg and Ebola viruses in Kenya and Uganda, respectively [31,32]. The viruses have been detected if not isolated from bats and experimentally in rodents. However, the role played by these animals as reservoir hosts and in natural ecology is not well understood. Nevertheless, filovirus infections in humans continue being a threat. To detect and identify animal species harboring filovirus in Laikipia North sub-County, Kenya, we tested 477 samples from 2

species of bats and 8 species of rodents. The aforementioned bats' species *Chaerephon pumilus* and bat family Vespertilionidae have tested positive for Bombali ebola virus by PCR (Sierra Leone) and serologically (Guinea, Cameroon and the DRC), respectively [15,33]. Although in our study all were negative, this is an important addition to the existing data on the epidemiology of filovirus in Kenya.

Filovirus ribonucleic acid (RNA) detection using molecular tools is rare. In a study by De Nys (2018) on bats, filovirus (Ebola viruses) viral RNA was not detected [33]. In experimental studies where Egyptian fruit bats (*Rousettus aegyptiacus*) were inoculated with Ebola virus strains (Sudan, Reston, Bundibugyo and Tai Forest) and Marburg virus, the bats did not shed EBOV while Marburg virus was shed through the oral and rectal route [34]. Similarly, in an experimental study, Paweska (2016) inoculated Egyptian fruit bats with EBOV but was not able to isolate the virus from oral or rectal swabs from any of the experimental animals, implying that the bats were not shedding the virus [35]. In nature, filovirus (Ebolavirus and Marburg virus) RNA has been detected by molecular tools in a couple of studies in fruit bats and insectivorous bats [12,36]. This is due to low viral load in the rectal and oral swabs [37]. Conducting concurrent serological assays would probably have indicated exposure of the animals to filoviruses.

In this study, samples were collected from *Chaerephon pumilus* (little free-tailed bat) and *Scotophilus dinganii* (Yellow African bat) which have been listed as a potential filovirus reservoir hosts [15]. In as much as we were not able to isolate filoviruses using molecular tools, we cannot rule out the possibility of these viruses circulating in bats from Laikipia North sub-County, Kenya because we only conducted molecular assays. Serological assays targeting antibodies would have demonstrated previous and current exposure of the animals to filoviruses in serum or plasma. In a review conducted by Olival (2014), many studies were not able to detect EBOV through PCR but were able to detect antibodies [38]. The negative molecular results in this study could have been due to low levels of detectable viral copies within the bats species, clearing of the virus by the bats naturally due to a robust immune response or the bats were never infected at all as argued by Lacroix 2021 [37].

Rodents have been suspected as being reservoir hosts to filovirus (EBOV) though not normally susceptible. In an experimental study by Morgan (1999), they demonstrated that EBOV can be isolated from experimentally infected rodent organs after several serial passages of the virus [39]. In addition, studies conducted by Pappalardo and co-workers, showed a few mutations at viral protein 24, VP24 (K_{142}, L26F and L147P) and glycoprotein ($S_{65}$and D_{49}) genes which are conserved in Ebolaviruses. These genes may contribute to the functionality of the virus and adaptation of the virus in rodents. This suggests that there is a probability of the viruses adapting to new rodent hosts, which may result in novel pathogenic human Ebolaviruses [40]. Our findings are similar to studies by Spengler (2015) where viral RNA from oral swabs from Guinea pigs model was not detected in ante-mortem animals [41]. In our study, we only sampled live animals.

Conclusion

Our findings show that animals sampled in Laikipia North sub-County in May 2018, did not harbor filoviruses. This may not be true due to the small sample size and a few bats and rodents' species we captured. A combination of capture techniques e.g. the use of both acoustic sampling, mist nets and turtle traps have been shown to capture large numbers of bats from different species. We believe that continued surveillance studies of filovirus should be conducted in small mammals longitudinally. The role of bats and rodents as reservoir hosts and in filovirus transmission still remains unclear, due to the fact that viral RNA detection is very rare. The identification of the reservoirs of filoviruses would aid in the

development of strategies to prevent human outbreaks and reduce the impact of the viruses on animal species such as great apes, whose populations have been greatly threatened in endemic regions.

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What is known about this topic

- *Bats are reservoirs of filovirus;*
- *Ebolavirus antibodies and RNA has been detected in regions that have not reported filovirus outbreaks.*

What this study adds

- *The study describes the distribution of filovirus;*
- *The study suggests that rodents may not be the reservoir hosts of filoviruses;*
- *The study adds the different species of bats and rodents found in Laikipia County, Kenya.*

Competing interests

The authors declare no competing interests.

Authors' contributions

All the authors have read and agreed to the final manuscript.

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We thank the different teams that facilitated sample collection.

Table and figure

Table 1: animal species and types of samples collected

Figure 1: map of Kenya (sampling sites)

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Table 1: animal species and types of samples collected					
	Species	**Family**	**Sample type**		
			Blood	Oral swab	Rectal swab
Bats	*Chaerephon pumilus*	Molossidae	80	80	80
	Scotophilus dinganii	Vespertilionidae	20	20	20
Total			100	100	100
Rodents	*Mus spp*	Muridae	3	3	3
	Grammomys dolichoros	Muridae	10	10	10
	Saccostomus mearnsi	Nesomyidae	1	1	1
	Acomys kompi	Muridae	17	17	17
	Aethomys hindei	Muridae	4	4	4
	Acomys percivali	Muridae	3	3	3
	Gerbilliscus robustus	Muridae	15	15	15
	Grammomys spp	Muridae	6	6	6
Total			59	59	59
Spp: Denotes to species					

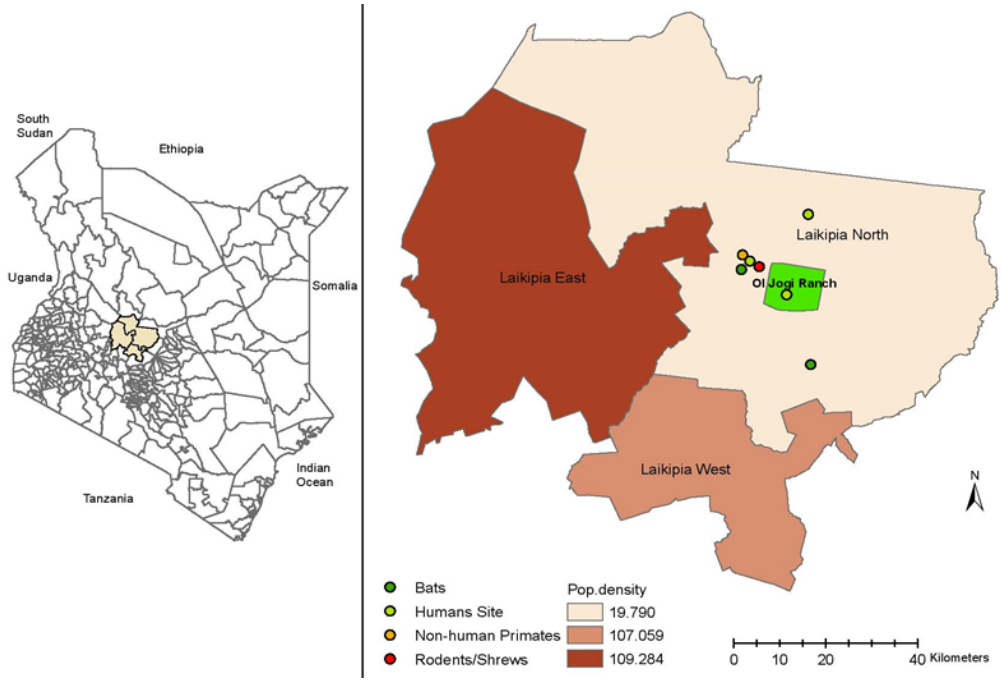

Figure 1: map of Kenya (sampling sites)