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Original Article **Open Access**

Investigation of rodent reservoirs of emerging pathogens in Côte d'Ivoire, West Africa

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Abstract:

Background: One of the main health problems in West Africa remains upsurge of emerging pathogens. Ebola virus disease outbreak occurred in 2014 in Liberia, Guinea and Sierra Leone, Monkeypox virus in Nigeria in 2017 and most recently Lassa virus in Nigeria, Togo and Benin in 2018. These pathogens have animal reservoirs as vectors for transmission. Proper investigation of the pathogens in their rodent vectors could help reduce and manage their emergence and spread.
Methodology: This study was conducted with an approval from the Côte d'Ivoire Bioethics Community. Small mammal trappings were carried out in 9 sites within three zones namely, peri-urban, peri-rural and protected areas. Liver, lung and kidney tissues from trapped small mammals were sampled in accordance with the recommended conditions of biosafety and bioethics. The organs were transported in liquid nitrogen to the laboratory. Molecular tests were used to detect pathogens. Orthopoxviruses and Monkeypox virus were detected in the organs by PCR using consensus primers targeting the virus surface membrane haemagglutinin (HA) genes, while *Leptospira* species were detected by PCR using primers targeting the *rrs* and *lfb1* genes.
Results: Out of 4930 night-traps, 256 (5.19%) small mammals were trapped including *Crocidura*, *Rattus, Lophuromys, Praomys, Mus* and *Mastomys*. *Leptospira* species were detected in 6 genera from 7 study sites and the infected small mammals accounted for 13.3%. *Leptospira* sp was detected mainly in the rodent vector genera *Rattus* (32.3%), *Lophuromys* (29.0%), and *Praomys* (16.1%). Three species of *Leptospira* were detected and *Leptospira interrogans* was the most common frequent species (74.2%). Monkeypox virus was not detected from studied small mammals.
Conclusion: The initial data from our investigation indicates the presence of *Leptospira* sp in rodent vectors, *Rattus, Lophuromys* and *Praomys*, which are the potential small mammalian reservoirs of this pathogen in Cote d'Ivoire.

Keywords: Environment; reservoir; emerging pathogen; Côte d'Ivoire; West Africa

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Investigation des rongeurs réservoirs d'agents pathogènes émergents en Côte d'Ivoire, Afrique de l'ouest

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Résumé:

Contexte: L'un des principaux problèmes de santé en Afrique de l'Ouest reste la recrudescence des pathogènes émergents. En 2014, une épidémie de maladie à virus Ebola s'est produite au Libéria, en Guinée et en Sierra Leone, Monkeypox virus au Nigeria en 2017 et plus récemment Lassa virus au Nigeria, au Togo et au Bénin en 2018. Ces agents pathogènes ont des réservoirs animaux dont les rongeurs impliqués dans la transmission à l'homme. Une investigation appropriée de ces réservoirs pourrait aider à réduire et à gérer la propagation de ces agents pathogènes émergents.
Méthodologie: Cette étude a été menée avec l'approbation du comité bioéthique de Côte d'Ivoire. Des piégeages

de petits mammifères ont été effectués sur 9 sites répartis en trois zones, à savoir les zones périurbaines, périrurales et protégées. Des tissus du foie, du poumon et des reins de petits mammifères capturés ont été prélevés conformément aux recommandations en biosécurité et de bioéthique. Les tissus prélevés ont été transportés dans de l'azote liquide jusqu'au laboratoire. Des tests moléculaires ont été effectués pour détecter les agents pathogènes. Monkeypox virus a été recherché dans les tissus par PCR à l'aide d'amorces consensus *Orthopoxvirus* ciblant les gènes de l'hémagglutinine et puis à l'aide d'amorces spécifiques ciblant des gènes de la membrane de surface du Monkeypox virus. *Leptospira* a été recherché par PCR à l'aide d'amorces ciblant les gènes rrs et lfb1.

Résultats: Pour 4930 nuit piège, 256 petits mammifères ont été capturés. Le taux de succès de capture était de 5,19%. Les rongeurs capturés étaient du genre *Crocidura, Rattus, Lophuromys, Praomys, Mus et Mastomys*. *Leptospira* a été détecté chez 13,3% des rongeurs capturés. Cette bactérie a été isolée sur 7 sites sur 9. *Leptospira* sp a été détecté principalement chez les rongeurs du genre *Rattus* (32,3%), *Lophuromys* (29,0%) et *Praomys* (16,1%). Trois espèces de *Leptospira* ont été détectées dont *Leptospira interrogans*, l'espèce la plus fréquente (74,2%) isolée. Monkeypoxvirus n'a pas été détecté chez les petits mammifères capturés.

Conclusion: Les premières données de notre investigation indiquent la présence de *Leptospira sp*, chez les rongeurs *Rattus, Lophuromys et Praomys*, qui sont les potentiels réservoirs de ce pathogène en Côte d'Ivoire.

Mots clés: Environnement; réservoir; agent pathogène émergent; Côte d'Ivoire; Afrique de l'Ouest

Introduction:

The new challenge of West Africa region on health was the fight against emerging pathogens. The recent events are worrying. In 2014, Ebola virus disease occurred in Guinea, Liberia and Sierra Leone for up to 20,000 cases (1,2). After the isolation of Monkeypox virus in a dead primate in the Tai-forest of Cote d'Ivoire in 2012 (3), Monkeypox virus epidemic disease went up to 80 cases in 2017 in Nigeria (4). Lassa fever outbreak, caused by a haemorrhagic fever virus, also occurred in Benin, Togo and Nigeria in 2018 (5,6). In addition to these viruses, the threat of *Leptospira* bacteria is real. Studies have shown the presence of these bacteria in humans suspected with viral fever (7,8). These pathogens have animal reservoirs as vectors for transmission (9-13).

The contact among people and animals is frequent for various reasons including urbanization, agriculture or hunting. This contact promotes pathogen transmission and spread in the human population. Cote d'Ivoire, a West Africa country with more than 20 million inhabitants remain under the threat of these pathogens. The largest population movements in the region are towards Cote d'Ivoire. It is paramount to develop the different strategies taking into account the reservoir animal vectors to cope with threats. Proper investigation of the pathogens in their animal vectors could help reduce and prevent their emergence and spread. Therefore, the local investigation scientist group for emerging pathogens, composed of physicians, veterinarians, microbiologists and epidemiologists, was created, with the aim of identifying the main animal reservoir vectors.

Materials and method:

Study sites

The investigated sites were chosen according to bibliography data (14-16). Several emerging pathogens such as *Leptospira*, Monkeypox virus, and haemorrhagic fever viruses were investigated. For biosafety level, *Leptospira* and Monkeypox virus were investigated in the first phase. Human serology test results for *Leptospira* and Orthopoxviruses in Cote d'Ivoire were obtained (8,17). Small mammal trappings were carried out at 9 sites within three zones namely; peri-urban, peri-rural and protected areas (Fig. 1).

Bioethics approval

This study was conducted with an approval from the Côte d'Ivoire Bioethics community (Number of bioethics agreement 024/ MSLS/CNER-dkn in 2015). Biodiversity protection, ecology protection, biosafety and bioethics remain the preoccupation of the investigating group.

Small mammals trapping and autopsy:

Rodents trapping were made by Sherman trap, Chauvancy trap, locally made trap and Pitfalls trap. Traps used in this study were all livetraps. The laying of traps was done at 5 pm and checked in the morning at 6 am. The traps were arranged in line of 20 separated by 5 meters for 3 successive days. The traps containing rodents were retrieved and replaced with a new one. All trapped small mammals were transported to the autopsy sites under biosafety conditions.

The rodents were anaesthetized with isofloran. After measurements and phenotypic identifications were made, autopsy was performed, and the liver, lung, kidney and other organs were harvested in accordance with the recommended conditions of biosafety and bioethics. Three to four tissue samples were removed per organ. Samples were transported in liquid nitrogen to the laboratory. Carcasses were preserved in 2% formalin. All infectious wastes produced during autopsies were incinerated. Fig 2 shows the workflow for sample processing.

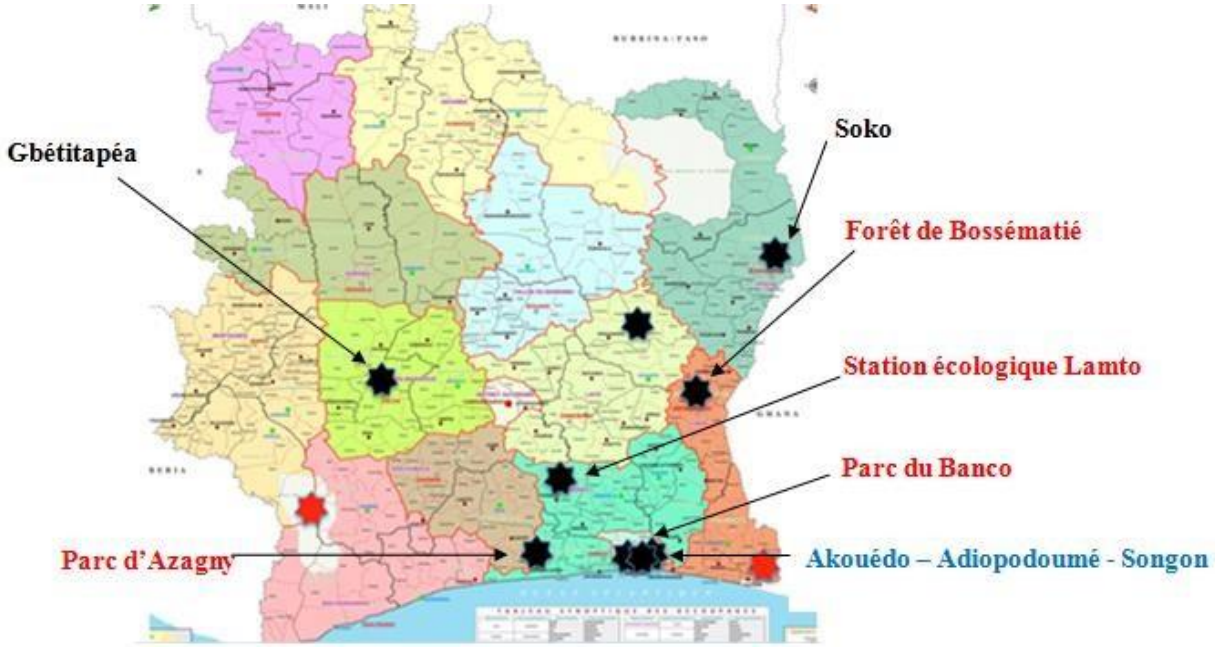

Fig 1: Study sites investigated for rodent reservoirs of emerging pathogens in Cote d'Ivoire
(The black stars are the different sites, the sites written in blue are peri-urban, in black are peri-rural areas and in red are protected areas)

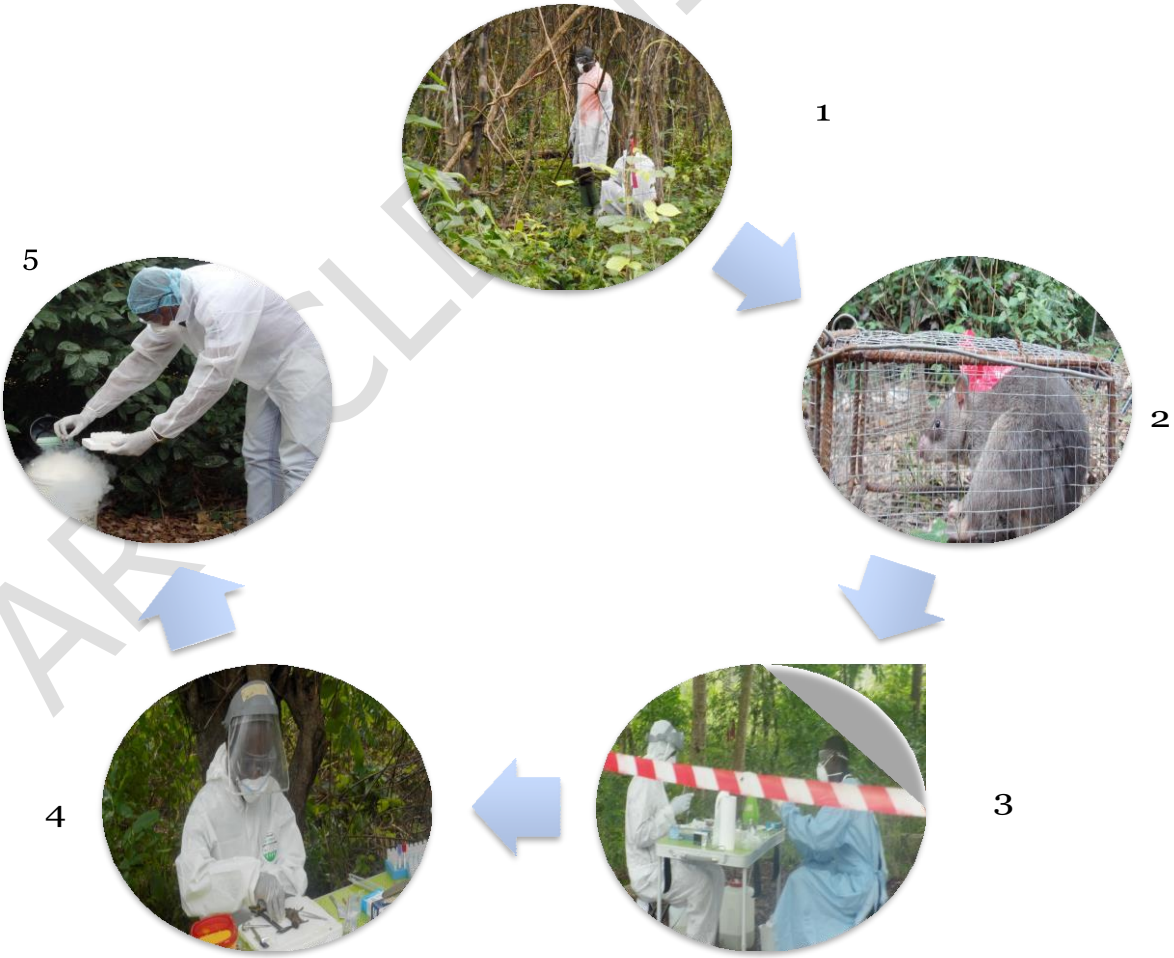

Fig 2: Workflow process of trapping and autopsy of rodents

1 = Inspection of trapping sites, 2= specimen trapping (here a local trap), 3 = Autopsy delimited zone, 4 = equipment wearing for autopsy, 5 = liquid nitrogen for transport sample.

Rodent identification:

Identification of rodents was based on morphological characteristics. The measurements (general length, body/tail percentage, head-to-body length, tail length, posterior leg length (soles), length of the ears) were determined as previously described by Herbreteau (18). The weight and coat inspection were taken into account.

Tissues preparation

About 25g of kidney, liver or lung were ground in a sterile mortar with a sterile pestle. The ground material obtained was dissolved in 1 ml of 1 x PBS. This mashed potato-like material obtained was used for the extraction of DNA

DNA extraction:

The DNeasy Blood and Tissue Kit (Qiagen® Cat No./ID: 69506) was used for DNA extraction from the tissues. To 200 µl broth of tissue, 180 µl of ATL buffer and then 20 µl of proteinase K, were added. The whole solution was incubated at 56°C for 1 hour in a thermomixer, then 200 µl of buffer AL and 200 µl of absolute ethanol were added for the precipitation of the DNA. DNA was collected on a silicate spin column and then washed with AW1 and AW2 solution. A volume of 200 µl of eluate buffer AE was used to collect the DNA

Molecular detection of Orthopoxvirus and Monkeypox virus by PCR assay

Molecular detection of Orthopoxvirus and Monkeypox virus was done by PCR using consensus primers of Orthopoxviruses designed from the virus surface membrane HA gene (forward: EACP1: 5' ATG ACA CGA TTG CCA ATA C 3', and reverse: EACP2: 5' CTA GAC TTT GTT TTC TG 3') with expected PCR product band size of 942 bp (19). For Monkeypox virus, the primers used were forward: MPV1: 5' CTG ATA ATG TAG AAG AC 3' and reverse: MPV2: 5' TTG TAT TTA CGT GGG TG 3' with expected PCR product of 406 bp size (19).

The GoTaq G2 Flexi DNA polymerase kit (Promega Corporation, USA) was used for the PCR mixes, containing 0.2 µM of each primer, 1.5 µM, $MgCl_2$, 0.1 µM dNTPs, 1unit Taq polymerase, 1 x of buffer and 5 µl of DNA template for a final volume of 50 µl. Amplification conditions for the detection of Orthopoxviruses were; 94°C for 5 min (1 cycle), 94°C for 30 sec, 48°C for 1 min, 72°C for 1 min (36 cycles), and then 72°C for 8 min (1 cycle). The PCR products were viewed on a GelDoc Bioanalyzer (BioRad®) after electrophoresis on 1.5% agarose gel.

Molecular detection of *Leptospira* by PCR

Molecular detection of *Leptospira* was performed by PCR targeting the *rrs* and *lfb1* genes. PCR targeting *rrs* gene used primers previously published by Merien (20) (LA:5'-GGCGGCGCGTCTTAAACATG-3' and LB:5'-TTC CCCCCATTGAG CAA GATT-3'), which amplify a 331 bp PCR product. The Taq DNA Polymerase kit (New England BioLabs®) was used for the PCR mixes containing 50 mM KCl, 10 mM Tris-HCl (pH 8.4), 2 mM $MgCl_2$, 1 µM of each oligo, 200 µM of each dNTPs and 1 UI of Taq DNA polymerase. Five microlitres of DNA template was used for a final volume of 50µl. Amplification conditions were as follows; 94°C 3 min (1 cycle), 94°C 1 min, 63°C 1 min 30 sec, 72°C 2 min (30 cycles), and 72°C 10 min (1 cycle). The PCR products were viewed on a GelDoc Bioanalyzer (BioRad®) after electrophoresis on 1.5% agarose gel stained by SYBR safe (ThermoScientific®). Bench Top 100 bp DNA Ladder (Promega®) was used as the size marker.

Real time PCR targeting the *lfb1* gene used primers previously published by Merien (21) (LFB1-F: 5'-CATTCATGTTTCGAATCATTT CAAA-3' and LFB1-R: 5'-GGCCCAAGTTCCTTC TAAAAG-3'. The Maxima SYBR Green qPCR Master Mix (Sigma®) was used for the PCR mixes with 0.8 µM of each oligos, 0.5 µL of 1/10 dilution of Rox dye and 5 µL of DNA template for a final volume of 25 µL. Amplification conditions were as follows; 95°C 10 min (1 cycle), 95°C 15 sec, and 57°C 1 min (49 cycles). This stage was followed by Tm analysis with 0.5°C increase each 30 seconds between 75 to 90°C.

Data analysis

Statistical analysis of data was performed using EPI INFO 7.2 software and the Chi-square test was used to compare the variables with significance level of *p* fixed below 5%.

Results:

The number of night-trap was 4930 for a total catch of 256 specimens, with trapping success or yield of 5.19% (range of 1.35% to 8.75% depending on the trapping area). Overall, the yield was 2.9% in protected areas, 4.5% in peri-rural areas and 8.2% in peri-urban areas (protected areas vs peri-urban areas X^2=57,96, p<0.00001, peri-rural vs peri-urban areas X^2=11,48, p<0.001, protected areas vs peri-rural areas X^2=4.7, p<0.01) (Table 1). Success rate was higher in the residential of peri-urban areas, and diversity was more in forest and bush of protected area.

Table 1: Small rodents trapping success by area and sites

Capture site	Night-trap number (n = 4930)	Specimen captured number (n = 256)	Trapping success efficiency (%) (5.19%)	X^2	*p* value
Protected areas					
Azagny	590	8	1.32		
Banco	840	27	3.2		
Bossématié	600	17	2.83		
Lamto	240	14	5.8	4.18 (a)	<0.04*(a)
Sub-total	2,270	66	2.90	11.48 (b)	<0.001*(b)
Peri-rural areas				57.96.33 (c)	<0.00001*(c)
Soko	500	25	5.0		
Gbetitapea	300	11	3.6		
Sub-total	800	36	4.5		
Peri-urban areas					
Adiopodoumé	840	70	8.33		
Akouédo	720	63	8.75		
Songon	300	21	7.0		
Sub-total	1,860	154	8.2		

(a): comparison of trapping success-protected areas and peri-rural areas; (b): comparison of trapping success-peri-urban areas and peri-rural area; (c): comparison of trapping success-protected areas and peri-urban area; X^2= Chi square; * = statistically significant

Table 2: Micromammals captured

Order	Family	Genus	Number (n = 256)	Percentage (%)
Insectivora (Eulipotyphia) (n= 60)	Soricidae	*Crocidura*	60	23.4
Rodentia (n=195)	Scuiridae	*Funisciurus*	5	2.0
	Gerbillidae	*Tatera*	3	1.1
	Cricetomydae	*Cricetomys*	3	1.1
	Muridae	*Rattus*	70	27.34
		Lophuromys	28	10.9
		Praomys	25	9.7
		Mus	20	7.8
		Mastomys	20	7.8
		Lemniscomys	5	2.0
		Uranomys	4	1.5
		Myomys	3	1.1
		Hylomuscus	2	0.7
		Hybomys	1	0.4
		Nanomys	1	0.4
		Malacomys	1	0.4
		Not identified	5	2.0

A total of 256 small mammals were trapped including 76.2% (n=195) rodents and 23.8% (n=60) insectivora. The insectivora consists mainly of the genus *Crocidura*, with species such as *Crocidura olivieri* (90.2%), *Crocidura occidentalis* (1.6%), *Crocidura obscuruor* (3.3%) and Crocidura sp (3.0%). Concerning rodents, four families were trapped including *Muridae, Sciuridae, Gerbillidae* and *Cricetomydae* with 94.4% being *Muridae* with mainly genera; *Rattus, Lophuromys, Praomys, Mus* and *Mastomys* (Table 2). Diversity of trapped animals was seen in protected areas but abundance was more in urban areas (Table 3)

Liver, kidney and lung were analysed by molecular test to detect Monkeypox virus and *Leptospira* bacteria. One (0.39%) *Crocidura olivieri* liver was positive for Orthopoxvirus but Monkeypox virus negative, and was a rodent of urban area. *Leptospira* DNA was detected in 31 (12.1%) small mammals, which included 6 genera; *Rattus* sp (32.3%, n=10), *Lophuromys* sp (29.0%, n= 9), *Praomys* sp (16.1%, n=5), *Mastomys* sp (9.7%, n=3), *Mus* sp (6.5%, n=2) and *Crocidura* sp (6.5%, n=2).

Of the *Leptospira* infected small mammals, *Lophuromys* sp was the most infected (36.0%), followed by *Praomys* sp (20.8%), *Rattus* sp (16.7%), *Mastomys* sp (12.0%), *Mus* sp (9.5%) and *Crocidura* sp (3.8%). Three *Leptospira* species were detected, *Leptospira interrogans* was commonest (74.2%), followed by *Leptospira borgpetersenii* (16.1%) and *Leptospira kirshneri* (9.7%). Fig 3 shows distribution of infected small mammals in the 9 trapping sites.

Table 3: Micromammal captured by area and sites

Species of micromammal	**Peri-rural area Abundance = 36 Diversity = 11**		**Protected area Abundance = 66 Diversity = 14**				**Peri-urban area Abundance = 154 Diversity = 11**		
	Soko	**Gbetitapea**	**Azagny**	**Lamto**	**Bossématié**	**Banco**	**Adiopodoumé**	**Songon**	**Akouédo**
Crocidura olivieri (n=55)	2	3		2		5	23	7	13
Crocidura occidentalis (n=1)	1								
Crocidura obscuruor (n=2)			2						
Crocidura sp (n=2)			1		1				
Mastomys natalensis (n=15)	9	3					3		
Mastomys erythrolocus (n=5)	2		2				1		
Mus mucuslus (n=20)	3						16		1
Myomys daltoni (n=3)	3								
Praomys sp (n=25)	4		3		12	5	1		
Hylomuscus sp (n=2)			2						
Cricetomys sp (n=3)			1	2					
Lophuromys sp (n=27)		1	1		1	7	10	7	
Lophuromys sicapusis (n=1)			1						
Rattus rattus (n=16)		1	5				3		7
Rattus norvegicus (n=24)			4				10	2	8
Rattus sp (n=31)							31		
Hybomys sp (n=1)			1						
Malacomys longipes (n=1)			1						
Tatera sp (n=3)		3							
Lemniscomys sp (n=5)			3				2		
Uranomys ruddi (n=4)			4						
Funisciurus carruthesis (n=5)							5		
Nanomys sp (n=1)							1		
Not identified (n=4)	1						3		
Abundance	25	11	8	14	17	27	70	21	63
Species wealth	8	5	5	5	4	5	7	5	4

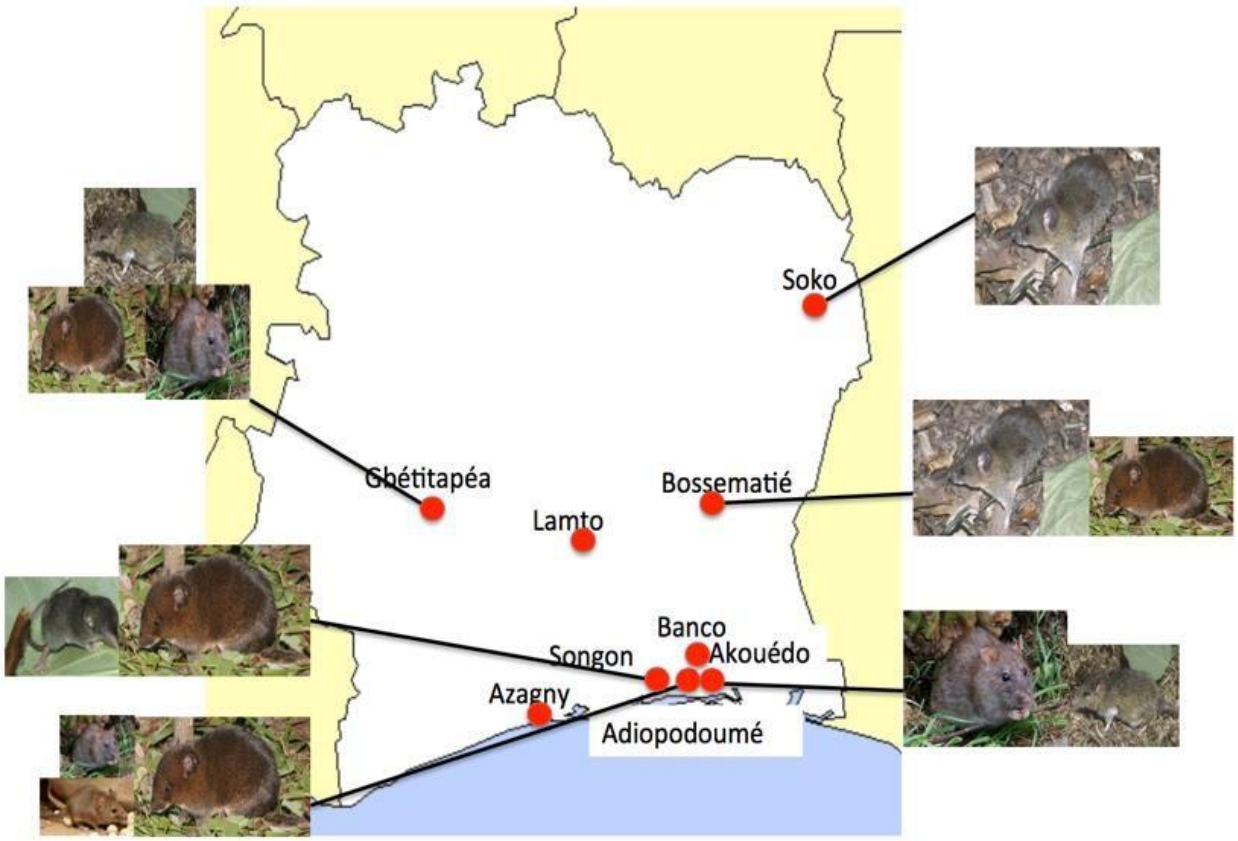

Fig 2: Distribution of infected small mammals by trapping sites

Discussion:

Success rate of trapping in our study was similar to Duplantier in Madagascar during an investigation of a plague epidemic (22) and he had linked this rate to a significant death of the murine population due to the plague epidemic. He also noted a high rate of success capture in homes, which was due to the fact that they are food storage areas attracting rodents. It is an important factor of contact between humans and rodents. On the other hand, Le Quilliecet (23) reported a lower rate 1%, which was attributed to the low rodent density in the trapping area and the model of trap used. Thus, several factors could influence the success rate including rodent density in the area and the type of trap used. Diversity of rodents captured was important in protected area. This is due to the absence or reduction of the human impact on protected areas.

Several genus including *Crocidura, Rattus, Mastomys, Mus, Myomys, Praomys, Hylomuscus, Hybomys, Lophuromys, Malacomys, Terara, Lemniscomys, Uranomys*, and *Nanomys* were captured in this study. In Côte d'Ivoire, several studies had already underlined this diversity (24-28) and this was also confirmed in other West African regions (28). The involvement of rodents captured in the transmission of certain diseases is well known (9-13).

However, for pathogen like Monkeypox virus, doubts still exist for the true reservoir. The most suspected reservoir vector would be *Funisciurus anerythrus* (30,31). In the first phase of this study, Monkeypox virus was not isolated, however, the presence of Orthopoxvirus or the presence of anti-Orthopoxvirus antibodies in different studies in Africa (11,32,33) indicate the need to maintain this surveillance in order to prevent epidemic as occurred in Nigeria in 2017 (4).

In this study, *Leptospira* were detected in 6 genera from 7 trapping sites. These finding suggest a large distribution of these bacteria in small mammals in Côte d'Ivoire. *Leptospira* is able to infect a wide range of mammals and the large distribution of *Leptospira* in small mammals is now well known (34). Regarding infection rate of the small mammal, *Lophuromys* sp, *Praomys* sp and *Rattus* sp were the most infected. With respect to the geographic distribution of these infected small mammals, the most infected are *Lophuromys* sp, *Praomys* sp and *Rattus* sp in the southern peri-urban sites, eastern peri-urban sites and Abidjan urban sites respectively. These small mammals could play major role in maintenance of bacteria and their spread in the environment at their local level. Three species of *Leptospira* were found in our study, with *L.*

interrogans being the most frequently detected, followed by *L. borgpetersenii* and *L. kirshneri*. These 3 species are the ones reported in published studies from Africa, and *L. interrogans* is the most frequent specie reported (35, 36,37).

Conclusion:

The initial data from our investigation indicated the presence of *Leptospira* sp in the rodent vectors, *Rattus, Lophuromys* and *Prao mys* as the potential small mammalian reservoirs in Cote d'Ivoire. Further exploration of the tissue samples taken from these reservoirs will continue, in our bid to search for other emerging pathogens.

Contribution of authors:

MS was responsible for conceptualization, investigation, methodology and writing of original draft; KofKS was involved in investigation and writing of the original draft; KouKS was involved in investigation and writing of original draft; CKJ, KKE, SA and SY were involved in resources and visualization; FH and DM were involved in supervision of the study and validation of the manuscript

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Conflict of interest:

No conflict of interest is declared

References:

1. Levy, B., and Odoi, A. Exploratory investigation of region level risk factors of Ebola Virus Disease in West Africa. Peer J. 2018; 6: e5888 http://doi.org/10.7717/peerj.5888
2. World Health Organization. Ebola Situation Reports: Archive. 2016. http://www.who.int/csr/disease/ebola/situation-reports/archive/en/. Accessed 27 January 2019.
3. Radonić, A., Metzger, S., Dabrowski, P. W., et al. Fatal Monkeypox in Wild-Living Sooty Mangabey, Côte d'Ivoire, 2012. Emerg Infect Dis. 2014; 20 (6): 1009 – 1011
4. Durski, K. N., McCollum, A. M., Nakazawa, Y., et al. Emergence of Monkeypox — West and Central Africa, 1970–2017. MMWR. 2018; 67 (10): 306 - 310
5. Akhiwu, O., Yiltok, E. S., Ebonyi, A. O., et al. Lassa fever outbreak in adolescents in North Central Nigeria: report of cases. J Virus Eradication. 2018; 4: 225–227
6. Attinsounon, O. A., Ossibi Ibara, B. R., Alassani, A., et al. Report of a fatal case of Lassa fever in Parakou in 2018: clinical, therapeutic and diagnostic aspects. BMC Infect Dis. 2018; 18: 667. https://doi.org/10.1186/s12879-018-3587-6
7. Zida, S., Kania, D., Sotto, A., et al. Leptospirosis as Cause of Febrile Icteric Illness, Burkina Faso. Emerg Infect Dis. 2018; 24 (8): 1569-1572
8. Koffi, S. K., Meite, S., Ouattara, A., et al. Geographic distribution of anti-Leptospira antibodies in humans in Côte d'Ivoire, West Africa. Eur J Clin Microbiol Infect Dis. 2018; 37 (11): 2177-2180. doi: 10.1007/s10096-018-3359-7.
9. Kouadio, L., Nowak, K., Akoua-Koffi, C., et al. Lassa Virus in Multimammate Rats, Côte d'Ivoire. Emerg Infect Diss 2013; 21 (8): 1481 – 1483
10. Fichet-Calvet, E., Becker-Ziaja, B., Koivogui, L., and Gunther, S. Lassa Serology in Natural Populations of Rodents and Horizontal Transmission. Vector-Borne Zoon Dis. 2014; 14 (9): 665 - 674
11. Orba, Y., Sasaki, M., Yamaguchi, H., et al. Orthopoxvirus infection among wildlife in Zambia. J Gen Virol. 2015; 96: 390–394
12. Olivero, J., Fa, J. E., Real, R., et al. Mammalian biogeography and the Ebola virus in Africa. Mammal Rev. 2017; 47; 24–37.
13. Mgode, G. F., Machangu, R. S., Mhamphi, G. G., et al. Leptospira Serovars for Diagnosis of Leptospirosis in Humans and Animals in Africa: Common Leptospira Isolates and Reservoir Hosts. PLoS Negl Trop Dis. doi: 10.1371/journal.pntd.0004251
14. Nakazawa, Y., Mauldin, M. R., Emerson, G. L., Reynolds, M. G., Lash, R. R., and Gao, J. A. Phylogeographic Investigation of African Monkey pox. Viruses. 2015; 7: 2168-2184
15. Levine, R. S., Peterson, A. T., Yorita, K. L., Carroll, D., Damon, I. K., and Reynolds, M. G. Ecological niche and geographic distribution of human monkeypox in Africa. PLoS One. 2007; 2 (1): e176.
16. Levine, R. S., Yorita, L. K., Walsh, C. M., and Reynolds, G. M. A method for statistically comparing spatial distribution maps. Int J Hlth Geog. 2009; 8: 7
17. Meite, S., Koffi, K. S., Ouattara, A., et al. Stigmate immunologique de la circulation des Orthopoxvirus chez des nés après l'éradication de la variole en Côte d'Ivoire, Afrique de l'ouest. Rev Cames Sante. 2017; 5 (1):
18. Herbreteau, V., Jittapalapong, S., Rerkamnuaychoke, W., Chaval, Y., Cosson, J. F., and Morand, S. (eds). Protocols for field and laboratory rodent studies. Retrieved from CERoPath project, 2011.
19. Ropp, S. L., Jin, Q., Knight, J. C., Massung, R. F., JJ Esposito, J. J. PCR strategy for identification and differentiation of smallpox and other Orthopoxviruses. J Clin Microbiol. 1995; 33 (8); 2069-2076
20. Merien, F., Amouriaux, P., Perolat, P., Baranton, G., and Saint Girons, I. Polymerase Chain Reaction for Detection of *Leptospira* spp in Clinical Samples. J Clin Microbiol. 1992; 2219–2224.
21. Merien, F., Portnoi, D., Bourhy, P., Charavay, F., Berlioz-Arthaud, A., and Baranton, G. A Rapid and Quantitative Method for the Detection of *Leptospira* Species in Human Leptospirosis. FEMS Microbiol Lett. 2005;
22. Duplantier, J. M., Duchemin, J. B., Ratsitorahina, M., Rahalison, L., and Chanteau, S. Résurgence de la peste dans le district d'Ikongo à Madagascar en 1998. Réservoirs et vecteurs impliqués. Bullettin Sociologie Pathologie Exotique. 2001; 94 (2): 119-122
23. Le Quilliec, P., and Croci, S. Piégeage de micromammifères Une nouvelle boîte-dortoir pour le piège non vulnérant Inra. 2006. Le Cahier des Techniques de l'Inra. Le milieu vivant. Edition spéciale, 125 – 28
24. Gautun, J-Cl., Bellier, L., and Heim De Balsac, H. Liste préliminaire des Rongeurs de la savane de Dabou (Côte-d'Ivoire). J West Afr Sci Assoc. 1969; 14: 219-223
25. Gautunm J-Cl., and Petter, F. Liste préliminaire des Rongeurs d'une savane centrale de Côte-d'Ivoire: Foro-Foro. Mammalia. 1972; 36: 634-

637.
26. Hunkeler, P. Les Cestodes parasites des petits mammifères (Rongeurs et Insectivores) de Côte-d'Ivoire et de Haute-Volta Extrait de la Rev. Suisse Zoology 1973, Tome 80, fascicule 4
27. Tranier, M., and Dosso, H. Recherches caryotypiques sur les rongeurs de Côte d'Ivoire: résultats préliminairespour les milieux fermés. Ann Université Abidjan, Série E (Ecologie), Tome XII; 1979: 181-183
28. Koyaté, B., and Dosso, H. Données sur le peuplement des rongeurs muridés des champs de riz pluvial dans la région de Taï (Côte d'Ivoire) Ann Université Abidjan, Serie E (Ecologie), Tome XIV, 1981: 119-134
29. Fichet-Calvet, E., Becker-Ziaja, B., Koivogui, L., and Gunther, S. Lassa Serology in Natural Populations of Rodents and Horizontal Transmission. Vector-Borne Zoon Dis. 2014; 14 (9): 665 - 74
30. Khodakevich, L., Szczeniowski, M., Manbu-ma-Disu., Jezek, Z., Marennikova, S., Nakano, J., and Messinger, D. Le rôle des écureuils dans le maintien de la transmission du virus simien. Trop Geog Med. 1987; 39 (2): 115 – 122
31. Reynolds, M. G., Caroll, D. S., Olson, V. A., et al. A silent enzootic of an orthopoxvirus in Ghana, West Africa: Evidence for multi-species involvement in the absence of widespread Human Disease. Am J Trop Med Hyg. 2010; 82 (4): 746-754
32. Salzer, J. S., Carroll, D. S., Rwego, I. B., et al. Serologic evidence for circulating Orthopoxviruses in peri-domestic rodents from rural Uganda. J Wildlife Dis. 2013; 49: 125–131
33. Diagne, C. A., Charbonnel, N., Henttonen, H., Sironen, T., and Brouat, C. Serological Survey of Zoonotic Viruses in Invasive and Native Commensal Rodents in Senegal, West Africa. Vector-Borne Zoon Dis. 2017; 17 (10): 730 – 733.
34. Allan, K. J., Biggs, M. H., Halliday Jo, E. B., et al. Epidemiology of Leptospirosis in Africa: A Systematic Review of a Neglected Zoonosis and a Paradigm for 'One Health' in Africa. PLoS Negl Trop Dis. doi: 10.1371/journal.pntd.0003899
35. Gauthier, D., Garba, M., Tatard, C., et al. Urban Market Gardening and Rodent-Borne Pathogenic *Leptospira* in Arid Zones: A Case Study in Niamey, Niger. PLoS Negl Trop Dis. doi: 10.1371/journal.pntd.0004097
36. Halliday Jo, E. B., Knobel, D. L., Allan, K. J., et al. Urban Leptospirosis in Africa: A Cross-Sectional Survey of *Leptospira* Infection in Rodents in the Kibera Urban Settlement, Nairobi, Kenya. Am J Trop Med Hyg. 2013; 89 (6): 1095– 1102
37. Houemenou, G., Ahmed, A., Libois, R., and Hartskeerl, R. A. *Leptospira* spp. prevalence in small mammal populations in Cotonou, Benin. ISRN Epidemiol. 2013: 1–8.