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1 # Serological evidence of high pathogenicity virus infection in *Eidolon helvum* fruit bats in
2 Nigeria

3 Diego Cantoni, Martin Mayora-Neto, Bethany Auld, Kelly Da Costa, Joanne Del Rosario,
4 Veronica O. Ameh, Claude T. Sabeta, Mariliza Derveni, Arran Hamlet, Edward Wright, Simon
5 Scott, Efstathios S. Giotis[1], Ashley Banyard[1], Nigel Temperton[1].

6

7 Author Affiliations: Universities of Kent & Greenwich, Chatham, United Kingdom; and
8 University of Glasgow, Glasgow, United Kingdom (D. Cantoni); Universities of Kent and
9 Greenwich (M. Mayora-Neto, K. Da Costa, J. Del Rosario, S. Scott and N. Temperton);
10 University of Sussex, Brighton, United Kingdom (B. Auld, M. Derveni, E. Wright); Federal
11 University of Agriculture, Makurdi, Nigeria and University of Pretoria, Pretoria, South Africa
12 (V.O. Ameh); University of Pretoria, Pretoria, South Africa, and Agricultural Research
13 Council, Onderstepoort, South Africa, (V.O. Ameh, C.T Sabeta); Imperial College London,
14 London, United Kingdom (A. Hamlet, E.S. Giotis); University of Essex, Colchester, United
15 Kindgom (E.S. Giotis); Animal and Plant Health Agency, Weybridge, United Kingdom (A.
16 Banyard).

17 [1]These senior authors were co-principle investigators

18 **Running title:** Serological screening for highly pathogenic viruses in *E. helvum* bat samples

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21 **Article summary line:** The detection of neutralizing antibodies against henipavirus GH-M74a
22 virus, Nipah virus, and H17N10 virus in *Eidolon helvum* bat sera from Nigeria using
23 pseudotyped viruses suggests a potential risk of zoonotic spillover.

24 ## Abstract

25 The *Eidolon helvum* fruit bat is the most widely distributed fruit bat in Africa and is known to
26 be a reservoir for several pathogenic viruses that can cause disease in humans. To assess the

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risk of zoonotic spillover, we conducted a serological survey of 304 serum samples from *E. helvum* bats that were captured for human consumption in Makurdi, Nigeria. Using pseudotyped viruses, we screened the samples for neutralising antibodies against viruses from the *Coronaviridae, Filoviridae, Orthomyxoviridae* and *Paramyxoviridae* families. We report the presence of neutralising antibodies against henipavirus lineage GH-M74a virus (odds ratio 6.23; $p<0.001$), Nipah virus (odds ratio 4.04; $p=0.00031$), bat influenza H17N10 virus (odds ratio 7.25; $p<0.001$) and no significant association with Ebola virus (odds ratio 0.56; $p=0.375$) in the bat cohort. The data suggest a potential risk of zoonotic spillover including the possible circulation of highly pathogenic viruses in *E. helvum* populations. These findings highlight the importance of maintaining sero-surveillance of *E. helvum* to monitor changes in virus prevalence and distribution over time and across different geographic locations.

Introduction

Throughout the course of human history, viral zoonotic spillover events have been sporadic yet catastrophic, resulting in several highly lethal pandemics. With approximately 60-75% of all human infectious diseases arising from zoonotic transmission (*1*), it is crucial to remain vigilant in identifying and monitoring potential sources of zoonotic spillover, such as wildlife reservoirs, in order to prevent future outbreaks. Over the past four decades, bats have been identified as a significant source of zoonotic events that have sparked major outbreaks of viruses with considerable implications for human health. As the second most diverse mammalian order, bats have been linked to the transmission of a range of viruses, including coronaviruses, filoviruses, lyssaviruses, and henipaviruses, among others (*1,2*). With over 12,000 bat-derived virus sequences spanning 30 viral families having relevance to both

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50 veterinary and medical sectors, the need for surveillance of bat populations is critical to assess
51 and mitigate the risk of potential zoonotic spillover events.

52 *Eidolon helvum*, the straw-coloured fruit bat, is one of the most widely distributed fruit
53 bats in Africa (Figure 1A). It has extensive migratory patterns of over 2000 kilometres and is
54 hypothesised to migrate based on availability of fruits to increase reproductive success (*3*). It
55 is often hunted for either its bushmeat (*4,5*) or for pest control (*6*). Due to the overlap of
56 territories shared between *E. helvum* and humans (Figure 1B), there is ample opportunity for
57 zoonotic spillover. According to the DBatVir database, more than 900 viruses have been
58 sequenced from *E. helvum* (Figure 1C). Furthermore, the presence of neutralising antibodies
59 have been detected against multiple viruses in serum samples of *E. helvum* (*7*).

60 Serological evidence of neutralizing antibodies in bat sera is not definitive proof of
61 active virus infection in bats, but it does suggest that bats have been exposed to the virus and
62 have mounted an immune response. Screening for neutralising antibodies in bats using highly
63 pathogenic viruses requires use of high containment facilities, which can be circumvented by
64 using pseudotyped viruses (PVs) in neutralization assays. The use of PVs in neutralization
65 assays is considered safe for handling under biosafety level 2 conditions. Pseudotyped virus
66 neutralization assays (PVNA) have gained widespread usage for detecting neutralizing
67 antibodies due to their high sensitivity and robust correlation with live virus neutralization
68 assays (*8–10*). In this study, we screened 304 serum samples from *E. helvum* bats that were
69 captured for human consumption in Nigeria using PVs expressing the viral glycoproteins of
70 several viruses known to pose high public health risks (Table 1). Due to limited volumes of
71 sera, we prioritised the order of screening based on the viruses' potential risk to animal and
72 human health (*11–13*). Our findings revealed the presence of multiple virus-specific
73 neutralizing antibodies, suggesting the circulation of highly pathogenic viruses with pandemic
74 potential in colonies of *E. helvum* in Nigeria.

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75 **Table 1**. List of viruses pseudotyped for screening *E. helvum* samples in this study.

Virus Family	Genus	Species	
Coronaviridae	*Betacoronavirus*	Severe Acute Respiratory Virus 1	(SARS-CoV-1)
		Severe Acute Respiratory Virus 2	(SARS-CoV-2)
		Bat coronavirus RaTG13	(RaTG13)
Filoviridae	*Ebolavirus*	Zaire Ebolavirus	(EBOV)
	Marburgvirus	Marburg Virus	(MARV)
Orthomyxoviridae	*Alphainfluenzavirus*	Bat influenza H17N10	(H17N10)
Paramyxoviridae	*Henipavirus*	Nipah Virus	(NiV)
		Ghanaian henipavirus M74a	(GH-M74a)

76

77 ## Materials and Methods

78 ### Bat sera collection

79 All sera were collected from terminally bled straw-coloured fruit bats (*Eidolon helvum*)
80 that were captured for human consumption in Makurdi, Benue State Nigeria (7°44’25.7’’N
81 8°31’52.8’’E). The bats in Makurdi were collected from roosts in trees in and around the Benue
82 State Government House and on trees in private residences close to the government house. Bats
83 were also sampled from roosts on trees in private residences where permissions were gained.
84 Sampling was done for two consecutive seasons (November 2017 – March 2018 and November
85 2018 – March 2019). Blood samples were processed for sera and stored at -20°C until required.

86 ### Pseudotype virus production

87 Lentiviral (HIV) based pseudotypes used for this study were generated as described in
88 detail here (*14,15*). Briefly, 1.0 µg of p8.91 plasmid encoding the HIV gag-pol was mixed with
89 1.5 µg of pCSFLW reporter gene and 1 ug the surface viral glycoproteins as required (Table
90 2). After mixing the plasmids in 200 µL of Opti-MEM (ThermoFisher, Woolwich, UK),
91 Fugene HD (Promega, Southampton, UK) transfection reagent was added at a 1:3

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92 (plasmid:Fugene HD) ratio and incubated for 15 minutes prior to adding the transfection
93 complexes to HEK293T/17 cells in T-75 cell culture flasks. Harvesting of pseudotypes were
94 carried out at 48- and 72-hours post transfection, whereby culture media was removed from
95 the flasks and filtered through a 0.45 µM cellulose acetate filter (Corning, Deeside, UK).
96 Samples were aliquoted and frozen at -80ºC for long term storage prior to use.

97 **Table 2.** Plasmids used to generate pseudotyped viruses. HEK293T Cells were transfected with
98 Angiotensin-converting enzyme 2 (ACE2) and Transmembrane protease, serine 2
99 (TMPRSS2). CHO: Chinese hamster ovary cells. MDCK: Madin-Darby canine kidney cells.
100 For this study we used the G and F genes from a Bangladeshi NiV isolate (NiVb)

Viral Glycoprotein	Accession	Vector	Target Cells
Coronaviridae			
SARS-CoV S (Tor2)	NC_004718.3	pcDNA 3.1+	HEK293T ACE2+TMPRSS2
SARS-CoV-2 S (Wuhu1)	NC_045512.2	pcDNA 3.1+	HEK293T ACE2+TMPRSS2
RaTG13 S	QHR63300.2	pcDNA 3.1+	HEK293T ACE2+TMPRSS2
Filoviridae			
EBOV Mayinga 76 GP	EU224440	pCAGGS	CHO
MARV Angola 05 GP	DQ447660	pCAGGS	CHO
Orthomyxoviridae			
IAV-like H17N10 H17	AFC35438.1	pI.18	MDCK II
Paramyxoviridae			
NiVb G and F	JN808864.1	pCAGGS	BHK
GH-M74A G and F	HQ660129	pCAGGS	BHK

101 **Pseudotype virus titration**

102 Pseudotyped viruses were titrated by serially diluting filtered pseudotypes with fresh
103 DMEM starting from a 1:2 to a final 1:512 dilution using white 96-well flat bottom plates
104 (ThermoFisher, Woolwich, UK). Then appropriate target cells were added (Table 1) and plates
105 were returned to the incubator. After 48 hours, cells were lysed with Bright-Glo reagent

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106 (Promega, Southampton, UK), and luciferase expression levels were assessed using a GloMax
107 plate reader (Promega, Southampton, UK).

108 **Sera screen and neutralisations**

109 Sera were initially screened at single point dilution (1:100, except NiV and GH-M74a
110 at 1:50). Each plate had wells containing PV only to determine maximum pseudotype entry.
111 Samples where a 1 log decrease in RLU compared to the no-serum virus-only control was
112 observed were then selected for cytotoxicity assay using Cell Titre Glo kit (Promega,
113 Southampton, UK) and light microscopy to verify viability of cells prior to undertaking PVNA
114 (data not shown). Neutralisation assays were carried out by mixing bat sera and cell culture
115 media at a starting input of 1:40 ratio (sera: cell culture media) and diluted either 4-fold (GH-
116 M74a and NiV) to 1:5000 or 8-fold (all other viruses) to 1:5120. PVs were added to the plates
117 at a minimum RLU titre of 10^5/mL, incubated for 1 hour at 37°C, followed by addition of target
118 cells at a density of 10^4 cells per well, except for NiV and GH-M74a where a cell density of
119 20^4 cells per well. Plates were incubated at 37°C and 5% CO_2 for 48 hours prior to lysis using
120 Bright-Glo reagent according to manufacturer's protocol and monitoring of luciferase
121 expression using a GloMax plate reader. Regression curves were fitted using GraphPad Prism
122 8 software (San Diego, CA, USA) as described previously (*16*).

123 **Statistical analysis**

124 Data were analyzed using STATA version 16 (StataCorp, College Station, TX, USA).
125 Descriptive statistics were used to summarize the distribution of the variables. Bivariate logistic
126 regression was used to determine the association between the presence or absence of
127 neutralizing antibodies against each virus. Multivariable logistic regression was used to adjust
128 for confounding factors. The data were coded as binary variables (1 = positive, 0 = negative).
129 Logistic regression was used to determine the association between the presence or absence of

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130 neutralizing antibodies against each virus and the dependent variable was the presence or
131 absence of neutralizing antibodies against each virus. Crude and adjusted odds ratios (OR) were
132 estimated with 95% confidence intervals (CI) to measure the strength of association between
133 each variable and the presence or absence of neutralizing antibodies.

134 **Results**

135 Our PVNA screening has revealed the presence of neutralising antibodies against
136 several of the pseudotyped viruses tested (Figure 2A and 2B, Table 3). However, no
137 neutralization was observed against the *Coronaviridae* members SARS-CoV-2, SARS-CoV,
138 and the bat coronavirus RaTG13. Although neutralizing antibodies against EBOV PVs were
139 detected in a single sample (n=1/278), no neutralization was observed against MARV. We also
140 found several samples positive for neutralizing antibodies against influenza A virus H17N10
141 PVs (n=26/304), indicating the presence of positive or cross-reactive H17 neutralizing
142 antibodies. Additionally, positive samples were detected for Nipah virus (NiV) (n=16/54) and
143 GH-M74a (n=36/54) from the *Paramyxoviridae* PVs.

144 **Table 3.** Results of pMN assays with each PV. *Note: sample sizes decreased due to limited*
145 *sera volumes.*

Viruses	Positive Samples	Negative	Percentage
Coronaviridae			
SARS-CoV-1	0	304	0.00%
SARS-CoV-2	0	290	0.00%
RaTG13	0	290	0.00%
Filoviridae			
EBOV	1	278	0.36%
MARV	0	279	0.00%
Orthomyxoviridae			
H17N10	26	304	8.55%
Paramyxoviridae			
NiV	16	54	29.63%
Gh-M74a	36	54	66.67%

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155 Our logistic regression analysis shows that the presence of neutralizing antibodies is
156 significantly associated with virus type, as indicated by the p-values for the virus coefficients.
157 Specifically, the odds of having neutralizing antibodies are significantly higher for H17 (odds
158 ratio = 7.25, $p < 0.001$), NiV (odds ratio = 4.04, p = 0.00031), and GH-M74a (odds ratio =
159 6.23, $p < 0.001$) compared to the reference category of SARS-CoV-2. EBOV did not show a
160 significant association with neutralizing antibody status (odds ratio = 0.56, p = 0.375).

161 We further analyzed the data to investigate the presence of samples positive for
162 neutralizing antibodies against multiple viruses (Figure 2C). Our analysis revealed one sample
163 positive for only H17, two samples positive for H17 and GH-M74a, and 13 samples positive
164 for both NiV and GH-M74a. These findings suggest that multiple viruses may have circulated
165 within the same bat or that antibodies to one of the three viruses may cross-neutralise against
166 multiple viruses.

167 **Discussion**

168 The study investigated the seroprevalence of neutralizing antibodies against several
169 highly pathogenic viruses in *E. helvum* bats in Nigeria. We report a high level of seroprevalence
170 against GH-M74a (66.7% of samples) and NiV (29.6%). This suggests that these viruses may
171 be prevalent in the bat population, which is concerning given the high mortality rates (50-
172 100%) associated with NiV infection in humans (*17*). Henipavirus antibodies have been
173 frequently detected with high seroprevalence rates in *E. helvum* (18–20). We considered that
174 the antibodies generated by GH-M74a infection are also cross-neutralizing the NiV viruses as
175 the two viruses share a key conserved region in their attachment protein (*18*). Nonetheless, the
176 detection of three unique samples that neutralized only NiV and not GH-M74a PV implies that
177 NiV may indeed have been in circulation within this bat population. Currently no vaccines that
178 can prevent nor treat NiV infection and disease have been licenced, though experimental

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179 mRNA-based vaccines are currently in development for NiV and are under phase 1 clinical
180 trials. The study found a high level of detection of the GH-M74a virus in *E. helvum* bats,
181 indicating that it may be circulating widely in the population. This suggests a risk of zoonotic
182 spillover, potentially through secondary hosts such as pigs and horses, even if not directly to
183 humans.

184 We also observed seropositivity towards H17N10, a bat Influenza A-like virus that
185 contains unconventional HA and NA proteins (*21*). The evolutionary distinct H17N10 along
186 with H18N11 virus (not screened for in this study) were originally recovered from
187 asymptomatic fruit bats of the Neotropic bat family *Phyllostomidae* (*Sturnira lilium* and
188 *Artibeus planirostris*) in several countries of Central and South America (*21–23*). These viruses
189 have attracted considerable attention following reports that their entry in host cells is mediated
190 by the trans-species conserved MHC-II proteins, suggesting zoonotic potential *(24,25*). So far,
191 bats in Central and S. America, but not in Central Europe (*26*), have been found seropositive
192 for H17N10 and H18N11, and to our knowledge this is the first report of H17-seropositive
193 samples in non-Neotropical bat species. Considering the lack of a validated serological assay
194 to screen *E. helvum* bat sera specifically for H17N10, we cannot exclude serological cross-
195 reactivity with heterologous H17-like antigens. Nonetheless, the potential presence of
196 undiscovered H17 or H17-like IAV species in *E. helvum*, such as the ones described in
197 *Phylostomidae* is tantalising, cannot be ruled out and should be further investigated by unbiased
198 approaches *i.e.*, metagenomics.

199 Although we only detected a single sample positive for neutralising antibodies against
200 EBOV, similar studies have also reported very low seroprevalence rates in *E. helvum;* 1 out of
201 262 samples from Ghana (*27*), and 19 out of 748 samples from Zambia (*28*). On the other hand,
202 a report detected much higher seroprevalence of EBOV antibodies in *E. helvum* from
203 Cameroon, with 107 out of 817 positive samples (*29*). At the time of writing this manuscript,

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204 the reservoir host of EBOV is yet to be determined, though bats have been heavily implicated
205 (*30*).

206 Our study detected seroprevalence of neutralising antibodies against several viruses,
207 but we cannot determine whether these viruses had sustained a prolonged infection in the bats,
208 since RNA extractions were not carried out. Nonetheless, this raises the question as to whether
209 the immune system of *E. helvum* is able to clear these viruses quickly, and how long the
210 neutralising antibodies persist after infection. Ultimately, ongoing research on the dynamics of
211 immune responses in bats (*31–33*) suggests that sero-surveillance studies provide a reliable
212 assessment of potential reservoirs for these viruses.

213 In summary, our serological screening of *E. helvum* sera obtained from bats has
214 revealed the presence of antibodies against several highly pathogenic viruses with epidemic
215 potential. The capture and preparation of these bats for human consumption suggests a potential
216 for direct exposure to bat bodily fluids, thereby elevating the risk of cross-species transmission
217 of the viruses identified in this study. Given that human settlements are encroaching into areas
218 known to harbour large bat colonies, the risk of zoonotic spillover will continue to increase.
219 Therefore, monitoring of bat viruses especially in the large bat populations in Sub-Saharan
220 Africa is crucial in order to better understand the prevalence and transmission of these viruses
221 and to mitigate the risk of potential spillover events.

222

223 **Author Contributions**

224 DC, AB, ESG and NT conceptualized the study. VOA and CTS collected and processed serum
225 samples. DC, MN, BA, KDC, JDR, MD and SS carried out PVNA and data processing and
226 analysis. AH generated figures and with AB and ESG analyzed the processed data, and

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227 provided expert critical feedback. All authors contributed significantly to the article and
228 approved the final submitted version.

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238 **Biographical Sketch**

239 Dr Cantoni, currently at the MRC-Centre for Virus Research, University of Glasgow, is
240 interested mostly in SARS-CoV-2 and neglected tropical viruses; from both a serosurveillance
241 and molecular biology perspective.

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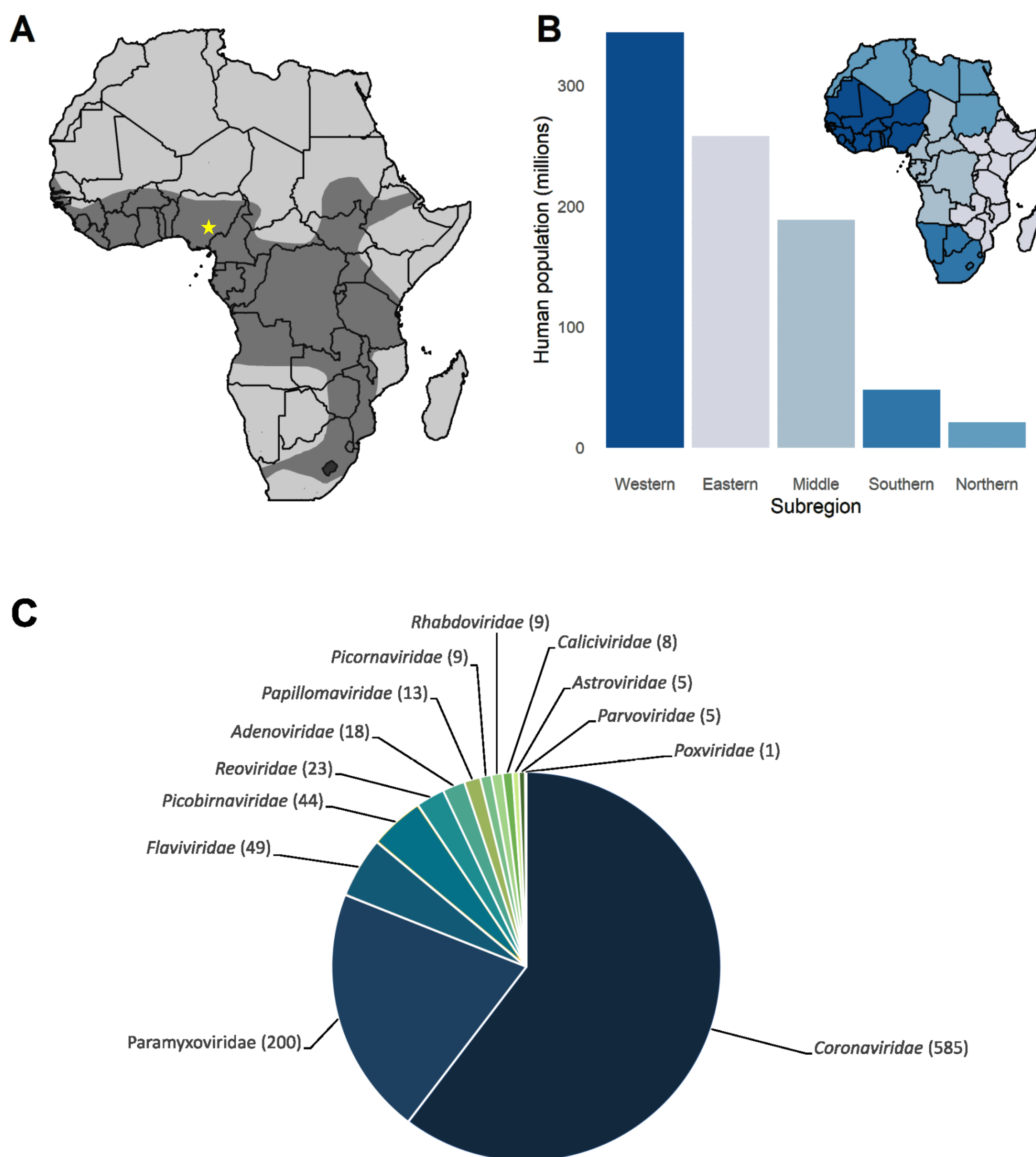

330

331 **Figure 1. Profile of Straw-coloured *E. helvum* fruit bat.**
332 Species distribution within Africa coloured in dark grey, with yellow star denoting
333 location in Nigeria from which the samples were obtained (A). Human population
334 density within each subregion of Africa (B). Viruses reported in *E. helvum* samples in
335 DBatVir database (http://www.mgc.ac.cn/cgi-bin/DBatVir/).
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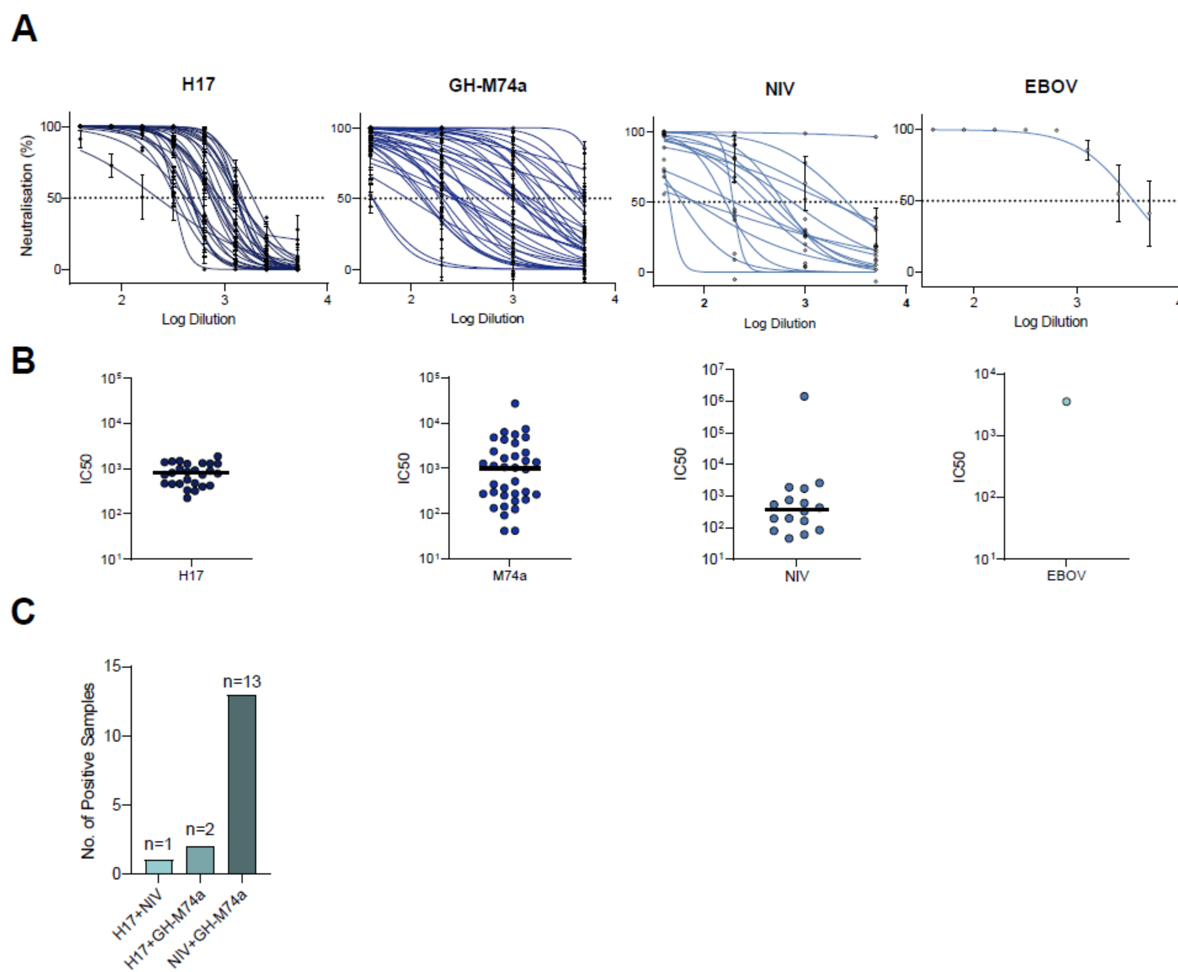

339

340 **Figure 2. Positive detection of neutralising antibodies in *E. helvum* by pMN**
341 **assay.**
342 Neutralisation curves derived from pMN assays against positive samples that were
343 selected from the initial screening (A). IC50s calculated by pMN assays against each
344 PV (B). Number of samples positive for neutralising antibodies against multiple
345 viruses (C).
346