

Supplementary information: Bat coronavirus phylogeography in the Western Indian Ocean

Léa Joffrin, Steven M. Goodman, David A. Wilkinson, Beza Ramasindrazana, Erwan Lagadec, Yann Gomard, Gildas Le Minter, Andréa Dos Santos, M. Corrie Schoeman, Rajendraprasad Sookhareea, Pablo Tortosa, Simon Julienne, Eduardo S. Gudo, Patrick Mavingui and Camille Lebarbenchon

Research permits for the collection of biological materials

Reunion Island

Samples were collected as part of a previous study on lyssavirus infection in bats in the Indian Ocean (1), under a research permit delivered by the Préfecture de La Réunion: “Arrêté préfectoral du 11 Février 2013 and Arrêté préfectoral du 11 Septembre 2014 (N°2014-07)”.

Mauritius

Samples were collected as part of a previous study on lyssavirus infection in bats in the Indian Ocean (1), under a memorandum of agreement for the supply of biological material by Government of Mauritius (delivered by the National Park and Conservation Service for authorization of Mauritius), signed 17 December 2010 and 09 January 2013. CITES permit from the Mauritian national authority was issued for tissue export (permit MU120933) to Reunion Island.

Mayotte

Samples were collected as part of a previous study on lyssavirus infection in bats in the Indian Ocean (1), under a research permit delivered by the “Préfecture de Mayotte: Arrêté N°158/DEAL/SEPR/2014”.

Madagascar

Samples were collected as part of previous studies on infectious agents in Malagasy wildlife (1–4), under the following research permits delivered by the “Direction du Système des Aires Protégées and Direction Générale de l’Environnement et des Forêts”; Madagascar National Parks: 350/10/MEF/SG/DGF/DCB.SAP/SCB, 032/12/MEF/SG/DGF/ DCB.SAP/SCBSE, 067/12/MEF/SG/DGF/DCB.SAP/SCBSE, 194/12/ MEF/SG/DGF/DCB.SAP/SCB, N°283/11/MEF/SG/DGF/DCB.SAP/SCB, N°077/12/MEF/SG/DGF/DCB.SAP/SCBSE, 238/14/MEEF/SG/ DGF/DCB.SAP/SCB and 268/14/MEEF/SG/DGF/DCB.SAP/SCB. A CITES permit from the Malagasy national authority was issued for tissue export (permit 243C-EA06/MG12) to Reunion island.

Seychelles (Mahe)

Samples were collected as part of a previous study on lyssavirus infection in bats in the Indian Ocean (1), under the following research permit delivered by the Direction of Wildlife, Trade and Conservation Section and the Ministry of Environment and Energy of Republic of Seychelles: Export permits Agreement of 5 March 2014. CITES permit from the Republic of Seychelles was issued for export (permit N°1772) to Reunion Island.

Mozambique (Inhassoro district, Inhambane province)

Samples were collected as part of a previous study on astrovirus infection in bats in the Indian Ocean (5), under a research permit delivered by the Ministry of Health (N°S/N/SDI/0233/15).

Type of collected samples

Organs were collected before 2014, stored in liquid nitrogen in the field and then at -80°C upon arrival at the laboratory (Appendix Table 1). From 2014 onward, feces, rectal, and oral swabs were collected (Appendix Table 1) and placed in 1mL brain heart infusion medium (Conda, Madrid, Spain) supplemented with penicillin G (1,000 units/mL), streptomycin (1 mg/mL), kanamycin (0.5 mg/mL), gentamicin (0.25 mg/mL) and amphotericin B (0.025 mg/mL). These samples were also stored in liquid nitrogen in the field and at -80°C upon arrival at the laboratory (Appendix Table 1).

REFERENCES

1. Mélade J, McCulloch S, Ramasindrazana B, Lagadec E, Turpin M, Pascalis H, et al. Serological Evidence of Lyssaviruses among Bats on Southwestern Indian Ocean Islands. *PLoS One*. 2016;11(8):e0160553.
2. Wilkinson DA, Mélade J, Dietrich M, Ramasindrazana B, Soarimalala V, Lagadec E, et al. Highly Diverse Morbillivirus-Related Paramyxoviruses in Wild Fauna of the Southwestern Indian Ocean Islands: Evidence of Exchange between Introduced and Endemic Small Mammals. *J Virol*. 2014;88(15):8268–77.
3. Gomard Y, Dietrich M, Wieseke N, Ramasindrazana B, Lagadec E, Goodman SM, et al. Malagasy bats shelter a considerable genetic diversity of pathogenic *Leptospira* suggesting notable host-specificity patterns. *FEMS Microbiol Ecol*. 2016;92(4):fiw037.
4. Mélade J, Wieseke N, Ramasindrazana B, Flores O, Lagadec E, Gomard Y, et al. An eco-epidemiological study of Morbilli-related paramyxovirus infection in Madagascar bats reveals host-switching as the dominant macro-evolutionary mechanism. *Sci Rep*. 2016;12(6):23752.
5. Hoarau F, Minter G Le, Joffrin L, Schoeman MC, Lagadec E, Ramasindrazana B, et al. Bat Astrovirus in Mozambique. *Virol J*. 2018;15(104):1–5.

Supplementary tables

Table S1. Number of positive samples and sequences generated with the Real-Time PCR detection system, and the classic PCR system used to generate sequences for phylogenetic analyses.

Location	Sampling year	Bat family	Bat species	Detection system			Phylogeny system	
				N tested	N detected	N sequences	N tested	N sequences
Madagascar	2012	Molossidae	<i>Mops midas</i>	21	4	4	4	0
		Rhinonycteridae	<i>Triaenops menamena</i>	21	2	0	2	0
	2013	Molossidae	<i>Mormopterus jugularis</i>	63	10	7	10	5
		Pteropodidae	<i>Rousettus madagascariensis</i>	45	6	6	6	2
	2014	Rhinonycteridae	<i>Triaenops menamena</i>	13	2	2	2	0
Mayotte	2014	Molossidae	<i>Chaerephon sp</i>	4	1	1	1	1
			<i>Chaerephon pusillus</i>	61	7	7	7	5
Mozambique	2015	Hipposideridae	<i>Hipposideros caffer</i>	59	10	6	10	3
		Miniopteridae	<i>Miniopterus mossambicus</i>	21	4	4	4	4
		Molossidae	<i>Mops condylurus</i>	54	11	11	11	4
		Nycteridae	<i>Nycteris thebaica</i>	14	4	3	4	4
		Rhinolophidae	<i>Rhinolophus lobatus</i>	9	6	6	6	4
			<i>Rhinolophus rhodesiae</i>	30	9	9	9	9
			<i>Rhinolophus sp</i>	2	1	1	1	1
Rhinonycteridae	<i>Triaenops afer</i>	51	9	8	9	8		
Reunion	2015	Molossidae	<i>Mormopterus francoismoutoui</i>	50	2	2	2	1
					88	77	88	51

Table S2. Nucleotide sequence similarity obtained with BLAST.

ID	species	% identity	GENBANK Sequence	Accession GENBANK ID
MAY004	<i>Chaerephon pusillus</i>	92%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486
MAY015	<i>Chaerephon pusillus</i>	92%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486
MAY025	<i>Chaerephon pusillus</i>	92%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486
MAY027	<i>Chaerephon pusillus</i>	92%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486
MAY033	<i>Chaerephon pusillus</i>	92%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486
MAY051	<i>Chaerephon sp</i>	92%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486
RB369	<i>Mormopterus francoismoutoui</i>	90%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486
SMG18218	<i>Mormopterus jugularis</i>	90%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486
SMG18223	<i>Mormopterus jugularis</i>	90%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486
SMG18225	<i>Mormopterus jugularis</i>	90%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486
SMG18231	<i>Mormopterus jugularis</i>	90%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486
SMG18238	<i>Mormopterus jugularis</i>	90%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486
SMG18866	<i>Rousettus madagascariensis</i>	98%	Kenya bat coronavirus BtKY89 RNA-dependent RNA polymerase gene, partial cds	GU065433

SMG18930	<i>Rousettus madagascariensis</i>	98%	Kenya bat coronavirus BtKY89 RNA-dependent RNA polymerase gene, partial cds	GU065433
SMG18951	<i>Hipposideros caffer</i>	96%	229E-related bat coronavirus strain BtKY229E-8	KY073748
SMG18958	<i>Triaenops afer</i>	98%	NL63-related bat coronavirus strain BtKYNL63-15, complete genome	KY073746
SMG18970	<i>Nycteris thebaica</i>	88%	Betacoronavirus BtCoV/KW2E-F93/Nyc_spec/GHA/2010 RNA-dependent RNA polymerase gene, partial cds; and spike glycoprotein gene, complete cds	JX899383
SMG18971	<i>Nycteris thebaica</i>	88%	Betacoronavirus BtCoV/KW2E-F93/Nyc_spec/GHA/2010 RNA-dependent RNA polymerase gene, partial cds; and spike glycoprotein gene, complete cds	JX899383
SMG18972	<i>Nycteris thebaica</i>	88%	Betacoronavirus BtCoV/KW2E-F93/Nyc_spec/GHA/2010 RNA-dependent RNA polymerase gene, partial cds; and spike glycoprotein gene, complete cds	JX899383
SMG18987	<i>Hipposideros caffer</i>	96%	229E-related bat coronavirus strain BtKY229E-8, complete genome	KY073748
SMG18990	<i>Rhinolophus lobatus</i>	87%	Kenya bat coronavirus BtKY83 RNA-dependent RNA polymerase gene, partial cds	GU065427
SMG18991	<i>Rhinolophus lobatus</i>	87%	Kenya bat coronavirus BtKY83 RNA-dependent RNA polymerase gene, partial cds	GU065427
SMG18992	<i>Rhinolophus lobatus</i>	87%	Kenya bat coronavirus BtKY83 RNA-dependent RNA polymerase gene, partial cds	GU065427
SMG18993	<i>Triaenops afer</i>	98%	NL63-related bat coronavirus strain BtKYNL63-15, complete genome	KY073746
SMG18996	<i>Triaenops afer</i>	98%	NL63-related bat coronavirus strain BtKYNL63-15, complete genome	KY073746
SMG18997	<i>Triaenops afer</i>	98%	NL63-related bat coronavirus strain BtKYNL63-15, complete genome	KY073746
SMG19002	<i>Hipposideros caffer</i>	96%	229E-related bat coronavirus strain BtKY229E-8, complete genome	KY073748

SMG19004	<i>Miniopterus mossambicus</i>	98%	Miniopterus bat coronavirus/Kenya/KY27/2006 polyprotein (ORF1ab) gene	HQ728484
SMG19006	<i>Miniopterus mossambicus</i>	97%	Miniopterus bat coronavirus/Kenya/KY27/2006 polyprotein (ORF1ab) gene	HQ728484
SMG19013	<i>Miniopterus mossambicus</i>	98%	Miniopterus bat coronavirus/Kenya/KY27/2006 polyprotein (ORF1ab) gene	HQ728484
SMG19015	<i>Miniopterus mossambicus</i>	98%	Miniopterus bat coronavirus/Kenya/KY27/2006 polyprotein (ORF1ab) gene	HQ728484
SMG19020	<i>Rhinolophus rhodesiae</i>	87%	Kenya bat coronavirus BtKY83 RNA-dependent RNA polymerase gene, partial cds	GU065427
SMG19024	<i>Rhinolophus rhodesiae</i>	98%	Miniopterus bat coronavirus/Kenya/KY27/2006 polyprotein (ORF1ab) gene	HQ728484
SMG19026	<i>Rhinolophus sp</i>	87%	Kenya bat coronavirus BtKY83 RNA-dependent RNA polymerase gene, partial cds	GU065427
SMG19027	<i>Rhinolophus rhodesiae</i>	87%	Kenya bat coronavirus BtKY83 RNA-dependent RNA polymerase gene, partial cds	GU065427
SMG19028	<i>Rhinolophus rhodesiae</i>	87%	Kenya bat coronavirus BtKY83 RNA-dependent RNA polymerase gene, partial cds	GU065427
SMG19029	<i>Rhinolophus lobatus</i>	82%	Rhinolophus bat coronavirus BtCoV/4307-2 RNA-dependent RNA polymerase (RdRp) gene, partial cds	KP876528
SMG19030	<i>Rhinolophus rhodesiae</i>	87%	Kenya bat coronavirus BtKY83 RNA-dependent RNA polymerase gene, partial cds	GU065427
SMG19031	<i>Rhinolophus rhodesiae</i>	87%	Kenya bat coronavirus BtKY83 RNA-dependent RNA polymerase gene, partial cds	GU065427
SMG19032	<i>Rhinolophus rhodesiae</i>	87%	Kenya bat coronavirus BtKY83 RNA-dependent RNA polymerase gene, partial cds	GU065427
SMG19037	<i>Rhinolophus rhodesiae</i>	87%	Kenya bat coronavirus BtKY83 RNA-dependent RNA polymerase gene, partial cds	GU065427
SMG19038	<i>Rhinolophus rhodesiae</i>	82%	Rhinolophus bat coronavirus BtCoV/4307-2 RNA-dependent RNA polymerase (RdRp) gene, partial cds	KP876528
SMG19141	<i>Mops condylurus</i>	98%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486

SMG19154	<i>Triaenops afer</i>	98%	NL63-related bat coronavirus strain BtKYNL63-15, complete genome	KY073746
SMG19174	<i>Triaenops afer</i>	98%	NL63-related bat coronavirus strain BtKYNL63-15, complete genome	KY073746
SMG19183	<i>Triaenops afer</i>	98%	NL63-related bat coronavirus strain BtKYNL63-15, complete genome	KY073746
SMG19185	<i>Triaenops afer</i>	98%	NL63-related bat coronavirus strain BtKYNL63-9a	KY073744
SMG19207	<i>Mops condylurus</i>	98%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486
SMG19210	<i>Mops condylurus</i>	98%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486
SMG19217	<i>Mops condylurus</i>	98%	Chaerephon bat coronavirus/Kenya/KY22/2006 polyprotein (ORF1ab) gene	HQ728486

Table S3. Number of tested and positive samples, per location, bat family, species, samples types, and collection year.

Location	Bat family	Bat species	Collection Year	N tested	N tested per type of samples				N positive (%)				
					Organs		Feces	Swabs	Organs		Feces	Swabs	
					Lung	Intestine			Lung	Intestine			
Madagascar	Hipposideridae	<i>Macronycteris commersoni</i>	2012	19		19							
	Miniopteridae	<i>Miniopterus manavi</i>	2012	19		19							
		<i>Miniopterus gleni</i>	2012	14		14							
				2014	2				2				
		<i>Miniopterus griveaudi</i>	2014	28				28					
		<i>Miniopterus mahafaliensis</i>	2012	8		8							
		<i>Miniopterus sororculus</i>	2012	8		8							
	Molossidae	<i>Chaerephon atsinanana</i>	2013	29		29							
		<i>Chaerephon leucogaster</i>	2018	45				45					
		<i>Mops leucostigma</i>	2018	94				94					
		<i>Mops midas</i>	2012	19		19				4 (21.1)			
				2018	2				2				
		<i>Mormopterus jugularis</i>	2013	22		22				10 (45.5)			
				2014	41				41				
		<i>Otomops madagascariensis</i>	2012	12		12							
			2014	6				6					
	Pteropodidae	<i>Rousettus madagascariensis</i>	2014	45				45					6 (13.3)
	Rhinonycteridae	<i>Paratriaenops furculus</i>	2014	32				32					
		<i>Triaenops menamena</i>	2012	21		21				2 (9.5)			
				2014	13				13				2 (15.4)
Vespertilionidae	<i>Myotis goudoti</i>	2014	17				17						
	<i>Neoromicia malagasyensis</i>	2013	2		2								

		<i>Neoromicia matroka</i>	2013	4		4					
		<i>Pipistrellus hesperidus</i>	2014	5			5				
Mauritius	Molossidae	<i>Momopterus acetabulosus</i>	2012	6		6					
	Pteropodidae	<i>Pteropus niger</i>	2012	48	48						
	Emballonuridae	<i>Taphosius mauritianus</i>	2014	2			2				
Mayotte	Molossidae	<i>Chaerephon sp</i>	2014	4			4			1 (25.0)	
		<i>Chaerephon pusillus</i>	2014	60			60			7 (11.6)	
	Pteropodidae	<i>Pteropus seychellensis comorensis</i>	2014	22			22				
Mozambique	Hipposideridae	<i>Hipposideros caffer</i>	2015	59			59				10 (16.9)
	Miniopteridae	<i>Miniopterus mossambicus</i>	2015	21			21				4 (19.0)
	Molossidae	<i>Mops condylurus</i>	2015	54			54				11 (20.4)
	Nycteridae	<i>Nycteris thebaica</i>	2015	14			14				4 (28.6)
	Rhinolophidae	<i>Rhinolophus lobatus</i>	2015	9			9				6 (66.7)
		<i>Rhinolophus mossambicus</i>	2015	20			20				
		<i>Rhinolophus rhodesiae</i>	2015	30			30				9 (30.0)
		<i>Rhinolophus sp</i>	2015	2			2				1 (50.0)
	Rhinonycteridae	<i>Triaenops afer</i>	2015	51			51				9 (17.7)
	Vespertilionidae	<i>Neoromicia nana</i>	2015	2			2				
	<i>Scotophilus viridis</i>	2015	2			2					
Reunion	Molossidae	<i>Mormopterus francoismoutoui</i>	2015	50			50				2 (4.0)

Seychelles	Pteropodidae	<i>Pteropus seychellensis</i> <i>seychellensis</i>	2014	50	50							
				TOTAL	1013	98	183	64	668	16	8	64

Table S4. Cytochrome *b* gene sequences accession numbers from western Indian Ocean bats. *: sequences generated in this study.

Bat family	Bat species	Cytb GenBank accession ID
Hipposideridae	<i>Hipposideros caffer</i>	FJ347980
Miniopteridae	<i>Miniopterus mossambicus</i>	MN273501*
Molossidae	<i>Chaerephon pusillus</i>	GQ489154
	<i>Mops condylurus</i>	GQ489181
	<i>Mormopterus francoismoutoui</i>	MN273499*
	<i>Mormopterus jugularis</i>	KR606332
Nycteridae	<i>Nycteris thebaica</i>	MN273500*
Pteropodidae	<i>Rousettus madagascariensis</i>	GU228727
Rhinolophidae	<i>Rhinolophus lobatus</i>	MN273497*
	<i>Rhinolophus rhodesiae</i>	MN273498*
Rhinonycteridae	<i>Triaenops afer</i>	EU798748

Supplementary figures

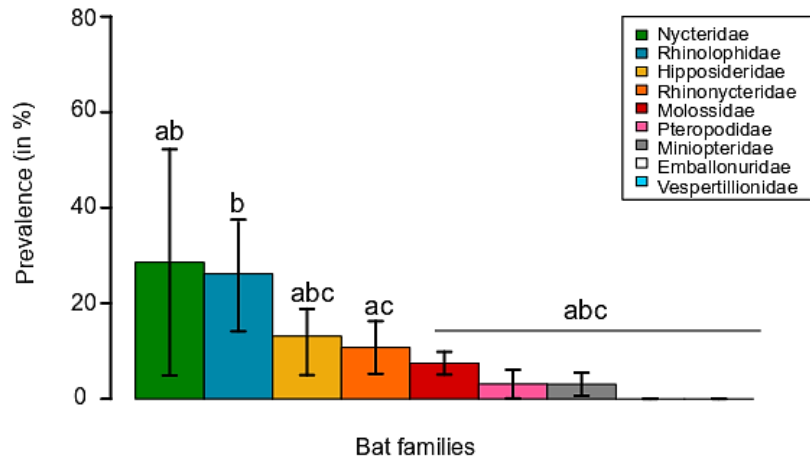


Figure S1. Mean CoV prevalence (\pm 95% confidence interval) as function of the bat family. Letters a–c above the bars refer to significantly different averages based upon a Pairwise test. Bars can have more than one letter to reflect the “overlap” between them.

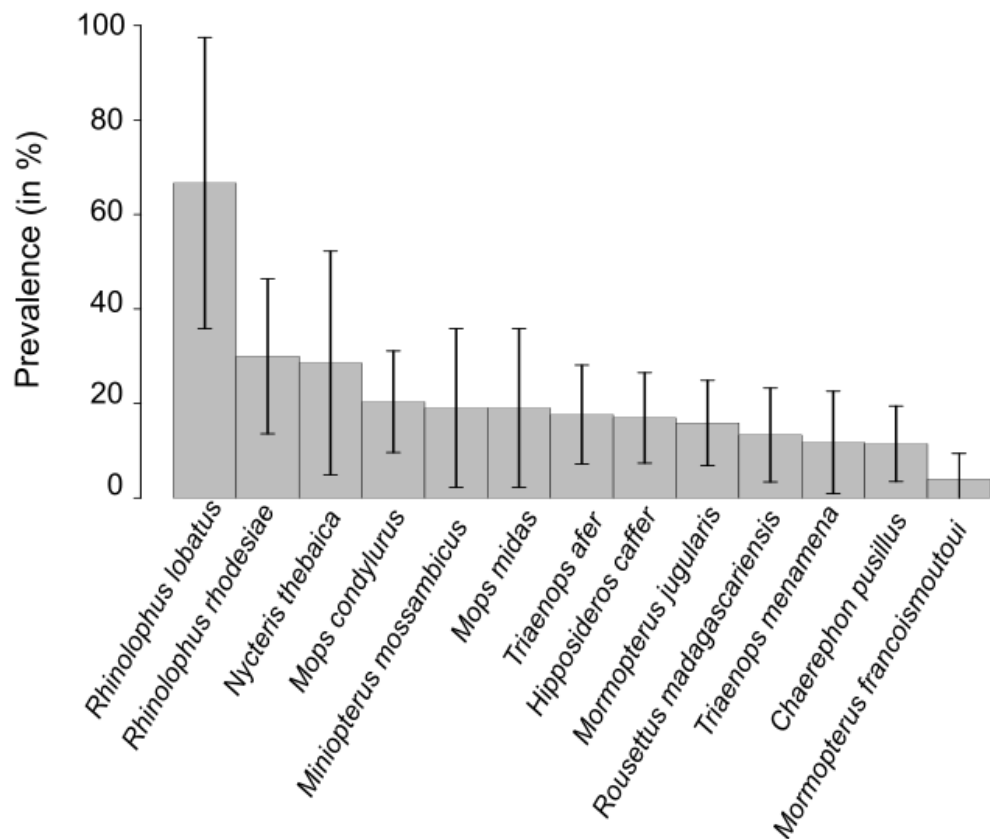


Figure S2. Mean CoV prevalence (mean \pm 95% confidence interval) as function of the bat species.

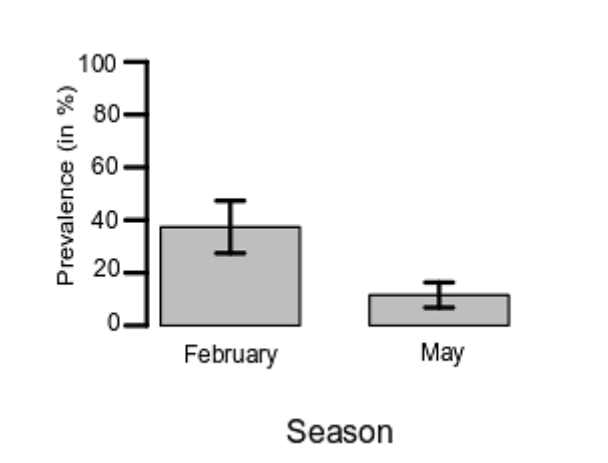


Figure S3. Mean CoV prevalence (mean \pm 95% confidence interval) as function of the bat sampling season in Mozambique.