THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

KOFFI Kouassi Eugène et al, The Experiment, 2019, Vol 47 (4), 2695-2704

Prevalence and characteristics of Salmonella isolated to smals mammals in Cote D'Ivoire: case of rodents and bats.

Abstract:
Salmonella sp was detected on specimens of rodents and bats caught in various localities in Côte d'Ivoire. The samples were made up of stool samples. The bacteria were isolated on selective agar and serotyping was carried out by agglutination technique. The study of antibiotic sensibility of bacteria was carried out by the standard method of diffusion in agar. The objective of this study was to determine the prevalence of *Salmonella* in small mammals in Côte d'Ivoire and to characterize these bacteria. The results show that 57 rodents specimens and 53 bats specimens were collected, sampled and analyzed. Two (2) strains of *Salmonella* with a frequency of 3.5% are isolated at *Rattus rattus* in Yopougon K17 and Akeoudo. Three (3) strains were isolated at bats species with a frequency of 5.6%, whose two (2) of whichin Lamto station at Epomops franqueti, Hipposideros caffer and one strain in the classified forest of Mabi at *Nycteris thebaica*. Two (2) differents serotypes are detected, including *Salmonella Mikawasima* at *Epomops franqueti* and *Salmonella Bochum* at *Hipposideros caffer*. Antibiotic resistance was 50% to Amoxicillin; 16.67% to Tetracyclin for rodent strains, whereas those isolated from bats were 100% sensitive to all molecules.
Keywords: *Salmonella*, rodents, bats, zoonosis

I. introduction:

Micro-mammals are mainly represented by rodents, soricomorphs and bats. The rodent order is characterized by its great biological diversity and its ability to colonize different natural and anthropized environments [1, 2]. It represents 44% of mammals. The order of chiropterans (bats) is the most diversified after that of rodents and represents about 20% of current mammals. It is composed of two sub-orders corresponding to two distinct guilds (fruit bats and insect-eating bats). All these micro-mammals with their biological diversity contribute significantly to the ecological balance. However, the continuous and marked changes in their environment have encouraged a closer relationship between humans and these little mammals. Indeed, these environmental changes have allowed bats to develop ecological niches in human dwellings and also lead to outbreaks of rural rodents following changes in agricultural land use. These disruptions have significant consequences for human health through the exponential emergence or re-emergence of infectious diseases, in particular diseases transmitted by these animals (zoonoses). Whether it is the chiropterans [3] than rodents and insectivores [4]. they have been identified as hosts of pathogenic viruses such as Hantavirus, Lassavirus...Apart from these viruses, some rodent species may harbourenterobacteriaceae responsible for infections such as salmonellosis in their intestines [5]. Also, many Salmonella serotypes have been isolated from chiropterans, living in contact with humans [6]. In view of these epidemiological data, these small mammals could constitute an important reservoir of Salmonella, living in the vicinity of humans and playing an active role in the spread of this bacterium. Today, these data concerning Côte d'Ivoire remain insufficient. The objective of this study was to determine the prevalence of salmonella in micro mammals in Côte d'Ivoire and to characterize these isolated bacteria.

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Figure 1: Prevalence of *Salmonella sp* detected to rodents and bats

II. Materials and methods:

2.1 Description of the study sites:

This collection took place at different sites depending on the type of animal for three months (March to April 2019). For rodents, samples were taken from different localities represented by: the Akouedo village landfill in Cocody commune (Abidjan), the km17 market in Yopougon commune (Abidjan), Gbétitapia village (Daloa), Agban village (Songon). For bats, samples were taken from the following sites: Azagny National Park (Great Lahou), Mabi classified forest (Azopé), Bossematié classified forest (Abengourou) and Ecotone of the Lamto ecological station (Toumodi). The GPS (Global Positioning System) coordinates of the various sites were recorded (Fig 1).

2.2 Capture of small mammals and collection of biological samples:

Sherman traps have been used to catch rodents. The characteristic of these types of traps is that they allow animals to be captured without killing them (figure). These traps are set every evening and visited every morning during the study period. Bats were caught using 12-metre long, very fine-meshed Japanese nets of 20 x 20 mm (Figure 4). These nets were deployed from 18:00 to 4:00 and checked every 30 minutes. The captured animals are removed from the nets and placed in cotton bags. The morphometric data of all captured individuals are determined. The animals were anaesthetized with

ISOFLURAN solution and then autopsied. The digestive contents of these animals were collected for bacteriological analysis.

2.3 Bacteriological analysis:

Bacteria of the genus Salmonella sp were detected in the digestive contents of rodents and bats. The search for Salmonella was carried out in accordance with The pre-enrichment with Buffered Peptone Water for 24 h at 37°C, followed by selective enrichment in Rappaport de Vassiliadis broth at 37°C for 24 h. Subsequently, Salmonella sp was cultured on Hektoen selective agar incubated at 37°C for 18 to 24 hours in a normal atmosphere. The identification focused on morphological characteristics (Gram-negative bacillus), cultural characteristics (blue-green colonies with or without a black centre) and biochemical characteristics (reduced Leminor rack). The serotyping of the isolated strains was carried out according to the Kauffman-White scheme and completed by Le Minor. This is a test that determines the antigenic formula of an enterobacteria. It consisted in identifying the Vi, O and H antigens of these bacteria, in order to precisely determine the antigenic formula, thanks to direct active agglutination reactions on the slide.

2.4. Study of the antibiotic sensibility

The sensitivity of strains to antibiotics was studied by the method of diffusion in agar medium according to the recommendations of the Antibiogram Committee of the French Society of Microbiology, Veterinary Recommendations 2018. Marker antibiotics were tested for each of the bacteria: Amoxicillin (25 µg), Amoxicillin / clavulanic acid (20/10 µg), Ceftiofur (30 µg), Gentamicin (15 µg), Nalidixic acid (30 µg), Enrofloxacin (5 µg), Tetracycline (30 IU).

Table 1: Total number of rodents caught and biological samples collected

Kind of rodent	Daloa Gbetitapia	Yopougon (KM17)	Cocody (Akouedo)	Songon	Total N(%)
Mastomys sp	0	1	1	3	5(8,7)
Mus musculus		10	8		18(31,5)
Rattus norvegicus	2	1	1	0	4(7)
Rattus rattus	1	2	1	2	6(10,5)
Cricetomy ssp	1	0	0	0	1(1,7)
Praomys sp	4	0	0	1	5(8,7)
Lophuromys sikapusi	0	7	2	6	15(26,3)
Nannomys sp	0	0	0	1	1(1,7)
Hylomyscus sp	2	0	0	0	2(3,5)
Total	**10**	**21**	**13**	**13**	**57**

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Table 2: Total number of bats caught and biological samples collected

Location Kind of bat	Bossematié (Abengourou)	Mabi (Azopé)	Lamto (Toumodi)	Azagny (G.Lahou)	Total N(%)
Epomop sburtikoferi	-	-	1	-	1(1,8)
Epomops franqueti	-	1	18	-	19(35,8)
Epomopssp	-	1	-	2	3(5,6)
Megaloglossus azagnyi	-	1	1	2	4(7,5)
Nanonycteris velkampi	-	1	1	-	2(3,7)
Roussettussp	-	-	4	1	5(9,4)
Scotonycteris zenkeri	1	4	-	-	5(9,4)
Hipposideros caffer	-	1	1	-	2(3,7)
Hipposideros ruber	-	3	-	-	3(5,6)
Hipposidero ssp	1	2	2	1	6(11,3)
Nycteris grandis	-	1	-	-	1(1,8)
Nycteris thebaica	-	1	-	-	1(1,8)
Rhinolophus sp	1	-	-	-	1(1,8)
Total	**3(5,6)**	**16(3,0)**	**28(52,8)**	**6(11,3)**	**53**

Table 3: Prevalence of *Salmonella* detected to rodents

number of species collected		***Positive to Salmonella***	***Prevalence (%)***
Mastomys sp	5	0	0
Mus musculus	18	0	0
Rattus norvegicus	4	0	0
Rattus rattus	6	2	33,3
Cricetomys sp	1	0	0

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Praomy ssp	5	0	0
Lophuromys sikapusi	15	0	0
Nannomys sp	1	0	0
Hylomyscus sp	2	0	0
Total	**57**	**2**	**3,5**

Table 4: Prevalence of *Salmonella* detected to bats.

number of species collected		***Positive to Salmonella***	***Prevalence (%)***
Epomops burtikoferi	4	0	0
Epomops franqueti	37	1	2,7
Epomops sp	3	0	0
Megaloglossus azagnyi	38	0	0
Nanonycteris velkampi	21	0	0
Roussettus sp	10	0	0
Scotonycteris zenkeri	23	0	0
Hipposideros caffer	7	1	14,2
Hipposideros ruber	2	0	0
Hipposideros sp	28	0	2
Nycteris grandis	10	0	0
Nycteris thebaica	2	1	50
Rhinolophus sp	3	0	0
TOTAL	**53**	**3**	**5,6**

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Table 5: Different serotypes *of Salmonella* isolated

Serotype of *Salmonella* sp	Animal species	Isolation site	Percentage of typable strain
Salmonella Mikawasima	Bat *Epomops franqueti*	Lamto Ecology Station	66,66%
Salmonella Bochum	Bat *Hipposideros caffer*	Lamto Ecology Station	
1 non-serotypable strain	Bat *Nycteris thebaica*	Mabi Classified Forest	
1 non-serotypable strain	Rodent *Rattus rattus*	Akouédo landfill site	0%
1 non-serotypable strain	Rodent *Rattus rattus*	Market of km 17	

3. Results and Discussion:

3.1 Specimens captured and biological samples collected

All the rodents collected in the different localities were composed of 57 specimens represented by (09) species, Mastomys sp 5 (8.7%), *Mus musculus* 18 (31), 5%), *Rattus norvegicus* 4(7%), *Rattus rattus* 6(10.5%), *Cricetomys sp* 1(1.7%), *Praomys* sp 5(8.7%), Lophuromys sikapusi 15(26.3%), *Nannomys* sp 1(1.7%), *Hylomyscus* sp 2(3.5%). These results show that *Mus musculus* is the dominant species (Table 1). From each specimen, one sample was collected and analyzed, for a total of 57 digestive content samples. This study provides an overview of the diversity and abundance of Rodent and Bald Mouse Communities at the different sites. It confirms the presence of nine rodent species, the majority of which are known to be the main small mammals in African cities [7, 8]. In this study, the high rate (31.5%) of *Mus musculus* showed that this species was the most abundant at all four sites. Indeed, this species is part of the communities of small terrestrial mammals frequently reported in urban areas in terms of abundance [9], especially in large cities in West Africa. This is the case for the cities of Cotonou in Benin ([10], Niamey in Niger [11] and Makurdi in Nigeria [12].

As for bats, 53 specimens were collected and sampled (Table 2). A proportion of 75.4% of fruit bats caught was represented by (07) species, *Epomops burtikoferi*, *Epomops franqueti*, *Epomops sp*, *Megaloglossus azagnyi*, *Nanonycteris velkampi*, *Rousettus sp*, *Scotonycteris zenkeri* and 24.5% insectivorous bats represented by 05 species, *Hipposideros caffer*, *Hipposiderosruber*, *Hipposidero ssp*, *Nycteris grandis*, *Nycteris thebaica*. The species *Epomops franqueti* was dominant with 19 speciemens.

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The majority of animals were collected at LAMTO PN with a proportion of 52.8% (Table 2). For these species of Chiropter, it is described that this fauna contains more species of microchiropterans than megachiropters [13]. On the other hand, in this study, the proportion of megachiroptera (75.4%) is three times that of microchiroptera (24.5%) obtained. The low capture rate of microchiroptera is due to the fact that they detect the presence of nets in their path through their echolocation system [14]. When caught in nets, they cut the nets quickly enough with their sharp teeth to escape [15]. Of all the bats caught, the species *Epomops franqueti* was the most abundant. These results are different from those obtained by [13], who reported that *Megaloglossus azagnyi* was more abundant in Banco National Park

3.2 Prevalence of *Salmonella sp:*

Two (2) strains are isolated from 57 samples, representing a frequency of 3.5%. These strains were isolated only from the species *Rattus rattus* in the Yopougon K17 market and in samples from the Akéoudo landfill in the municipality of Cocody (Abidjan) (Fig1). These results confirm the presence of this bacterium in these animals in Côte d'Ivoire. Here, the origin of these bacteria could be either environmental because a strain has been isolated from the *Rattus rattus* species collected at the household landfill in Akouedo. Indeed, this site is the largest garbage dump in the country. It receives hospital, household and other waste from the city of Abidjan. It is also the place of economic activity of certain populations who will sort and recycle certain objects to market them. On this site, market gardening is also practiced. All these breeds would encourage the presence of rodents and also contact with several microbial agents, including salmonella. The second strain isolated from the same *Rattus rattus* species collected on the market would reflect the biscuit nature of this bacterium. It could therefore develop in several types of environment. Also, the market nature of poultry, humans and other activated animals would develop the presence of this bacterium. The presence of this bacterium in the *Rattus rattus* species is thought to be related to the animal's living environment. Indeed, this rat is described as a living animal in the sewers which constitute a best environment for the development of many microorganisms including bacteria.

In bat samples, three (3) strains isolated from 53 samples, representing a frequency of 5.6%. Two (2) strains have been reported on Lamto station in the species *Epomops franqueti*, *Hipposideros caffer* and one strain in *Nycteris thebaica* in Mabi (Fig 1). This would confirm the reservoir nature of these animals as described by various authors [16]. Here these bacteria have been isolated both from fruit-eating species (*Epomops franqueti*) and insectivores (*Hipposideros caffer, Nycteris thebaica* in different types of vegetation. These were the primary forest of Azagny National Park, the ecotone of the Lamto Ecology Station, and the classified forest of Mabi. These data show that the type of bat and the living environment would not influence the presence of Salmonella.

The presence of Salmonella in these healthy animals could indicate that these animals are asymptomatic carriers. Although the level of this isolated bacterium in this study is low, it may pose a health risk to the population that consumes and handles them. These animals would also be sources of environmental contamination through the spread of these bacteria.

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3.3 Different serotype of *Salmonella:*

The different salmonella serotypes isolated are presented in Table 5. In rodents, the serotype of the isolated strains was not detected. As for the strains isolated from bats, 2 out of 3 were serotyped with a rate of 66.6%. The isolated serotypes are *Salmonella Mikawasima* at *Epomops franqueti* and *Salmonella Bochum* at *Hipposideros caffer* species. The serotype of the strain isolated at *Nycteris thebaica* in the classified Mabi forest has not been determined. These serotypes are different from those obtained by [16].

3.4. Antibiotic sensibility:

The Salmonella strains isolated in rodents were sensitive for all the molecules tested except for a few observed resistances of 50% for Amoxicillin and 16.67% for Tetracycline. All Salmonella strains isolated from bats were susceptible to antibiotics. This sensitivity to different antibiotics could mean that they are wild strains [17]. However, some resistance was observed for Amoxicillin and Tetracycline molecules on strains isolated from rodents. These strains, which are resistant to these families of antibiotics (beta-lactam antibiotics and Tetracyclin), could be considered as strains that have undergone mutations. The potential changes in these antibiotic-resistant strains could suggest that they are strains of human origin on the one hand, due to the resistance observed to Amoxicillin. Indeed, this molecule is the first-line antibiotic used in human medicine in Côte d'Ivoire. Thus, a strain resistant to this molecule isolated from rodents in the landfill would be linked to contacts between human strains and those of these rodents, knowing that the Akouedo landfill receives both medical and household waste. However, these tetracycline-resistant strains are believed to be due to contact with strains from livestock, as this molecule is more commonly used in livestock farming in Côte d'Ivoire. Knowing that this strain has been isolated from market rats, it would then be in contact with avian strains from these markets where many poultry products (chickens, feed, eggs) are sold. It is already noted that several resistances are observed with this molecule in Côte d'Ivoire in these poultry products.

Conclusion:

This study determined the prevalence of salmonella in small mammals in Côte d'Ivoire. This frequency was 3.5% in rodents and 5.6% in bats. This bacterium was isolated from the species *Rattus rattus* for rodents at the Yopougon K7 market and at the Akouedo landfill. In bats, this bacterium has been reported in the fruit-eating (*Epomops franqueti*) and insectivorous (*Hipposideros caffer*, *Nycteris thebaica*) species living in Azagny National Park and the ecotone of the Lamto Ecological Station and the classified Mabi Forest. The observed resistance profile shows that the Salmonella strains isolated in rodents were sensitive for all the molecules tested except for a few observed resistances which were 50% for Amoxicillin, and 16.67% for Tetracycline. The serotypes isolated from bats were Salmonella Mikawasima in *Epomops franqueti* and Salmonella Bochum detected in *Hipposideros caffer*, all sensitive to the molecules tested.

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Reference:

1. Gabrey SW: 1997. Bird and small mammal abundance at four types of wastemanagement facilities in northeast Ohio. Landscape and Urban Planning 37: 223-233.
2. Happold DCD: 2013. Mammals of Africa, Volume III: Rodents, Hares and Rabbits. Bloomsbury Publishing, London, 784 pp.
3. klempa, B., Koulemou, K., Auste, B., Emmerich, P., Thomé-Bolduan, C., Gunther, S., Koivogui, L., Kruger, D. H. & Fichet-Calvet, E., 2013- Seroepidemiological study reveals regional co-occurrence of Lassa and Hantavirus antibodies in Upper Guinea, West Africa. *Tropical Medecine International Health*, 18: 366-371.
4. Kang, H. J., Kadjo, B., Dubey, S., Jacquet, F. & Yanagihara, R., 2011- Molecular evolution of Azagny virus, a newfound hantavirus harbored by the west African pygmy shrew (*Crocidura obscurior*) in Côte d'Ivoire. *Virology Journal,* 8: 373.
5. Letellier A., Messier S and Quessy S., "Prevalence of *Salmonella* and *Yersinia enterocolitica* in finishing swine at Canadians abattoirs". J Food prot. 62: (1999) 22-25.
6. Clegg (F. C), Heath (P. J.). *Salmonella* excretion bv terraoins and the associated hazard to human 12. health. *Vet.&Rec.*, 1975, 96 (4) : 90-91.
7. Granjon L. and Duplantier JM: 2009. Les rongeurs de l'Afrique sahélosoudanienne. Collection Faune et flore Tropicale 43. IRD éditions ; MNHN, Marseille, France. 215pp.
8. Adamou-Djerbaoui M, Labdelli F, Djelaila Y, Oulbachir K, Adamou MS. and Denys C: 2015 : Inventaire des Rongeurs dans la région de Tiaret (Algérie). Travaux de l'Institut Scientifique, Série Générale 8 : 105-112
9. Akpatou Kouamé Bertin, Bohoussou Kouakou Hilaire, Ahissa Laurent, Kadjo Blaise, Diversité et abondance des Rongeurs et Soricomorphes dans différents standings de la commune de Yopougon, Côte d'Ivoire.
10. Houémènou G, Kassa B. and Libois R : 2014a. Ecologie, diversité spécifique et abondance des petits mammifères de la ville de Cotonou au Bénin (Afrique de l'Ouest). International Journal of Biological and Chemical Sciences 8(3): 1202-1213.
11. Garba M, Kane M, Gagare S, Kadaoure I, Sidikou R, Rossi J-P. and Dobigny G: 2014. Local perception of rodentassociated problems in Sahelian urban areas: a survey in Niamey, Niger. Urban Ecosyst, 17 : 573.
12. Omudu EA. and Ati TT: 2010. A survey of rats trapped in residential apartments and their ectoparasites in Makurdi, Nigeria. Research Journal of Agriculture and Biological Sciences, 6 (2):144-149.
13. Eloi Anderson Bitty1,2*, Blaise Kadjo, Sery Gonedele bi, Modeste Okon Okon et Philippe Kouassi Kouassi. Inventaire de la faune mammalogique d'une forêt urbaine, le Parc National du Banco, Côte d'Ivoire Int. J. Biol. Chem. Sci. 7(4): 1678-1687, August 2013 ISSN 1991-8631 © *2013 International Formulae Group. All rights reserved.* DOI: http://dx.doi.org/10.4314/ijbcs.v7i4.23 *Original Paper* http://indexmedicus.afro.who.int

ISSN-2319-2119

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14. Britton ARC, Jones G. 1999. Echolocation behaviour and prey capture success in foraging bats: laboratory and field *E. A. Bitty et al. / Int. J. Biol. Chem. Sci. 7(4): 1678-1687, 2013* 1687 experiments on Myotis daubentonii. *J. Exp. Biol.*, 202: 1793-1801.
15. Decher J, Kadjo B, Abedi ML, Elhadji OT, Soumaoro K. 2005. Une étude rapide des petits mammifères (musaraignes, rongeurs et chiroptères) des forêts classées de la haute Dodo et du Cavally, Côte d'Ivoire. In *RAP Bulletin N° 34: A Rapid Biological Assessment of Two ClassifiedForests in South-Western Côte d'Ivoire*, Lauginie F, Rondeau G, Alonso LE (eds). Conservation International: Washington, D.C; 101-109.
16. M. P. Doutre et H. Sarrat :Sérotypes de salmonelles isolées chez les chiroptères frugivores et insectivores du Sénégal,Importance épidémiologique Rev. Elev. Méd. vét. Pays trop., 1973, 26 (3) : 219-87.
17. Koffi K. Eugène.1, Kouassi K. Stéphane.2, Saraka N Daniel.1 et Dosso Mireille 2.Etude bactériologique des escargots géants africains en Côte d'Ivoire, Journal of Animal & Plant Sciences, 2019. Vol.39, Issue 1: 6394-6402; Publication date 31/01/2019, http://www.m.elewa.org/JAPS; ISSN 2071-7024

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