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Role of Ectoparasites and Rodents in the Spread of Infectious Diseases Infectious Diseases in the Republic of Guinea

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Abstract
Parasites are organisms that adversely affect their host, either by modifying specific physiological functions or by multiplying and developing large populations within their host. The aim of this study is to demonstrate the role of ectoparasites of zoonotic rodents in the transmission of infectious diseases. 200 H.B. Sherman type traps, with dried fish as bait, were used to capture rodents, either in lines or spaced 4 metres apart. Rodents were identified using the classic method of Rosevear, D.R., Wilson, D.E. and Reeder, D.M. PCR and RT-PCR were carried out on samples of blood, organs, mite shreds and insects. The study took place from April 2020 to August 2022; 8 prefectures were chosen according to their ecosystems (scrubland, agricultural fields, villages, orchards, bushes, warehouses, riverbanks, etc.). A total of 1,265 rodents, divided into 18 species, were the subject of our work. The species most frequently encountered were: Rattus rattus (n=437), Mus musculus (n=185), Mus spp. (n=150) and Cricetomys gambianus (n=92). A total of 412 ectoparasites were identified, comprising 7 species including 3 mites and 4 insects. Analyses detected 2 cases of Mammarenavirus lassa, 42 cases of Borrelia spp. 5 cases of Anaplasma spp. 4 cases of Ehrlichia spp. 4 cases of Leptospira spp. and 1 case of Coxiella burnetii. Analysis of the results shows that N'Zérékoré and Kindia are the prefectures most at risk.

Keywords: Ectoparasites, Rodents, Zoonoses, Republic of Guinea

Introduction:

Understanding the impact of small mammals in epidemiological cycles, as primary or secondary hosts, requires an in-depth understanding of the relationships they maintain with other organisms (hosts and pathogens) and with their environment [1].

Recent studies on the impact of global changes in the environment on the dynamics of pathogens and their geographical distribution perfectly illustrate the relevance of large-scale studies to better understand the kinetics and persistence of micro-organisms in host populations, whether of human, animal or plant origin [2].

In ecological terms, infectious diseases are considered to be an extension of the host-parasite relationship. Parasites are generally small organisms that exploit their host both as a food resource and as a habitat. They adversely affect their host, either because they modify specific physiological functions or because they

multiply and develop large populations within their host; or individually, their effect is often very small. This explains why parasites have traditionally been ignored by ecosystem ecology: they are hidden within their host, and their impact on direct ecosystems is apparently negligible. However, their indirect influence on ecosystem dynamics can be significant through their interaction with their hosts [3].
Rodents have historically been known to transmit diseases, which is why attempts to eradicate wild rodents interacting with humans have been envisaged. Many methods are used, from mechanical traps to poison [1].
The aim of this study is therefore to prove the importance of rodents in contact with humans, in order to demonstrate the absolute necessity of finding new ways of combating rodents that carry zoonoses.
This approach thus lies at the junction of epidemiology, which examines diseases and health factors within a population, and ecology, which studies the interactions between various organisms and between organisms and their surrounding environment [1].
In this study, we are particularly interested in the carriage of pathogens by rodents. To this end, we proposed to study the carriage of bacterial and viral pathogens by ectoparasites collected from rodents in natural ecosystems, in order to assess the risk factors for transmission of these agents.

General objective
To assess the role of ectoparasites and rodents in the spread of infectious diseases in the Republic of Guinea in order to identify the associated health risks and propose appropriate prevention and control strategies.

Specific objectives
- Inventory and identify rodents;
- Collect ectoparasites of rodents encountered;
- Inventory and identify pathogens notified in ectoparasites and rodents;
- Identify zoonotic diseases in the regions under investigation.

Performance of the work and description of the methodology:
- Equipment
Adson 15 cm, Iris scissors 12 cm, Mosquito forceps 12. 5 cm stralght, Alcohol 70%, Scale, Gown, Goggles, Personal protective equipment, FFP mask, Surgical mask, Hard hat, Protective visor, Ruler, Cotton wool, 5cc syringe, Heparin tube, Tube, Alcohol lamp, Special labels, Hand magnifier, Gloves, Bib, Metal tray, Tweezers, Ikon D3500 camera, Fine needles, Soap, H. B. Sherman trap, Russian trap, Traditional trap, Register, Toothbrush, Clines, Chlorine, NaCl solution, RNeasy Plus kit (Qiagen, Hilden, Germany), Tissue Lyser LT (Qiagen, Hilden, Germany), Amplisens Ribo Prep.
Biomaterial: Mites, Insects and Rodents.

Sampling
Capture campaigns were carried out between April 2020 and August 2022 in several prefectures of Guinea.
The biomaterial was collected using a snowball effect (the first sample found was followed by others as they became available). In total, 1265 rodents were captured, from which 412 ectoparasites were collected.

Methodology
Capture and processing of rodents
Our study was conducted from April 2020 to August 2022 at the selected study sites and areas. Given the seasonal nature of our biological model, surveys in the study areas took place during the dry and rainy seasons. A series of sites was selected on the basis of their biodiversity. We adopted the transect method given the surface area and floristic characterisation (cover and composition).
To capture rodents, we chose three model plots in rural areas: the first in the bush, the second in the agricultural landscape (far from the village), and the third in the village and adjacent gardens. In these locations, we marked out where the traps would be set. The size of a site was 72 by 135 metres. The traps on the sites were placed at exactly the same distance from each other. All the traps were of the same type (Sherman or Russian trap, traditional trap) and numbered. The number of traps placed varied from 50 to 200 depending on the characteristics of the site. The animals were captured on the sites 3 times in succession, and the traps were checked twice a day in the morning and evening (with an interval of 12 hours). Each animal captured was given an individual (recognition) number.

The captured rodents were placed in breathable cotton bags, moistened beforehand to prevent dehydration. They were then transported to the laboratory where they were euthanised according to standard protocols.
We weighed and measured the animal, and determined its sex and species. All the information collected was recorded in the diary: date of capture, place of capture, animal identifier, trap number. Species identification was carried out using the classic method recommended by [4,5].

Collection of ectoparasites

Prior to dissection, ectoparasites were sought on all rodents captured, then immersed in 70% ethanol and stored at -20°C.
The method used to collect ectoparasites involved visually examining and brushing the entire body of the rodents. For ticks, however, it is necessary to collect by grasping, and to do this the rostrum is pulled out with a firm, sharp blow without lysing it, as it represents an important element for identification.

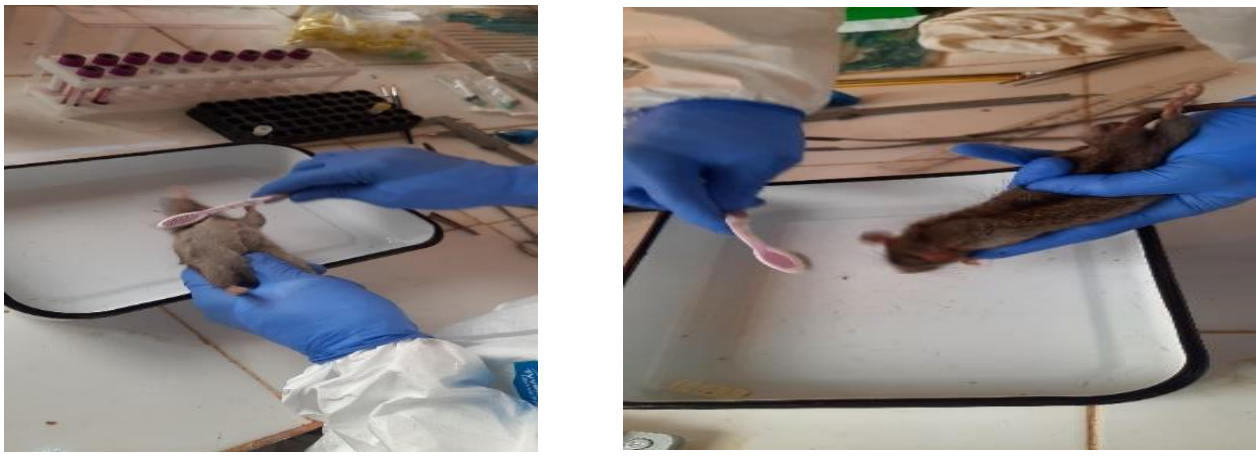

Fig. 1 Detection of ectoparasites in small mammals (Kolié 2021).

II-4.8 Viral detection

Detection of arenaviridae RNA was carried out using a commercial Amplisens Ribo Prep kit (Central Research Institute of Epidemiology, Moscow, Russia). Total RNA from skin homogenates, ectoparasites, oral swabs, blood plasma, urine and non-pooled body tissues (brain, liver, spleen, lung, kidney, lymph nodes and salivary glands) for RT-PCR positive animals was re-extracted using the same RNA preparation and extraction method (Central Research Institute of Epidemiology, Moscow, Russia) according to the manufacturer's instructions.

Presentation of the results

Out of a total of 1,265 rodents, 5 families (Muridae, Soricidae, Nesomyidae, Sciuridae and Gliridae) were recorded using the morphometric method. The distribution of the different species according to family is shown in tables 1 and 2.

Table 1: Inventory of rodents caught

order	Family	Species
Rodentia	*Muridae*	*Mus musculus* (Linnaeus, 1758) ou spp.
		Mus spp (Linné, 1758)
		Mus setulosus (Peters, 1876)
		Rattus rattus (Linnaeus, 1758)
		Lemniscomys zebra (Heuglin, 1864)
		Mastomys natalensis (Smith, 1834)
		Praomys daltoni (Thomas, 1892)
		Praomys rostratus (Miller, 1900)
		Dasymys rufulus (Meunier, 1900)
		Lemniscomys striatus (Linnaeus, 1758)
		Lophuromys sikapusi (Temminck, 1853)
	Soricidae	*Crocidura sp* (Walger, 1832)
	Nesomyidae	*Cricetomys gambianus* (Château d'eau, 1840)

	Sciuridae	*Xerus erytropus* (Desmarest, 1817)
		Heliosciurus gambianus (Ogilby, 1835)
		Funisciurus pyrropus (Cuvier, 1833)
		Epixerus ebii (Temminck, 1853)
	Gliridae	*Graphiurus kelleni* (Reuvens, 1890)

Of the 1,265 rodents caught, we distinguished five (5) families divided into 14 genera and 18 species.
The Muridae family was the most represented with 11 species out of the 18 species inventoried, 4 species characterise the Sciuridae family and the least represented families were the Soricidae, the Nesomyidae and the Gliridae with only one species in each family.

Table 2: Summary of ectoparasites collected

N°	order	Family	Species
1	Acariens	*Ixodidae*	*Ixodes spp.*
2		*Dermanyssidae*	*Ornithonyssus bacoti*
3		*Myobiidae*	*Radfordia ensifera*
4	Insectes	*Pulicidae*	*Ctenophalides felis*
5			*Xenopsylla cheopis*
6		*Ceratophyllidae*	*Nosopsyllus fasciatus*
7		*Haematopiridae*	*Polyplax spinulosa*

This table shows that 6 families of ectoparasites have been collected from rodents, comprising 7 species, divided into two (2) orders: mites and insects.

Table 3: Quantification of ectoparasites by rodent species

Species of rodents / Ectoparasite species	*Mus musculus*	*Ratus ratus*	*Cricetomys gambianus*	*Crocidura*	*Mastomis natalensis*	*Praomys daltoni*	*Xerus erytropus*	*Mus spp.*	*Mus setulosus*	*Lophuromys sikapusi*	*Dasymys rufulus*	*Lemniscomys striatus*	*Praomys rostratus*	Totaux
Ixodes spp.	-	15	10	-	-	-	5	-	-	10	-	-	-	40
Ornithonyssus bacoti	-	12	11	-	-	-	-	10	6	3	-	4	-	46
Radfordia ensifera	6	13	-	-	-	-	-	-	-	7	8	3	-	37
Ctenophalides felis	13	105	8	-	-	-	4	-	-	5	6	7	-	148
Nosopsyllus fasciatus	3	20	15	-	5	-	6	4	6	-	-	-	-	59
Polyplax spinulosa	-	15	12	-	-	-	-	-	-	-	-	-	-	27
Xenopsylla cheopis	-	55	-	-	-	-	-	-	-	-	-	-	-	55

Total	**22**	**235**	**56**	**-**	**5**	**-**	**15**	**14**	**12**	**15**	**24**	**14**	**-**	**412**

Legend: - absence of ectoparasites

A total of 412 ectoparasites were collected from rodents, with the highest number recorded in Rattus rattus, with 235 specimens collected.

Of the 7 parasite species identified, Rattus rattus was the most heavily infested rodent species. This can be explained by its presence in all the habitats studied, as well as by the high number of specimens captured.

Of the 7 species of ectoprasites, Ctenophalides felis was the most abundant with 148 specimens collected, followed by Nosopsyllus fasciatus with 59 individuals and the least represented species was Polyplax spinulosa with 27 individuals collected.

Table 4: Prevalence of parasite species

N°	Ectoparasite species	Rodent species	Number of rodents	Prevalence
1	*Ixodes spp.*	*Rattus rattus*	15	3,08
		Cricetomys gambianus	10	2,05
		Xérus erytropus	5	1.02
		Dasymys rufulus	10	2,05
2	*Ornithonyssus bacoti*	*Rattus rattus*	12	2,46
		Cricetomys gambianus	11	2,26
		Mus spp.	10	2,05
		Mus setulosus	6	1,23
		Lophuromys sikapusi	3	0,62
		Lemniscomys striatus	4	0,82
3	*Radfordia ensifera*	*Mus musculus*	6	1,23
		Rattus rattus	13	2,67
		Lophuromys sikapusi	7	1,44
		Dasymys rufulus	8	1,64
		Lemniscomys striatus	3	0,62
4	*Ctenophalides felis*	*Mus musculus*	13	2,67
		Rattus rattus	105	21,56
		Cricetomys gambianus	8	1,64
		Xérus erytropus	4	0,82
		Lophuromys sikapusi	5	1,03
		Dasymys rufulus	6	1,23
		Lemniscomys striatus	7	1,44
5	*Nosopsyllus fasciatus*	*Mus musculus*	3	0,62
		Rattus rattus	20	4,11
		Cricetomys gambianus	15	3,08
		Mastomys nantalensis	5	1,03

		Xérus erytropus	6	1,46
		Mus spp.	4	0,97
		Mus setulosus	6	1,32
6	*Polyplax spinulosa*	*Rattus rattus*	15	3,08
		Cricetomys gambianus	12	2,46
7	*Xenopsylla cheopis*	*Rattus rattus*	55	11,29
	Hemimerus Spp.	*Cricetomys gambianus*	75	15,40
Total			**487**	**100**

From this table, we can see that Rattus rattus was the most infested species with a percentage of 21.56 while Mus musculus, Lemniscomys striatus and Lophuromys sikapusi were the least infested species with 0.62% each.

Table 5: Positive cases according to the two pathogen analysis methods (PCR and RT-PCR) by rodent species

N°	Espèces	Number of samples	Pathogens	Number of positive cases	Percentage
1	*Mus musculus*	185	*Borrelia spp.*	12	0,95
2	*Mus spp.*	150	*Borrelia spp.*	9	0,71
3	*Rattus rattus*	437	*Borrelia spp.*	15	1,19
4	*Mastomys natalensis*	70	*Mamarenavirus lassa*	2	0,16
			Borrelia spp.	2	0,16
5	*Crocidura spp.*	62	*Anaplasma spp.*	3	0,24
6	*Lophuromys sikapusi*	68	-	0	0
7	*Dasymys rufulus*	63	*Borrelia spp.*	2	0,16
			Leptospira spp.	3	0,24
8	*Lemniscomys striatus*	53	*Anaplasma spp.*	2	0,16
			Leptospira spp.	1	0,08
9	*Cricetomys gambianus*	92	*Ehrlichia spp.*	4	0,32
10	*Xérus erytropus*	16	*Coxiella burnettii*	1	0,08
11	*Lemniscomys zébra*	14	*Borrelia spp.*	2	0,16
12	*Praomys daltoni*	7	-	0	0
13	*Praomys rostratus*	4	-	0	0
14	*Funisciurus pyrropus*	9	-	0	0
15	*Heliosciurus gambianus*	2	-	0	0
16	*Epixerus ebii*	1	-	0	0
17	*Graphiurus kelleni*	1	-	0	0
Total		**1265**	-	**58**	**4,58**

Analysis of the samples revealed 58 positive cases (4.58%) out of 1,265 samples taken from the various rodent species captured.
Brain tissue samples from Mastomys natalensis detected the presence of Lassa virus RNA in 2 Mastomys natalensis. Blood plasma samples from all rodents tested negative for the presence of hantavirus RNA, while blood sediments revealed the presence of Coxiella burnettii bacterial DNA in Xerus erythropus.
In addition, blood sediments were analysed for the detection of DNA from Anaplasma spp. and Ehrlichia spp. Ehrlichia spp. bacteria were detected in Cricetomys gambianus, while Anaplasma spp. DNA was identified in Lemniscomys striatus and Crocidura spp, and the presence of Borrelia spp. DNA with infections in various rodents captured in different prefectures indicating a potential circulation of Borrelia spp. among rodents in the Republic of Guinea. Finally, blood sediments taken from all the rodents were tested for the presence of bacterial DNA of Leptospira spp. 4 positive cases were detected.

DISCUSSION

The results indicate that our study areas are rich in ecosystems because they are home to a diverse and very interesting rodent fauna. On the whole, rodents are associated with the various plant formations.
The composition of the populations in the various study sites during the 3 years of research was as follows: 18 species found compared with 11 species listed by Inapogui et al. (2000). The greatest species richness was obtained in the Kindia region where we captured 16 different species: Mus musculus, Mus spp, Mus setulosus, Rattus rattus, Mastomys nantalensis, Crocidura sp, Lophyromys sikapusi, Dasymys rufulus, Lemniscomys struatus, Cricetomys gambianus, Xérus erytropus, Lemniscomys zébra, Praomys daltoni, Praomys rostratus, Funisciurus pyrropus and Heliosciurus gambianus against 12 species inventoried by [6].
All the ectoparasites in our host models are mites from three families: (Ixodidae, Dermanyssidae and Myobiidae) and insects (Ceratophyllidae, Haematopiridae and Pulicidae) belonging to the haematophagous parasite groups.
A total of 412 ectoparasites were collected, divided into 7 species as follows: Ixodes spp., Ornithonyssus bacoti, Radfordia ensifera, Ctenophalides felis, Nosopsyllus fasciatus, Polyplax spinulosa, Xenopsylla cheopis. These results differ from those of [7] who collected 539 ectoparasites divided into 4 species as follows: Ixodes spp., Anoploure, Xenopsylla cheopis, Hemimerus spp.
All the ticks were Ixodes spp. (40 ticks), represented by the three stages (larvae, nymphs and adults). This is in line with the work of [8] who noted that: the three developmental stages of ticks (larva, nymph and adult) as well as both sexes (male and female) were collected from small mammals in the Special Reserve of Ambohitantely, Madagascar.
The abundance of ectoparasites found in Rattus rattus is similar to the work of [7], who found in their study of fleas infecting small mammals in urban areas in Benin that Rattus rattus was more abundant, followed by Rattus norvegicus and Arvicanthis niloticus. Our results differ from those reported by [9] which show Rattus norvegicus as the most abundant instead of Rattus rattus as reported here.
Our results are quite significant, since a PCR carried out on blood and organ samples on the one hand, and on mite and insect crushed material on the other, has enabled us to detect 2 cases of Mammarenavirus lassa. This result confirms the declarations of a fatal case of lassa haemorrhagic fever and more than 30 contacts by the Guinean health authorities, which were recorded in the prefecture of Yomou during the month of May 2021.
On 20 April 2022, another case of lassa haemorrhagic fever was detected in a 17-year-old female patient from the sub-prefecture of Kassadou, Guéckédou prefecture [10]. All this shows that rodents are reservoirs of pathogens and a source of infection for ectoparasites and humans.
Rodents are just one example of the reservoirs of diseases that can be transmitted to humans. Other more common diseases, such as arboviroses, and in particular those transmitted by mosquitoes (dengue fever, chikungunya, etc.), are also closely linked to climatic and environmental conditions. These propagation conditions reflect human activities, disrupting ecosystems and eroding biodiversity.
An analysis of the results of our work shows that the areas at risk are the N'Zérékoré region and Kindia (the site of the survey and a site heavily populated by rodents).

Conclusion

At the end of our research work in the four natural regions of the Republic of Guinea on the role of rodents in the natural foci of zoonoses in the Republic of Guinea, several specimens were caught and divided into different families and species.

The collection of ectoparasites from rodents enabled us to identify species of haematophagous parasites, divided into two orders: mites and insects.
The PCR and RT-PCR results, combined with bacteriological analyses of samples of rodent tissues, organs and ectoparasites, enabled us to draw up a health assessment of rodent populations and to confirm the role of reservoir for certain zoonoses.
In the light of our results, the health status does not appear to be alarming, although potentially dangerous zoonoses are present, particularly for professionals.
These diseases spread from host to host and from species to species when optimal conditions are met. They are therefore linked to changes in ecosystems.
This would lead us to assume that diseases and vectors circulate in a borderless zone, putting humans at risk of contamination. As wild animals are the source or reservoir for many important zoonotic pathogens, a crucial aspect of pathogen transmission is knowledge of the different routes of zoonotic infection from wildlife to people.

Conflicts Of Interest

The authors have no competing interest in declaring that they are relevant to the content of this article.

Financial Interests

No funding was received for this study.

Contribution Of The Authors

The contribution of the authors (CONDE Y., DIALLO A. O. S., DIALLO M. G., BAH B. S. S, DIALLO S, BOIRO M. Y.) is not negligible and facilitated the drafting and correction for the publication of this article.

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