Seroprevalence of filovirus infection of *Rousettus aegyptiacus* bats in Zambia

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Abstract

Bats are suspected to play important roles in the ecology of filoviruses, including ebolaviruses and marburgviruses. A cave-dwelling fruit bat, *Rousettus aegyptiacus*, has been shown to be a reservoir of marburgviruses. Using an enzyme-linked immunosorbent assay with the viral glycoprotein antigen, we detected immunoglobulin G antibodies specific to multiple filoviruses in 158 of 290 serum samples of *R. aegyptiacus* bats captured in Zambia during the years 2014-2017. In particular, 43.8% of the bats were seropositive to marburgvirus, supporting the notion that this bat species continuously maintains marburgviruses as a reservoir. Interestingly, distinct peaks of seropositive rates were repeatedly observed at the beginning of rainy seasons, suggesting seasonality of the presence of newly infected individuals in this bat population. These data highlight the need for continued monitoring of filovirus infection in this bat species even in countries where filovirus diseases have not been reported.

Keywords: Filovirus; Ebolavirus; Marburgvirus; Antibody; Fruit bats; Ebola virus; Marburg virus; ELISA; *Rousettus aegyptiacus*

BACKGROUND

Marburgviruses and ebolaviruses belonging to the family *Filoviridae* cause severe hemorrhagic fever in humans and nonhuman primates. In contrast to the genus *Marburgvirus*, which contains only one known species, *Marburg marburgvirus* consisting of Marburg virus (MARV) and Ravn virus (RAVV), five distinct species are known in the genus *Ebolavirus*; *Zaire ebolavirus*, *Sudan ebolavirus*, *Taï Forest ebolavirus*, *Bundibugyo ebolavirus*, and *Reston ebolavirus*, represented by Ebola virus (EBOV), Sudan virus (SUDV), Taï Forest virus (TAFV), Bundibugyo virus (BDBV), and Reston virus (RESTV), respectively. In addition, a new filovirus named Lloviu virus (LLOV), as yet not known to cause any disease in humans or non-human primates, was discovered in bats and assigned to the new genus *Cuevavirus*, species *Lloviu cuevavirus* [1, 2].

While previous studies have suggested that these filoviruses infect several different species of animals, some species of fruit bats are suspected to be natural reservoirs of filoviruses [3]. The EBOV genome was detected in fruit bats (*Epomops franqueti*, *Hypsignathus monstrosus*, and *Myonycteris torquata*) [4], whereas infectious ebolaviruses have never been isolated from any species of fruit bats. However, some populations of *Rousettus aegyptiacus* bats (Egyptian fruit bats) have been shown to maintain both MARV and RAVV [5-7]. It was also reported that some of the bats were seropositive for EBOV [8]. Egyptian fruit bats are widely distributed within Africa, including in non-endemic countries like Zambia. Experimental infection of Egyptian fruit bats with MARV demonstrated viraemia, viral antigens in numerous tissues, seroconversion and horizontal transmission of the virus while they remained clinically healthy [9-13].

In the present study, we focused on cave-dwelling Egyptian fruit bats, which could potentially be infected with filoviruses, as suggested by previous studies [5-8]. We detected filovirus-specific immunoglobulin G (IgG) antibodies using viral glycoprotein (GP) antigens of all known filovirus species. This paper presents the results of a three-year genetic and serological survey for filoviruses in these bats in Zambia.

METHODS

Animals and sera

Serum and tissue samples were collected from wild healthy cave-dwelling Egyptian fruit bats (119 males and 171 females) caught by harp traps in Lusaka Province in Zambia from December 2014 to December 2017 (Supplementary Table 1). Captured bats were euthanized with diethyl ether, and samples were collected for antibody detection and reverse-transcription polymerase chain reaction (RT-PCR) assays. All these activities were performed under the aegis of the research project “Molecular and Serological Surveillance of Viral Zoonoses in Zambia” approved by the Department of National Parks and Wildlife (formerly Zambia Wildlife Authority), Ministry of Tourism and Arts of the Republic of Zambia (Act No. 12 of 1998).

Enzyme-linked immunosorbent assay (ELISA)

Filovirus envelope GP and nucleoprotein (NP)-based ELISA was performed as described previously [14, 15]. His-tagged soluble recombinant GPs of EBOV (Yambuku), SUDV (Nzara), TAFV (Pauléoula), BDBV (Butalya), RESTV (Philippines89), MARV (Angola), RAVV (Kitum Cave), and LLOV (Asturias) were purified from the supernatants of human embryonic kidney 293T cells transfected with pCAGGS expressing each GP. The recombinant NP of MARV (Mt. Elgon) was purified by ultracentrifugation through discontinuous CsCl gradient centrifugation using the lysate of 293T cells transfected with pCAGGS expressing NP as described previously [16]. ELISA plates (Nunc, Maxisorp) were coated with purified filovirus antigens (2 μg/ml in phosphate-buffered saline, 50 μl/well) overnight at 4°C, followed by blocking with 3% skim milk, and serum samples (diluted at

1:100) were added. Bound antibodies were visualized with a goat anti-bat IgG heavy and light chain antibody conjugated with horseradish peroxidase (Bethyl Laboratories, Inc.) and 3,3’,5,5’-tetramethylbenzidine (Sigma). The reaction was stopped by adding 1N phosphoric acid, and the optical density (OD) at 450 nm was measured. To offset the nonspecific antibody reaction, the OD value of the control antigen was subtracted from that of each sample. Assays were conducted in duplicate and averages were used for further data analyses.

Reverse transcription polymerase chain reaction (RT-PCR)

RT-PCR assays were performed as described previously [7, 15, 17, 18]. Briefly, total RNA was extracted from pooled 10% (w/v) spleen and liver homogenates of individual bats using a QIAamp Viral RNA Mini Kit (QIAGEN) according to the manufacturer’s instructions. One-step RT-PCR assays were carried out using a QIAGEN OneStep RT-PCR kit (QIAGEN) according to the manufacturer’s instructions. The filovirus-specific universal primers for detection of the NP gene, FiloNP-Fm, FiloNP-Rm, FiloNP-Fe, and FiloNP-Re, were used for the one-step RT-PCR program as described previously [18]. Nested RT-PCR assays for virus protein 35 (VP35) and NP genes of marburgvirus were also performed using primers F1 (forward-outside), F3 (forward-inside), R1 (reverse-outside), and R2 (reverse-inside) for the VP35 gene and MBG704F1 (forward-outside), MBG719F2 (forward-inside), MBG1248R1 (reverse-outside), and MBG1230R2 (reverse-inside) for the NP gene as described previously [7, 17].

Statistics

For the cutoff value, the Smirnov-Grubbs rejection test, which is widely used to detect significantly higher or lower values (i.e., outliers) that do not belong to the population consisting of all other values in the data set, were employed as described previously [15, 19]. Briefly, the highest OD value was first selected, and the statistical significance of the T value was calculated based on the critical values given by the Smirnov-Grubbs test. If it was considered to be an outlier, the *T*-value for the second highest OD value was then similarly tested without the highest one. These steps were repeated until the *T* value fell to below the level of statistical significance ($P < 0.05$) and the cutoff OD values were determined. The correlation coefficient was determined by Pearson's method.

RESULTS

Serological and genetic screening of Egyptian fruit bats for filovirus infection

Two hundred ninety Egyptian fruit bat serum samples were screened for IgG antibodies specific to all known species of filoviruses (Supplementary Figure 1), and the OD values obtained by the GP-based ELISA were analyzed statistically to determine the cutoff OD value as described in Methods. Samples that showed OD values above the cutoff value for at least one filovirus species were regarded as positive. When a sample showed cross-reactivity to GPs of multiple species, the OD values for each GP antigen were compared and the filovirus species that gave the highest OD value was selected for each positive sample [15, 19]. We found that 158 of 290 bats tested in this study were positive for anti-filovirus antibodies (Figure 1). However, viral RNA genomes were not detected in any of the tested samples.

Specificity of serum IgG antibodies detected in fruit bats

The specificity of the positive samples to each filovirus species is summarized in Table 1. Among the 158 ELISA-positive samples, 127 had the highest reactivity to the MARV GP antigen. The overall seroprevalence of MARV among the tested bat population was 43.8% (127 of 290 individuals). MARV-specific IgG antibodies were constantly detected in Egyptian fruit bats in Zambia during the research period (2014-2017). Serum samples reactive to the other filovirus GPs were also found except for EBOV GP. In total, 3.8% (11 of 290), 2.8% (8 of 290), 1.0% (3 of 290), 2.8% (8 of 290), and 0.3% (1 of 290) of the serum samples showed the highest reactivity to SUDV, TAFV, BDBV, RESTV, and LLOV antigens, respectively

(Table 1 and Supplementary Table 2). There was no significant difference between male and female bats for seropositivity (data not shown).

To further confirm the specificity to MARV, serum samples were also analyzed by NP-based ELISA (Figure 2A, B) and the correlation of the OD values given by GP and NP antigens was analyzed. We found that the OD values obtained in MARV GP and NP ELISA had a positive correlation (correlation coefficient r = 0.48). Since no correlation was observed between the OD values obtained in MARV GP and EBOV NP ELISA (r = 0.09), the positive correlation of the reactivity to MARV GP and NP antigens strongly suggested that these bats were predominantly infected with MARV. It was also found that most of the MARV GP-positive serum samples were less reactive to RAVV GP (Figure 2C), suggesting that the Egyptian fruit bat population in this cave might be mostly infected with MARV but not RAVV during the research period. However, due to the nature of this assay (i.e., potential differences in the specificity and sensitivity to each GP) and crossreactivity of anti-GP antibodies, specificity of detected antibodies could not be conclusively determined.

Seasonal patterns and maternal antibodies associated with the seroprevalence

To understand the seasonal pattern of filovirus infection related to the reproductive cycle of Egyptian fruit bats in Zambia, serological data were analyzed for each sampling date and body weight range (Figure 3). We found that the seroprevalence of filoviruses, including MARV, peaked in November to December and then decreased in January and February (Figure 3A, B). Interestingly, our field observations indicated that Zambian bats gave a birth once a year in November/December, as has been reported in South Africa [20], suggesting a possible link between the seroprevalence and reproductive cycle of Egyptian fruit bats in Zambia. Moreover, the high seroprevalence in bats weighing less than 50 g indicated that these pups passively acquired virus-specific maternal antibodies from seropositive mothers (Figure 3C, D). The seroprevalence was lowest in the bats with body weights ranging from 51 to 60 g and gradually increased as they grew, which likely reflects the disappearance of their maternal antibodies and subsequent infection of young bats.

DISCUSSION

Despite numerous epidemiological studies, the ecology of filoviruses, such as the natural reservoirs, transmission modes, and geographical distribution, remains largely unknown. Infectious ebolaviruses have never been isolated from any bat species, and there is only one report showing that a partial genome of EBOV was detected in 3 species of fruit bats in the Central African region [4]. In contrast, it has been shown that MARV and RAVV are maintained in large populations of Egyptian fruit bats in Uganda [7]. However, epidemiological information on filovirus infections outside endemic areas is quite limited. In the present study, we conducted serological investigations of cave-dwelling Egyptian fruit bats sampled in Zambia.

The overall seroprevalence of filovirus infection was very high (54.5%) in the bats tested in this study (Table 1). The majority of seropositive samples were specific to the MARV antigen. The high seroprevalence of MARV infection may suggest that this virus is circulating in Egyptian fruit bat colonies in Zambia. Although Zambia has never had a recognized human case of Marburg virus disease, it is not surprising that the virus has existed in the country, since Zambia lies within the modelled zoonotic niche of Marburg virus disease including neighboring countries with reported outbreaks of the disease such as Angola, Zimbabwe, and Democratic Republic of Congo [21]. Our data support the hypothesis that Egyptian fruit bats act as a natural reservoir of MARV even in the Southern African region.

There was a distinct peak in the seroprevalence of filoviruses, as well as to MARV specifically, at the beginning of every rainy season during the research period (Figure 3A, B). The repeated pulses of the seroprevalence in November/December suggest that MARV has been maintained in the Egyptian fruit bat population in Zambia. In a previous study in Uganda, Amman et al. [7] demonstrated that seasonal pulses of MARV infection in Egyptian fruit bats coincided with biannual reproductive seasons of the bats when the population of old juveniles (approximately 4-6 months old) increased in the colonies [7]. However, this transmission manner seems unsuitable for the Zambian population of Egyptian fruit bats that give a birth once a year in November/December. Experimental infection of the bats with MARV induced virus-specific IgG antibodies that peaked by 28 days post-infection and rapidly declined by 3 months [13]. Considering the 2-3 months duration of the presence of detectable virus-specific antibodies, it is speculated that active transmission of MARV in the studied bat colony likely occurs from August to October (i.e., a few months before the peak seropositivity) in Zambia. It is also worth noting that newborn pups might efficiently acquire maternal antibodies as shown in Figure 3. The increased proportion of immunologically naive bats due to the decline of maternal antibodies might trigger active transmission of the virus in the colony. Recently, Schuh et al. reported that MARV was horizontally transmitted from inoculated to contact Egyptian fruit bats 7 months after infection [13]. This long-term latent infection of the bats may partially explain how MARV could be maintained even in an Egyptian fruit bat population that has a single reproductive cycle per year. However, there is still another possibility that the virus is repeatedly introduced into the Egyptian fruit bat colonies in this cave from the same or different bat species migrating to this study area in some particular seasons. To understand the ecology of filoviruses, particularly MARV, in Zambia, longitudinal epidemiological studies of Egyptian fruit bats need to be continued.

It is of interest that IgG antibodies specific to RESTV and LLOV, which are thought to be Asian and European filoviruses, respectively [2, 22], were detected (Figure 1, Table 1). Our previous study also detected RESTV-specific antibodies in serum samples of migratory straw-colored fruit bats (*Eidolon helvum*) captured in Zambia [15]. These findings suggest that

RESTV, LLOV, and/or uncharacterized filoviruses antigenically similar to these viruses exist in Africa. Recently, partial genomes of unidentified filoviruses were detected in fruit bats in China [23]. IgG antibodies specific to African ebolaviruses such as EBOV were also detected in orangutans in Indonesia and fruit bats in Bangladesh [19, 24], while IgG antibodies to EBOV, SUDV, TAFV, and BDBV were recently found in fruit bats in Singapore [25]. These accumulated data on the filovirus ecology suggest that filoviruses are more divergent and widely distributed than currently assumed.

Though filovirus genomic RNA was not detected, the present study strongly suggests that Zambia is at risk for an outbreak of filovirus infection. This underlines the need for continued surveillance of these bats as well as monitoring of wildlife and domestic animals, and also for careful attention to residents living in close proximity to caves that Egyptian fruit bats inhabit in Zambia.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Figure legends

Figure 1. Filovirus-specific immunoglobulin G (IgG) antibodies detected in the sera collected from *Rousettus aegyptiacus* bats in Zambia.

Serum samples were tested (1:100 dilution) for IgG antibodies specific for glycoproteins of Ebola, Sudan, Taï Forest, Bundibugyo, Reston, Marburg, and Lloviu viruses (EBOV, SUDV, TAFV, BDBV, RESTV, MARV, and LLOV, respectively) by enzyme-linked immunosorbent assay. All optical density values (see Supplementary Figure 1) were subjected to the Smirnov-Grubbs rejection test. For the 158 positive samples, optical density values at 450 nm (OD_{450}) for the filovirus antigens are shown.

Figure 2. Reactivity of bat sera to marburgvirus antigens.

A and *B*, Serum samples positive for immunoglobulin G (IgG) antibodies specific for Marburg virus (MARV) glycoprotein (GP) were tested (1:100 dilution) for MARV (*A*) or Ebola virus (EBOV) (*B*) nucleoprotein (NP) by enzyme-linked immunosorbent assay (ELISA). A scatter plot of the optical densities at 450 nm (OD_{450}) obtained by GP- and NP-based ELISA is shown with the linear regression line and the coefficient of determination, R^2. *B*, Serum samples positive for IgG antibodies specific for MARV GP were tested (1:100 dilution) for Ravn virus (RAVV) GP by ELISA. A scatter plot of OD_{450} obtained by MARV and RAVV GP-based ELISA is shown.

Figure 3. Seropositive rates of the bats by sampling months or body weight ranges. *A* and *B*, Anti-glycoprotein (GP) immunoglobulin G positive rates for all filovirus species (*A*) and Marburg virus (MARV) (*B*) of the bats by sampling months, respectively. *C* and *D*, Seropositive rates to all filovirus species (*C*) and MARV (*D*) of the bats by body weight ranges, respectively.

Table 1

Table 1. Filovirus species-specificity of serum immunoglobulin G antibodies detected in *Rousettus aegyptiacus* in Zambia

Sampling	Seropositivity to the respective antigens, % (no. positive/total no.)[a]							
	EBOV	SUDV	TAFV	BDBV	RESTV	MARV	LLOV	Total
2014 Dec	0 (0/10)	10.0 (1/10)	20.0 (2/10)	0 (0/10)	0 (0/10)	60.0 (6/10)	0 (0/10)	90.0 (9/10)
2015 Jan	0 (0/66)	4.5 (3/66)	3.0 (2/66)	1.5 (1/66)	4.5 (3/66)	43.9 (29/66)	0 (0/66)	57.6 (38/66)
2015 Feb	0 (0/40)	0 (0/40)	0 (0/40)	0 (0/40)	2.5 (1/40)	27.5 (11/40)	0 (0/40)	30.0 (12/40)
2015 Jul	0 (0/26)	3.8 (1/26)	3.8 (1/26)	0 (0/26)	3.8 (1/26)	42.3 (11/26)	3.8 (1/26)	57.7 (15/26)
2015 Nov	0 (0/20)	0 (0/20)	0 (0/20)	0 (0/20)	5.0 (1/20)	85.0 (17/20)	0 (0/20)	90.0 (18/20)
2015 Dec	0 (0/22)	0 (0/22)	0 (0/22)	0 (0/22)	0 (0/22)	63.6 (14/22)	0 (0/22)	63.6 (14/22)
2016 Apr	0 (0/39)	2.6 (1/39)	2.6 (1/39)	0 (0/39)	2.6 (1/39)	33.3 (13/39)	0 (0/39)	41.0 (16/39)
2016 Dec	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)	50.0 (2/4)	0 (0/4)	50.0 (2/4)

2017 Jul	0 (0/31)	6.5 (2/31)	0 (0/31)	0 (0/31)	3.2 (1/31)	19.4 (6/31)	0 (0/31)	29.0 (9/31)
2017 Dec	0 (0/32)	9.4 (3/32)	6.3 (2/32)	6.3 (2/32)	0 (0/32)	56.3 (18/32)	0 (0/32)	78.1 (25/32)
Total	0 (0/290)	3.8 (11/290)	2.8 (8/290)	1.0 (3/290)	2.8 (8/290)	43.8 (127/290)	0.3 (1/290)	54.5 (158/290)

[a] The filovirus species for which each positive sample had the highest optical density value in the glycoprotein (GP)-based enzyme-linked immunosorbent assay was selected when a sample showed cross-reactivity to GPs of multiple species. Abbreviations, EBOV (Ebola virus), SUDV (Sudan virus), TAFV (Taï Forest virus), BDBV (Bundibugyo virus), RESTV (Reston virus), MARV (Marburg virus), and LLOV (Lloviu virus).

Figure 1

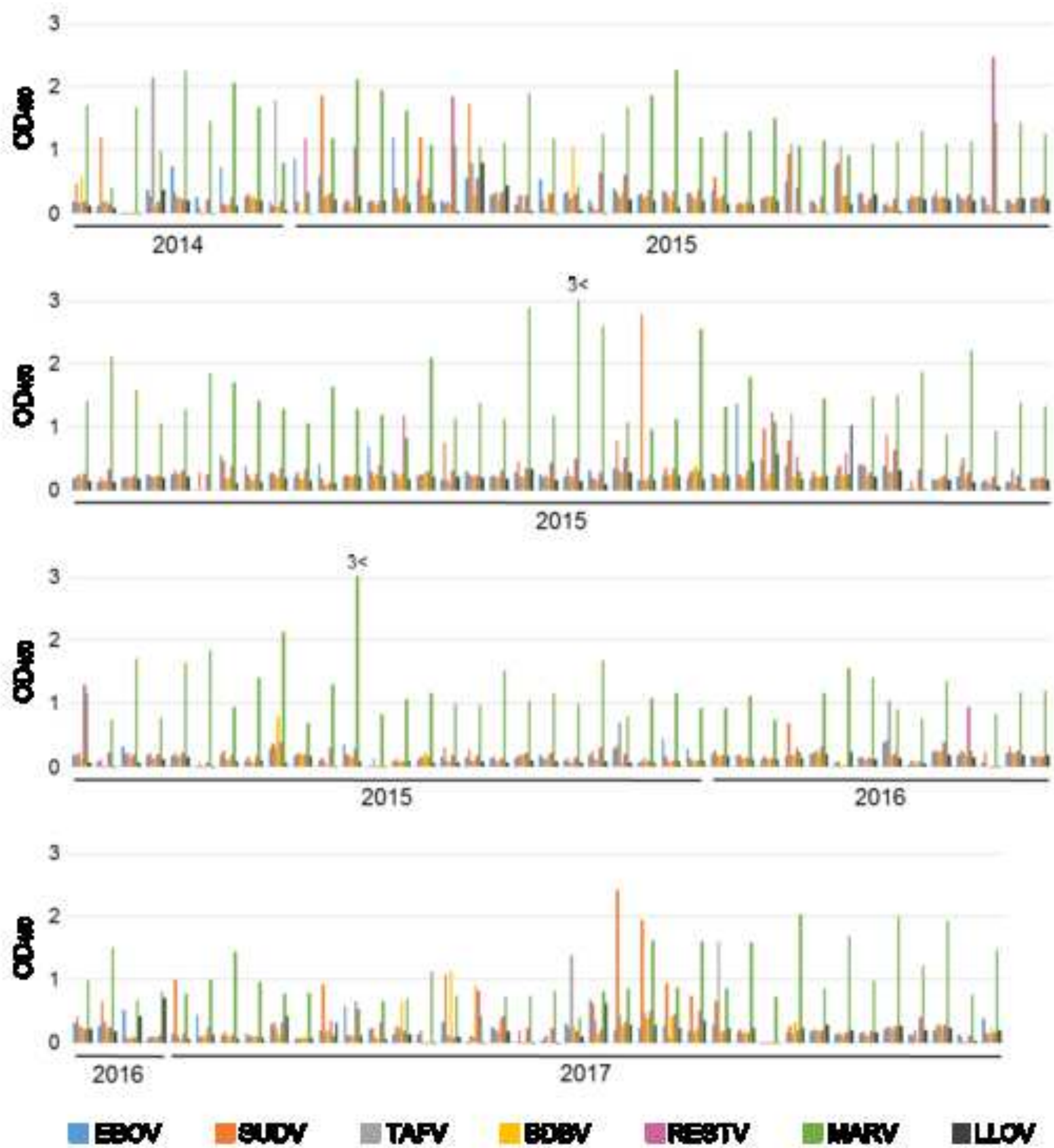


Figure 1

Figure 2

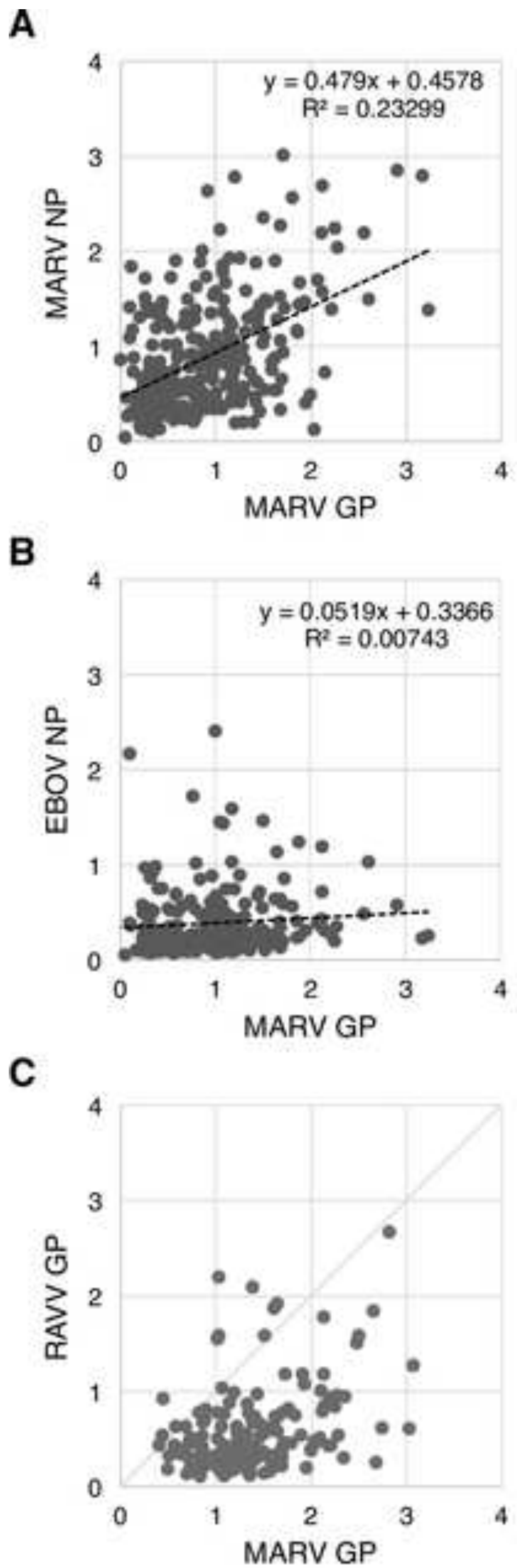


Figure 2

Figure 3

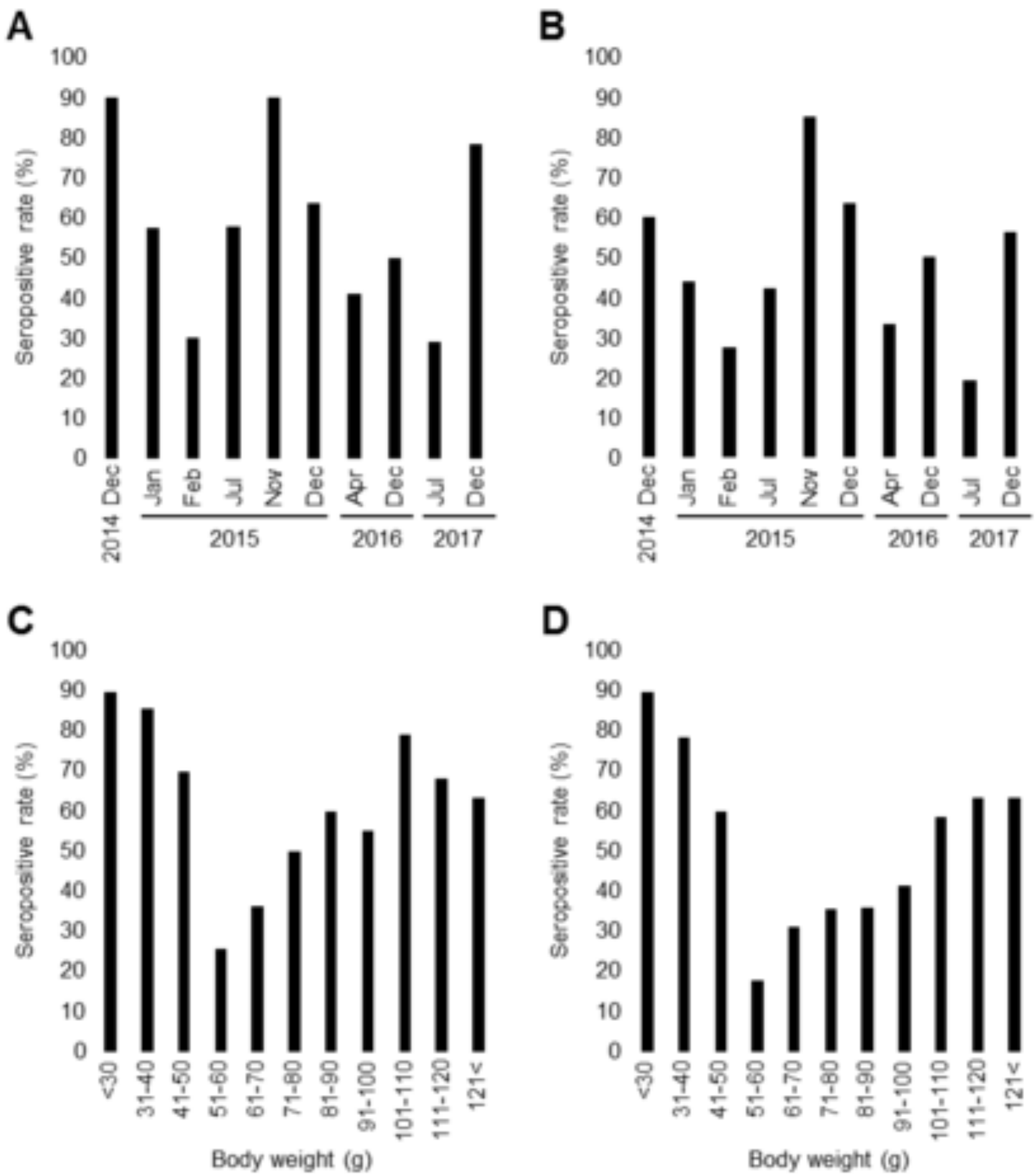


Figure 3

Supplementary Table 1. Summary of the fruit bat serum samples analyzed

			No. of bats analyzed		
Date	Province	District	Male	Female	Total
December 7, 2014	Lusaka	Chongwe	5	5	10
January 8, 2015	Lusaka	Chongwe	13	16	29
January 28, 2015	Lusaka	Chongwe	17	20	37
February 12, 2015	Lusaka	Chongwe	18	22	40
July 15, 2015	Lusaka	Chongwe	12	14	26
November 6, 2015	Lusaka	Chongwe	8	12	20
December 3, 2015	Lusaka	Chongwe	6	16	22
April 26, 2016	Lusaka	Kafue	6	10	16
April 28, 2016	Lusaka	Chongwe	7	16	23
December 7, 2016	Lusaka	Chongwe	1	3	4
July 31, 2017	Lusaka	Chongwe	12	19	31
December 4, 2017	Lusaka	Chongwe	14	18	32
Total			119	171	290

Supplementary Table 2

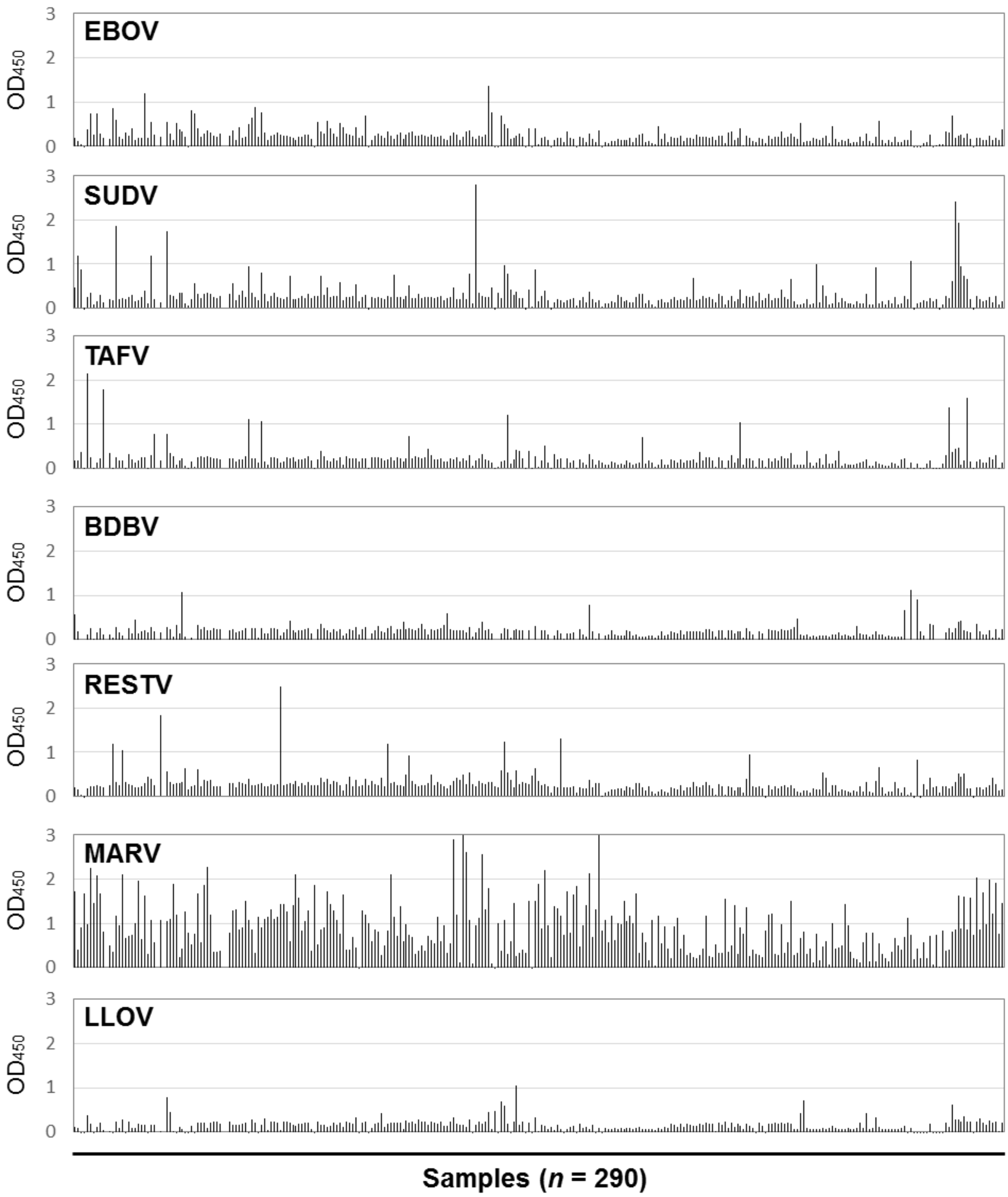
Sampling Date	Bat ID	Sex	Zaire	Sudan	Tai Forest	Bundibugy	Reston	Angola	LLOV	Highest OD	Filo Positive	Angola Positive
12/7/2014	ZFB14 #122	M	0.18105	0.46875	0.16985	0.5686	0.18955	1.71115	0.1066	Angola		
12/7/2014	ZFB14 #123	M	0.11825	1.1973	0.1795	0.1935	0.15055	0.3993	0.08235	Sudan		
12/7/2014	ZFB14 #124	F	0.02945	0.88735	0.35855	-0.06685	0.01225	0.90905	-0.1138			
12/7/2014	ZFB14 #125	M	-0.2862	-0.0253	-0.22965	-0.2605	-0.1086	1.67975	-0.4082	Angola		
12/7/2014	ZFB14 #126	F	0.36795	0.26135	2.15715	0.1048	0.1778	0.98045	0.37235	Tai Forest		
12/7/2014	ZFB14 #127	F	0.7353	0.34005	0.24725	0.2473	0.2196	2.2509	0.1923	Angola	8/10	6/10
12/7/2014	ZFB14 #128	F	0.2517	0.0802	-0.0756	0.0157	0.22095	1.45835	-0.16715	Angola		
12/7/2014	ZFB14 #129	M	0.7301	0.1489	0.1219	0.1496	0.2524	2.0747	0.11015	Angola		
12/7/2014	ZFB14 #130	F	0.2707	0.306	0.2315	0.26535	0.2168	1.67135	0.196	Angola		
12/7/2014	ZFB14 #131	M	0.19025	0.1225	1.79415	0.10265	0.20065	0.79745	0.0485	Tai Forest		
1/8/2015	ZFB15 #1	M				Sample missing						
1/8/2015	ZFB15 #2	M	0.163165	0.192165	0.341065	0.116115	0.239415	0.498615	0.020115			
1/8/2015	ZFB15 #3	F	0.862235	0.177985	-0.00301	0.028935	1.186485	0.342885	-0.07871	Reston		
1/8/2015	ZFB15 #4	M	0.58667	1.86177	0.25797	0.27792	0.32567	1.17757	0.22072	Sudan		
1/8/2015	ZFB15 #5	M	0.20929	0.20924	0.18384	0.14869	0.25469	0.95384	0.08929			
1/8/2015	ZFB15 #6	M	0.146845	0.223395	0.184195	0.096345	1.041795	2.118095	0.272595	Angola		
1/8/2015	ZFB15 #7	M	0.31279	0.20124	-0.00195	-0.09978	0.31724	0.66074	-0.11266			
1/8/2015	ZFB15 #8	F	0.239985	0.261035	0.310435	0.246335	0.276085	0.701335	0.218585			
1/8/2015	ZFB15 #9	F	0.392735	0.307785	0.198035	0.133935	0.242985	0.734735	0.084985			
1/8/2015	ZFB15 #10	M	0.143395	0.153095	0.136245	0.454895	0.210945	0.998195	0.094845			
1/8/2015	ZFB15 #11	F	0.18026	0.19126	0.17666	0.13886	0.20041	1.95166	0.19381	Angola		
1/8/2015	ZFB15 #12	F	0.17163	0.25248	0.23813	0.18543	0.21708	0.64558	0.14708			
1/8/2015	ZFB15 #13	F	1.200915	0.398265	0.255715	0.212065	0.290865	1.626115	0.164865	Angola		
1/8/2015	ZFB15 #14	F	0.19213	0.10718	-0.00354	0.16388	0.45228	0.30798	-0.13966			
1/8/2015	ZFB15 #15	F	0.53733	1.19228	0.30443	0.28428	0.38503	1.07568	0.16418	Sudan		
1/8/2015	ZFB15 #16	M	0.264425	0.208825	0.789575	0.181825	0.258275	0.567075	0.164475			9/29
1/8/2015	ZFB15 #17	F				Sample missing					13/29	
1/8/2015	ZFB15 #18	M	0.201155	0.141755	0.181055	0.150755	1.840655	1.062305	0.022355	Reston		
1/8/2015	ZFB15 #19	F				Sample missing						
1/8/2015	ZFB15 #20	F	0.551955	1.734705	0.785205	0.289505	0.560955	1.044455	0.788055	Sudan		
1/8/2015	ZFB15 #21	M	0.28576	0.30011	0.33691	0.21991	0.33086	1.10761	0.44136	Angola		
1/8/2015	ZFB15 #22	F	0.137685	0.264285	0.277985	0.070485	0.278285	1.892685	0.021785	Angola		
1/8/2015	ZFB15 #23	M	0.53013	0.21278	0.07088	0.33233	0.29388	1.19138	-0.03034	Angola		
1/8/2015	ZFB15 #24	F	0.383055	0.339555	0.164905	0.121855	0.298855	0.224005	0.118355			
1/8/2015	ZFB15 #25	F	0.32287	0.34952	0.22772	1.06657	0.30862	0.41422	0.05027	Bundibugyo		
1/8/2015	ZFB15 #26	F	0.20549	0.11784	0.04879	0.05514	0.63219	1.25484	-0.04548	Angola		
1/8/2015	ZFB15 #27	M	-2.75E-05	0.060745	-0.00808	-0.0122	0.140345	0.779295	-0.028			
1/8/2015	ZFB15 #28	F	0.79808	0.20103	0.14823	0.03253	0.23488	0.51948	0.13158			
1/8/2015	ZFB15 #29	M	0.72766	0.56746	0.04136	-0.0309	0.24546	0.75926	-0.08187			
1/8/2015	ZFB15 #30	F	0.392525	0.333325	0.254175	0.316775	0.608075	1.679075	0.214425	Angola		
1/8/2015	ZFB15 #31	M	0.20312	0.22427	0.28127	0.22287	0.23402	0.55597	0.20552			
1/8/2015	ZFB15 #32	F	0.277035	0.314185	0.249435	0.286385	0.364035	1.857635	0.204935	Angola		
1/28/2015	ZFB15 #33	F	0.357555	0.340305	0.269505	0.212205	0.351255	2.276605	0.089955	Angola		
1/28/2015	ZFB15 #34	F	0.31426	0.32046	0.25086	0.21456	0.37691	1.20386	0.20436	Angola		
1/28/2015	ZFB15 #35	M	0.22489	0.24569	0.21669	0.24609	0.22459	0.35634	0.21824			
1/28/2015	ZFB15 #36	M	0.21581	0.23601	0.21311	0.23651	0.22591	0.34311	0.21951			
1/28/2015	ZFB15 #37	F	0.279595	0.280495	0.210245	0.241895	0.224295	0.373795	0.186595			
1/28/2015	ZFB15 #38	M				Sample missing						
1/28/2015	ZFB15 #39	M				Sample missing						
1/28/2015	ZFB15 #40	F	0.23432	0.32362	0.21862	0.20812	0.30702	0.79282	0.22132			
1/28/2015	ZFB15 #41	F	0.350635	0.571485	0.227335	0.239735	0.285135	1.286585	0.153035	Angola		
1/28/2015	ZFB15 #42	F	0.132765	0.173165	0.154815	0.155715	0.197165	1.303065	0.147865	Angola		
1/28/2015	ZFB15 #43	F	0.429505	0.299205	0.205755	0.185205	0.326005	0.852255	0.146155			
1/28/2015	ZFB15 #44	F	0.188515	0.388365	0.207965	0.205415	0.293015	0.894265	0.181965			
1/28/2015	ZFB15 #45	M	0.21208	0.24623	0.26193	0.24403	0.26653	1.51098	0.19488	Angola		
1/28/2015	ZFB15 #46	M	0.502395	0.945345	1.107145	0.005045	0.395445	1.067545	-0.08138	Tai Forest		
1/28/2015	ZFB15 #47	M	0.65046	0.34301	0.22461	0.24966	0.25671	0.86331	0.28176			
1/28/2015	ZFB15 #48	M	0.87787	0.24457	0.23487	0.24467	0.24617	0.31637	0.20787			
1/28/2015	ZFB15 #49	F	0.19481	0.18361	0.12291	0.04751	0.26571	1.15421	-0.0422	Angola		
1/28/2015	ZFB15 #50	F	0.749035	0.797035	1.071535	0.260935	0.289135	0.905035	0.149735	Tai Forest		
1/28/2015	ZFB15 #51	M	0.31292	0.31332	0.15622	0.14342	0.22622	1.09142	0.30042	Angola		
1/28/2015	ZFB15 #52	M	0.123735	0.162485	0.089635	0.134585	0.236085	1.136435	0.033785	Angola	22/37	20/37
1/28/2015	ZFB15 #53	F	0.22179	0.27814	0.24329	0.25049	0.26294	1.30209	0.21904	Angola		
1/28/2015	ZFB15 #54	F	0.265965	0.352065	0.241265	0.265015	0.258015	1.098615	0.221565	Angola		
1/28/2015	ZFB15 #55	F	0.29557	0.25517	0.22167	0.23857	0.27677	1.13472	0.20722	Angola		
1/28/2015	ZFB15 #56	F	0.265875	0.227975	0.138875	0.087475	2.479425	1.444225	0.027325	Reston		
1/28/2015	ZFB15 #57	F	0.22061	0.20641	0.14116	0.15896	0.24031	1.43211	0.24061	Angola		
1/28/2015	ZFB15 #58	F	0.23624	0.25689	0.24274	0.23344	0.28319	1.25864	0.21959	Angola		
1/28/2015	ZFB15 #59	F	0.217955	0.726505	0.224405	0.415355	0.297305	0.584605	0.213605			
1/28/2015	ZFB15 #60	F	0.18301	0.21276	0.25011	0.20311	0.27151	1.42146	0.14981	Angola		
1/28/2015	ZFB15 #61	M	0.12584	0.19249	0.14354	0.15749	0.33899	2.11739	0.13114	Angola		
1/28/2015	ZFB15 #62	F	0.19572	0.22177	0.20442	0.20032	0.22622	1.58302	0.18737	Angola		
1/28/2015	ZFB15 #63	M	0.217155	0.281005	0.205455	0.207505	0.295705	0.823105	0.175405			
1/28/2015	ZFB15 #64	M	0.243055	0.230755	0.219805	0.221255	0.239655	1.057755	0.203305	Angola		
1/28/2015	ZFB15 #65	F	0.252905	0.318405	0.262805	0.259105	0.324505	1.291505	0.210805	Angola		
1/28/2015	ZFB15 #66	M	0.16312	0.23017	0.16547	0.14207	0.23817	0.37352	0.07072			
1/28/2015	ZFB15 #67	M	-0.085775	0.2814	-0.02755	-0.15358	0.25305	1.86785	-0.16178	Angola		
1/28/2015	ZFB15 #68	M	0.546645	0.272495	0.204295	0.223095	0.249145	0.518995	0.220045			

1/28/2015	ZFB15 #69	M	0.319475	0.733575	0.383475	0.347075	0.418775	0.845475	0.162375			
1/28/2015	ZFB15 #70	M	0.29014	0.29664	0.26084	0.24734	0.32924	0.90504	0.16519			
1/28/2015	ZFB15 #71	M	0.557315	0.466115	0.180115	0.202765	0.393365	1.717815	0.109915	Angola		
2/12/2015	ZFB15 #72	M	0.390665	0.250265	0.162665	0.165615	0.262315	1.424365	0.124415	Angola		
2/12/2015	ZFB15 #73	F	0.269025	0.280575	0.226225	0.220525	0.348025	1.282675	0.200275	Angola		
2/12/2015	ZFB15 #74	F	0.216365	0.278465	0.182765	0.185615	0.329715	1.065315	0.152765	Angola		
2/12/2015	ZFB15 #75	F	0.51091	0.57751	0.25671	0.22586	0.24136	0.75071	0.20326			
2/12/2015	ZFB15 #76	M	0.4254	0.17905	0.08575	0.09205	0.1377	1.6418	0.11025	Angola		
2/12/2015	ZFB15 #77	F	0.27067	0.24237	0.27522	0.13482	0.26742	0.39437	0.23397			
2/12/2015	ZFB15 #78	F	0.24797	0.24232	0.21797	0.23177	0.44352	0.40747	0.21327			
2/12/2015	ZFB15 #79	F	0.221225	0.278825	0.213875	0.208425	0.226425	0.696975	0.183325			
2/12/2015	ZFB15 #80	M	0.433205	0.543755	0.233855	0.289755	0.371855	0.435655	0.316805			
2/12/2015	ZFB15 #81	F	0.17255	0.16345	0.1563	0.1063	0.2342	-0.0132	-0.16785			
2/12/2015	ZFB15 #82	F	0.22332	0.25832	0.22032	0.22087	0.25457	1.29237	0.20797	Angola		
2/12/2015	ZFB15 #83	M	0.68578	0.30013	0.21683	0.26633	0.39808	1.19343	0.21898	Angola		
2/12/2015	ZFB15 #84	M	-0.035375	-0.06923	-0.04225	-0.13903	0.24915	1.0013	-0.25398			
2/12/2015	ZFB15 #85	F	0.130235	0.260485	0.240785	0.132485	0.351135	0.596285	0.083535			
2/12/2015	ZFB15 #86	F	0.228015	0.218165	0.248465	0.201765	0.268265	0.855615	0.184665			
2/12/2015	ZFB15 #87	M	0.282545	0.253595	0.236995	0.293895	0.257095	0.799945	0.214945			
2/12/2015	ZFB15 #88	M	0.223505	0.234255	0.220455	0.181605	0.412955	0.277205	0.426455			
2/12/2015	ZFB15 #89	M	0.18864	0.20789	0.17459	0.16284	0.22969	0.48649	0.11909			
2/12/2015	ZFB15 #90	M	0.3239	0.26885	0.188	0.25975	1.19065	0.8312	0.18665	Reston		
2/12/2015	ZFB15 #91	F	0.23128	0.25263	0.24363	0.29443	0.29433	2.10598	0.21473	Angola	12/40	11/40
2/12/2015	ZFB15 #92	F	0.169605	0.760055	0.169955	0.123555	0.315305	1.145855	0.210255	Angola		
2/12/2015	ZFB15 #93	F	0.249635	0.254135	0.240735	0.236435	0.247535	0.703235	0.202885			
2/12/2015	ZFB15 #94	M	0.30466	0.24291	0.22681	0.22751	0.23776	1.39186	0.20101	Angola		
2/12/2015	ZFB15 #95	M	0.160725	0.214525	0.147275	0.388775	0.222475	0.595425	0.067725			
2/12/2015	ZFB15 #96	F	0.25301	0.27661	0.22826	0.23576	0.49476	0.96556	0.25011			
2/12/2015	ZFB15 #97	F	0.29985	0.5219	0.7196	0.25645	0.9206	0.7391	0.21255			
2/12/2015	ZFB15 #98	F	0.31904	0.23914	0.23004	0.23164	0.33429	0.68734	0.23024			
2/12/2015	ZFB15 #99	F	0.232705	0.237655	0.267505	0.211155	0.275655	0.305555	0.184655			
2/12/2015	ZFB15 #100	M	0.22592	0.33457	0.23782	0.26027	0.23487	0.37382	0.27082			
2/12/2015	ZFB15 #101	F	0.2562	0.2327	0.22315	0.3399	0.2433	0.48515	0.21935			
2/12/2015	ZFB15 #102	F	0.23781	0.25301	0.24121	0.22271	0.25601	0.37931	0.23071			
2/12/2015	ZFB15 #103	M	0.19397	0.24882	0.44687	0.12147	0.29602	0.70992	0.16002			
2/12/2015	ZFB15 #104	M	0.24499	0.24644	0.28464	0.23499	0.50054	0.62429	0.22474			
2/12/2015	ZFB15 #105	F	0.21212	0.22112	0.21037	0.20592	0.24257	0.55107	0.21037			
2/12/2015	ZFB15 #106	M	0.20829	0.24954	0.20689	0.22124	0.30984	1.13499	0.17974	Angola		
2/12/2015	ZFB15 #107	M	0.221235	0.274535	0.220685	0.256835	0.279435	0.594735	0.220835			
2/12/2015	ZFB15 #108	F	0.16797	0.17957	0.15832	0.33032	0.23107	0.94427	0.13332			
2/12/2015	ZFB15 #109	F	0.13887	0.22382	0.14632	0.58692	0.17657	0.33697	0.13882			
2/12/2015	ZFB15 #110	M	0.22592	0.24567	0.22007	0.22822	0.25582	0.53287	0.22832			
2/12/2015	ZFB15 #111	M	0.290345	0.460595	0.210295	0.215995	0.352345	2.905945	0.336895	Angola		
7/15/2015	ZFB15 #112	M	0.2572	0.2068	0.2383	0.2007	0.42845	1.1915	0.17235	Angola		
7/15/2015	ZFB15 #113	M	0.12794	0.19769	0.15469	0.19589	0.37964	0.10854	0.16284			
7/15/2015	ZFB15 #114	F	0.21707	0.33692	0.22587	0.20147	0.49722	3.17572	0.15657	Angola		
7/15/2015	ZFB15 #115	F	0.316405	0.205805	0.186955	0.154005	0.280105	2.607155	0.101755	Angola		
7/15/2015	ZFB15 #116	M	0.35432	0.79037	0.29552	0.28227	0.52707	1.08417	0.27557	Angola		
7/15/2015	ZFB15 #117	M	0.194615	0.104615	0.056115	0.067415	0.275115	0.093765	-0.13727			
7/15/2015	ZFB15 #118	F	0.169545	2.801895	0.170645	0.152295	0.224945	0.964145	0.168745	Sudan		
7/15/2015	ZFB15 #119	M	0.234915	0.352765	0.227715	0.256415	0.354965	1.128665	0.229065	Angola		
7/15/2015	ZFB15 #120	F	0.201075	0.275975	0.318475	0.387575	0.295075	2.558475	0.174325	Angola		
7/15/2015	ZFB15 #121	M	0.25896	0.25121	0.20531	0.20731	0.28291	1.31831	0.23741	Angola		
7/15/2015	ZFB15 #122	F	1.36645	0.25635	0.18415	0.2237	0.31235	1.80305	0.44775	Angola		
7/15/2015	ZFB15 #123	F	0.75503	0.47068	0.13638	0.14218	0.31643	0.09503	0.07213			
7/15/2015	ZFB15 #124	F	-0.0926325	-0.05578	-0.06483	-0.16511	0.217435	-0.18398	0.466035		15/26	11/26
7/15/2015	ZFB15 #125	F	-0.1725175	0.343965	0.019215	-0.01297	0.199015	0.995015	-0.12754			
7/15/2015	ZFB15 #126	M	0.67669	0.22979	0.15684	0.14074	0.58969	0.36529	0.68309			
7/15/2015	ZFB15 #127	M	0.48469	0.98534	0.16499	0.26169	1.22859	1.08054	0.58074	Reston		
7/15/2015	ZFB15 #128	F	0.406225	0.791075	1.213525	0.220475	0.540125	0.302225	0.180725	Tai Forest		
7/15/2015	ZFB15 #129	F	0.152245	0.414095	0.107595	0.034445	0.376995	0.581795	-0.0807			
7/15/2015	ZFB15 #130	F	0.18959	0.30714	0.20759	0.21664	0.21119	1.45389	0.22584	Angola		
7/15/2015	ZFB15 #131	M	0.23484	0.36844	0.40609	0.23129	0.58709	0.25329	1.03879	LLOV		
7/15/2015	ZFB15 #132	M	0.28541	0.22831	0.38561	0.20926	0.28266	0.33336	0.15526			
7/15/2015	ZFB15 #133	F	0.201615	0.224215	0.229315	0.212415	0.321915	0.406265	0.231315			
7/15/2015	ZFB15 #134	M	-0.06679	-0.04752	-0.08449	-0.21529	0.29097	0.32757	-0.31907			
7/15/2015	ZFB15 #135	M	0.40279	0.41779	0.39364	0.21409	0.28069	1.49444	0.21489	Angola		
7/15/2015	ZFB15 #136	F	-0.021175	0.03315	-0.02625	-0.11565	0.4611	-0.12895	-0.3165			
7/15/2015	ZFB15 #137	F	0.38854	0.87409	0.27959	0.29939	0.64469	1.50249	0.32304	Angola		
11/6/2015	ZFB15 #173	F	0.03225	0.15545	0.0216	-0.0119	0.33915	1.8843	0.0141	Angola		
11/6/2015	ZFB15 #174	M	0.18335	0.266	0.18255	0.19925	0.24015	0.8783	0.1636	Angola		
11/6/2015	ZFB15 #175	F	0.21495	0.4048	0.5246	0.20135	0.2758	2.21495	0.1306	Angola		
11/6/2015	ZFB15 #176	M	0.1338	0.1898	0.13505	0.118	0.2136	0.9524	0.0707	Angola		
11/6/2015	ZFB15 #177	M	-0.29555	-0.245	-0.66585	-0.65865	0.0689	0.23215	0.1			
11/6/2015	ZFB15 #178	M	0.12455	0.1333	0.32995	0.09675	0.2297	1.3913	0.0376	Angola		
11/6/2015	ZFB15 #179	M	0.17615	0.19725	0.19795	0.2032	0.20715	1.33605	0.1689	Angola		
11/6/2015	ZFB15 #180	F	0.185	0.1842	0.22195	0.12325	1.307	1.1629	0.05905	Reston		
11/6/2015	ZFB15 #181	F	0.07565	0.1213	-0.0249	0.0123	0.21175	0.7452	-0.1375	Angola		
11/6/2015	ZFB15 #182	M	0.31725	0.1918	0.21845	0.12755	0.1898	1.71235	0.07045	Angola	18/20	17/20
11/6/2015	ZFB15 #183	F	0.1872	0.20785	0.12605	0.14355	0.19205	0.78345	0.1184	Angola		

11/6/2015	ZFB15 #184	F	0.16235	0.22445	0.16985	0.1611	0.2127	**1.6413**	0.14325	Angola		
11/6/2015	ZFB15 #185	F	-0.0454	0.0569	-0.10685	-0.06625	0.07055	**1.85155**	-0.20065	Angola		
11/6/2015	ZFB15 #186	M	0.20195	0.1853	0.19625	0.2375	0.2018	0.46675	0.1871			
11/6/2015	ZFB15 #187	F	0.17935	0.2427	0.11995	0.11705	0.18175	**0.9538**	0.07515	Angola		
11/6/2015	ZFB15 #188	F	0.08625	0.14055	0.0831	0.07155	0.1721	**1.4046**	0.10265	Angola		
11/6/2015	ZFB15 #189	F	0.2816	0.36845	0.32535	**0.78975**	0.3782	**2.14255**	0.0658	Angola		
11/6/2015	ZFB15 #190	M	0.16585	0.19715	0.20825	0.18285	0.1971	0.68565	0.1575	Angola		
11/6/2015	ZFB15 #191	F	0.09675	0.1232	0.0817	0.0244	0.2955	**1.3062**	-0.0664	Angola		
11/6/2015	ZFB15 #192	F	0.3509	0.1914	0.1649	0.14215	0.28655	**3.23435**	0.0881	Angola		
12/3/2015	ZFB15 #249	F	-0.02475	0.01345	0.1303	-0.0778	-0.00735	**0.83175**	-0.10355	Angola		
12/3/2015	ZFB15 #250	M	0.08675	0.10575	0.0683	0.0933	0.0799	**1.0609**	0.08005	Angola		
12/3/2015	ZFB15 #251	F	0.06785	0.0992	0.0769	0.1046	0.0984	0.5633	0.06225			
12/3/2015	ZFB15 #252	M	0.1053	0.14395	0.1416	0.2133	0.1547	**1.16315**	0.0708	Angola		
12/3/2015	ZFB15 #253	F	0.1113	0.13705	0.13345	0.1056	0.15085	0.6237	0.07455			
12/3/2015	ZFB15 #254	F	0.15265	0.30165	0.0877	0.08745	0.1866	**0.9967**	0.06435	Angola		
12/3/2015	ZFB15 #255	F	0.14315	0.2589	0.10005	0.09165	0.1764	**0.97465**	0.07395	Angola		
12/3/2015	ZFB15 #256	M	0.12535	0.1602	0.08715	0.0909	0.1276	**1.51505**	0.06595	Angola		
12/3/2015	ZFB15 #257	F	0.12425	0.17875	0.178	0.20525	0.22085	**1.0524**	0.09205	Angola		
12/3/2015	ZFB15 #258	F	0.17375	0.1248	0.1216	0.17365	0.20995	**1.16895**	0.08275	Angola		
12/3/2015	ZFB15 #259	F	0.09395	0.1317	0.07105	0.074	0.15155	**1.005**	0.0601	Angola	14/22	14/22
12/3/2015	ZFB15 #260	F	0.17825	0.2418	0.10795	0.1107	0.29805	**1.67885**	0.0803	Angola		
12/3/2015	ZFB15 #261	F	0.25115	0.3214	0.1217	0.06195	0.3024	0.3271	0.1096			
12/3/2015	ZFB15 #262	M	0.27545	0.3312	0.6964	0.0631	0.20135	**0.7942**	0.05785	Angola		
12/3/2015	ZFB15 #263	F	0.09155	0.10245	0.1146	0.0685	0.11585	0.5564	0.0656			
12/3/2015	ZFB15 #264	F	0.1113	0.1734	0.18455	0.0836	0.22735	0.1695	0.0685			
12/3/2015	ZFB15 #265	F	0.06605	0.08735	0.1325	0.07975	0.09225	**1.0735**	0.0612	Angola		
12/3/2015	ZFB15 #266	F	0.0424	0.0446	0.04245	0.0428	0.04455	0.04445	0.04325			
12/3/2015	ZFB15 #267	M	0.4359	0.16905	0.07825	0.0825	0.0973	**1.1626**	0.07455	Angola		
12/3/2015	ZFB15 #268	M	0.1544	0.21035	0.1921	0.1849	0.13985	0.53155	0.06305			
12/3/2015	ZFB15 #269	F	0.28485	0.12725	0.0869	0.089	0.10205	**0.9277**	0.1	Angola		
12/3/2015	ZFB15 #270	F	0.0804	0.1421	0.08095	0.09915	0.0761	0.4267	0.07615			
4/26/2016	ZFB16 #1	M	0.1941	0.1992	0.1927	0.1847	0.1903	0.2159	0.1792			
4/26/2016	ZFB16 #2	M	0.1919	0.258	0.171	0.164	0.1811	**0.9294**	0.1647	Angola		
4/26/2016	ZFB16 #3	F	0.1749	0.1826	0.1325	0.1242	0.1474	**1.1123**	0.1113	Angola		
4/26/2016	ZFB16 #4	F	0.2202	0.206	0.2	0.1996	0.2419	0.4178	0.1837			
4/26/2016	ZFB16 #5	F	0.1109	0.1713	0.1252	0.12	0.1299	0.7394	0.1213	Angola		
4/26/2016	ZFB16 #6	F	0.1773	0.243	0.1823	0.1877	0.1976	0.2751	0.1708			
4/26/2016	ZFB16 #7	F	0.1854	0.2099	0.1654	0.1721	0.2105	0.3249	0.1645			
4/26/2016	ZFB16 #8	F	0.1599	0.6911	0.1905	0.1833	0.3089	0.2292	0.138	Sudan	5/16	4/16
4/26/2016	ZFB16 #9	F	0.2576	0.1803	0.1398	0.1801	0.2248	0.2179	0.1403			
4/26/2016	ZFB16 #10	F	0.2039	0.201	0.361	0.1921	0.1965	0.2865	0.1754			
4/26/2016	ZFB16 #11	M	0.2157	0.2849	0.1858	0.168	0.2571	0.426	0.1488			
4/26/2016	ZFB16 #12	F	0.206	0.2162	0.2596	0.2203	0.3209	**1.1712**	0.2014	Angola		
4/26/2016	ZFB16 #13	M	0.1845	0.2478	0.2468	0.2421	0.2567	0.2639	0.1832			
4/26/2016	ZFB16 #14	M	0.2153	0.2108	0.1814	0.187	0.1839	0.2349	0.1772			
4/26/2016	ZFB16 #15	M	0.141	0.1295	0.0071	0.0575	0.0295	0.5131	0.0064			
4/26/2016	ZFB16 #16	F	0.2315	0.3303	0.2384	0.2045	0.2835	0.3215	0.2128			
4/28/2016	ZFB16 #17	F	0.2214	0.2645	0.1879	0.2167	0.2182	0.3324	0.1613			
4/28/2016	ZFB16 #18	M	0.0684	0.0851	-0.0163	0.0293	0.0152	**1.5506**	0.2312	Angola		
4/28/2016	ZFB16 #19	F	0.307	0.1796	0.1867	0.2074	0.2137	0.3502	0.089			
4/28/2016	ZFB16 #20	F	0.3177	0.2829	0.2899	0.2027	0.2039	0.4856	0.2019			
4/28/2016	ZFB16 #21	M	0.1401	0.1491	0.1201	0.1343	0.1366	**1.4105**	0.1145	Angola		
4/28/2016	ZFB16 #22	M	0.1844	0.1999	0.2278	0.1938	0.2114	0.2925	0.1843			
4/28/2016	ZFB16 #23	F	0.3915	0.4277	**1.0511**	0.1817	0.2011	**0.9137**	0.1294	Tai Forest		
4/28/2016	ZFB16 #24	M	0.0241	0.0964	0.0792	0.0278	0.0881	**0.7657**	0.052	Angola		
4/28/2016	ZFB16 #25	M	0.2271	0.2742	0.2334	0.2428	0.3815	**1.3532**	0.1903	Angola		
4/28/2016	ZFB16 #26	F	0.1902	0.2417	0.2251	0.1792	**0.9521**	0.2497	0.1411	Reston		
4/28/2016	ZFB16 #27	F	0.112	0.2755	0.1751	0.0985	0.2354	0.3882	0.0778			
4/28/2016	ZFB16 #28	F	0.0773	0.1461	0.0148	-0.0024	0.1955	0.3041	-0.0845		11/23	9/23
4/28/2016	ZFB16 #29	F	0.1916	0.3596	0.2271	0.1929	0.2222	0.2862	0.2002			
4/28/2016	ZFB16 #30	F	0.1566	0.1758	0.1651	0.139	0.1749	0.2365	0.1272			
4/28/2016	ZFB16 #31	F	0.0596	0.2273	0.0057	-0.0278	-0.068	**0.8302**	-0.1674	Angola		
4/28/2016	ZFB16 #32	F	0.2228	0.3147	0.2203	0.2217	0.2417	**1.1892**	0.1836	Angola		
4/28/2016	ZFB16 #33	M	0.1588	0.1731	0.1429	0.1982	0.1548	**1.2074**	0.1777	Angola		
4/28/2016	ZFB16 #34	F	0.2094	0.219	0.2282	0.2052	0.2355	0.2982	0.2017			
4/28/2016	ZFB16 #35	F	0.2027	0.2231	0.1861	0.1852	0.184	0.2711	0.1724			
4/28/2016	ZFB16 #36	F	0.3183	0.4215	0.2738	0.2252	0.2234	**0.979**	0.2167	Angola		
4/28/2016	ZFB16 #37	F	0.1897	0.2405	0.2169	0.2016	0.2465	0.3378	0.1767			
4/28/2016	ZFB16 #38	F	0.2178	0.2027	0.2198	0.2004	0.2049	0.568	0.1963			
4/28/2016	ZFB16 #39	M	0.2718	0.6546	0.3402	0.2412	0.2478	**1.5125**	0.1768	Angola		
12/7/2016	ZB16 #55	F	0.1954	0.1562	0.08635	0.28005	0.1839	0.275	0.0665			
12/7/2016	ZB16 #56	F	0.1649	0.0814	0.074	0.4749	0.09635	0.33795	0.0534		2/4	2/4
12/7/2016	ZB16 #57	F	0.52525	0.0798	0.06955	0.11365	0.0814	0.6667	0.42095	Angola		
12/7/2016	ZB16 #58	M	0.076	0.1012	0.09055	0.08725	0.12075	**0.81445**	0.7029	Angola		
7/31/2017	ZB17 #1	M	0.1	0.2117	0.3906	0.11735	0.13895	0.30445	0.07545			
7/31/2017	ZB17 #2	F	0.0999	0.0857	0.12335	0.0689	0.0847	0.41385	0.05685			
7/31/2017	ZB17 #3	F	0.17765	0.11695	0.0595	0.08255	0.17415	0.0986	0.0667			
7/31/2017	ZB17 #4	F	0.1558	**0.98965**	0.119	0.07035	0.1445	**0.77055**	0.0624	Sudan		
7/31/2017	ZB17 #5	F	0.1347	0.1303	0.22335	0.07825	0.15275	0.16595	0.0609			
7/31/2017	ZB17 #6	M	0.17705	0.52455	0.0876	0.07645	0.5445	0.46775	0.09115			

7/31/2017	ZB17 #7	F	0.2204	0.26875	0.3089	0.08485	0.41755	0.5819	0.05895			
7/31/2017	ZB17 #8	M	0.07035	0.0796	0.1156	0.06205	0.0785	0.06865	0.0829			
7/31/2017	ZB17 #9	M	0.43475	0.10325	0.1014	0.1059	0.2433	**0.9925**	0.13	Angola		
7/31/2017	ZB17 #10	M	0.1505	0.36035	0.17845	0.11365	0.24885	0.4174	0.08025			
7/31/2017	ZB17 #11	F	0.09335	0.1375	0.40185	0.16855	0.10365	0.44915	0.06595			
7/31/2017	ZB17 #12	M	0.1274	0.2333	0.05325	0.09495	0.1472	0.4841	0.05425			
7/31/2017	ZB17 #13	M	0.1189	0.1618	0.09925	0.1038	0.12345	**1.43915**	0.06955	Angola		
7/31/2017	ZB17 #14	F	0.15275	0.112	0.091	0.09305	0.0917	**0.961**	0.07755	Angola		
7/31/2017	ZB17 #15	F	0.07275	0.10295	0.0677	0.0733	0.07855	0.34785	0.05595			
7/31/2017	ZB17 #16	F	0.08795	0.09505	0.07935	0.08065	0.1397	0.2022	0.0622		9/31	6/31
7/31/2017	ZB17 #17	F	0.0854	0.14805	0.1067	0.29705	0.1101	0.1722	0.09445			
7/31/2017	ZB17 #18	M	0.2162	0.09715	0.11965	0.1327	0.231	0.1191	0.1978			
7/31/2017	ZB17 #19	M	0.1078	0.11975	0.1418	0.1072	0.11255	0.57105	0.0791			
7/31/2017	ZB17 #20	F	0.28375	0.32605	0.20775	0.11225	0.3239	**0.7833**	0.4273	Angola		
7/31/2017	ZB17 #21	F	0.1212	0.0772	0.06065	0.0614	0.11155	0.1359	0.0565			
7/31/2017	ZB17 #22	F	0.0582	0.0822	0.0584	0.10515	0.07065	**0.79205**	0.08705	Angola		
7/31/2017	ZB17 #23	F	0.2026	**0.9375**	0.1624	0.19095	0.34425	0.1223	0.3176	Sudan		
7/31/2017	ZB17 #24	F	0.57445	0.11965	0.10795	0.1182	**0.6565**	0.53165	0.11125	Reston		
7/31/2017	ZB17 #25	M	0.1226	0.1482	0.06985	0.115	0.19285	0.29125	0.067			
7/31/2017	ZB17 #26	M	0.07025	0.07365	0.05375	0.06115	0.06185	0.1976	0.0662			
7/31/2017	ZB17 #27	F	0.1257	0.18945	0.06315	0.07775	0.09925	0.1286	0.0552			
7/31/2017	ZB17 #28	M	0.091	0.1007	0.1339	0.05925	0.1005	0.3453	0.0616			
7/31/2017	ZB17 #29	F	0.21265	0.2404	0.10455	0.06335	0.3122	**0.6633**	0.0664	Angola		
7/31/2017	ZB17 #30	F	0.07995	0.0906	0.06075	0.06175	0.16645	0.48655	0.05625			
7/31/2017	ZB17 #31	F	0.089	0.0992	0.1951	0.06695	0.0876	0.3978	0.08415			
12/4/2017	ZB17 #73	M	0.1303	0.26945	0.2322	**0.6621**	0.18835	**0.6846**	0.12275	Angola		
12/4/2017	ZB17 #74	F	0.1278	0.1991	-0.16795	-0.18105	0.00675	**1.1272**	-0.10755	Angola		
12/4/2017	ZB17 #75	F	0.3386	**1.07785**	0.1164	**1.1257**	0.087	**0.74565**	0.09625	Bundibugyo		
12/4/2017	ZB17 #76	M	-0.162	-0.11595	-0.502	-0.4385	-0.0797	0.1926	-0.54515			
12/4/2017	ZB17 #77	M	-0.02845	0.1118	0.09255	**0.9115**	**0.83175**	0.42775	-0.1509	Bundibugyo		
12/4/2017	ZB17 #78	M	-0.1069	0.12735	-0.25285	0.1804	-0.174	0.21675	-0.01175			
12/4/2017	ZB17 #79	F	0.0497	0.18985	-0.0504	0.0816	0.27205	0.56655	-0.04155			
12/4/2017	ZB17 #80	M	0.0909	0.1625	0.0961	-0.15875	0.15625	0.20155	-0.0709			
12/4/2017	ZB17 #81	M	0.25735	0.2222	0.17385	0.3591	0.42595	**0.72135**	0.1886	Angola		
12/4/2017	ZB17 #82	M	-0.22005	0.1513	-0.6712	0.33	0.20605	0.0596	-0.57435			
12/4/2017	ZB17 #83	M	0.00655	0.20585	-0.06645	-0.1437	0.22665	**0.74655**	-0.01255	Angola		
12/4/2017	ZB17 #84	M	0.03325	-0.0323	-0.5075	-0.5911	0.0681	0.0006	-0.46645			
12/4/2017	ZB17 #85	F	0.0365	0.07375	0.10695	-0.1168	0.23225	**0.83335**	-0.10205	Angola		
12/4/2017	ZB17 #86	M	0.3281	0.2756	0.3026	0.16605	0.2141	0.36535	0.211			
12/4/2017	ZB17 #87	F	0.2942	0.2314	**1.38035**	0.2618	0.17375	0.4067	0.1028	Tai Forest		
12/4/2017	ZB17 #88	M	**0.6903**	**0.62**	0.364	0.14685	0.2238	**0.81025**	0.6168	Angola	25/32	18/32
12/4/2017	ZB17 #89	F	0.19335	**2.4266**	0.44105	0.26445	0.3282	**0.8613**	0.27985	Sudan		
12/4/2017	ZB17 #90	M	0.2391	**1.94285**	0.46805	0.3928	0.50805	**1.6209**	0.28285	Sudan		
12/4/2017	ZB17 #91	F	0.2656	**0.9425**	0.0885	0.41595	0.44955	**0.8812**	0.2377	Sudan		
12/4/2017	ZB17 #92	F	0.1852	**0.74235**	0.17425	0.21775	0.5091	**1.6111**	0.3442	Angola		
12/4/2017	ZB17 #93	F	0.2763	**0.65535**	**1.6006**	0.1868	0.1843	**0.85315**	0.2346	Tai Forest		
12/4/2017	ZB17 #94	F	0.16465	0.2144	0.15175	0.1644	0.16585	**1.58305**	0.23355	Angola		
12/4/2017	ZB17 #95	F	-0.07645	-0.04995	-0.12475	-0.08305	-0.04615	**0.7332**	-0.0065	Angola		
12/4/2017	ZB17 #96	F	0.17115	0.2646	0.1519	0.339	0.205	**2.03625**	0.23705	Angola		
12/4/2017	ZB17 #97	F	0.18525	0.21255	0.19095	0.18105	0.19775	**0.86245**	0.29165	Angola		
12/4/2017	ZB17 #98	F	0.13555	0.15845	0.12805	0.12045	0.15765	**1.68775**	0.20515	Angola		
12/4/2017	ZB17 #99	F	0.14115	0.1752	0.12415	0.09845	0.18845	**0.9781**	0.16685	Angola		
12/4/2017	ZB17 #100	M	0.2233	0.24245	0.2573	0.1987	0.25905	**1.9921**	0.26105	Angola		
12/4/2017	ZB17 #101	F	0.12875	0.12105	0.2038	0.0459	0.4089	**1.22385**	0.19935	Angola		
12/4/2017	ZB17 #102	F	0.19195	0.28025	0.2885	0.23395	0.27655	**1.9267**	0.2309	Angola		
12/4/2017	ZB17 #103	F	0.1387	0.08765	-0.05975	0.02705	0.12005	**0.75525**	0.0249	Angola		
12/4/2017	ZB17 #104	M	0.38415	0.1643	0.13235	0.2255	0.15805	**1.46435**	0.19975	Angola		

Numbers in red and orange are significantly higher OD values.



Supplementary Figure 1. Immunoglobulin G (IgG) antibodies detected in the sera collected from fruit bats in Zambia. Serum samples were tested (1:100 dilution) for IgG antibodies specific to EBOV, SUDV, TAFV, BDBV, RESTV, MARV, and LLOV GPs by enzyme-linked immunosorbent assay. All optical density (OD) values were subjected to the Smirnov-Grubbs rejection test to discriminate the positive (i.e. significantly higher OD values) from the negative population.