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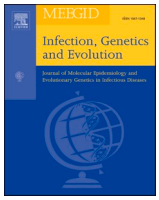

Investigation of caliciviruses and astroviruses in Gabonese rodents: A possible influence of national and international trade on the spread of enteric viruses

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ABSTRACT

Caliciviruses (*Caliciviridae*) and astroviruses (*Astroviridae*) are among the leading cause of non-bacterial food-borne disease and gastroenteritis in human. These non-enveloped RNA viruses infect a wide range of vertebrate species including rodents. Rodents are among the most important hosts of infectious diseases globally and are responsible for over 80 zoonotic pathogens that affect humans. Therefore, screening pathogens in rodents will be is necessary to prevent cross-species transmission to prevent zoonotic outbreaks. In the present study, we screened caliciviruses and astroviruses in order to describe their diversity and whether they harbor strains that can infect humans. RNA was then extracted from intestine samples of 245 rodents and retrotranscribed in cDNA to screen caliciviruses and astroviruses by PCRs. All the samples tested negative for caliciviruses and while astroviruses were detected in 18 (7.3%) samples of *Rattus rattus* species. Phylogenetic analyses based on the RdRp gene showed that all the sequences belonged to *Mamastrovirus* genus in which they were genetically related to *R. rattus* related AstVs previously detected in Gabon or in *Rattus* spp. AstV from Kenya and Asia. These findings suggested that transportation such as land and railway, as well national and international trade, are likely to facilitate spread of AstVs by the dissemination of rodents.

1. Introduction

Despite the progress of medicine, infectious diseases remain a significant challenge for humanity. One explanation for that is the frequent emergence of new infectious diseases (Watkins, 2018). While once rare, the frequency of infectious disease emergence has accelerated in the last 60 years. It is worth noting that approximately 75% of emerging infectious diseases known to affect humans originate from animals, with many of these diseases being related to wildlife species, including rodents (Taylor et al., 2001). Rodents belong to the order *Rodentia*, which is the most diverse mammalian group, with nearly 2552 species divided into 36 families (Burgin et al., 2018). Rodents are among the most important sources of infectious diseases globally, and carry over 80 zoonotic pathogens (Han et al., 2016). Therefore, surveillance of

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pathogens hosted by rodents in areas shared with humans is useful to detect pathogens that could cross the species barrier to infect humans leading to outbreaks.

Enteric viruses are transmitted via fecal-oral route, either directly or indirectly through contaminated food, water, and surfaces (De Graaf et al., 2017). These viruses reach the gastrointestinal tract and replicate in the enterocytes primarily. Most of the RNA enteric viruses including caliciviruses (CaVs) and astroviruses (AstVs) are non-enveloped, very stable in the environment and can survive for long periods of time under different conditions. They infect a large spectrum of hosts such as human, livestock, domesticated and wild animals. They are mainly responsible for gastroenteritis in humans and animals, but have also been associated with mild to severe extra-intestinal diseases such as respiratory diseases, nephritis and neurological disorders (Robilotti et al., 2015; Rosenberg, 2016; De Benedictis et al., 2011; Bank-Wolf, B. R, et al., 2010).

Caliciviruses belong to the family *Caliciviridae*, which contains 11 recognized genera, including *Bavovirus*, *Lagovirus, Norovirus, Salovirus* and *Sapovirus* (https://ictv.global/taxonomy). Only noroviruses (NoVs) and sapoviruses (SaVs) can infect humans. The reverse-zoonotic potential of CaVs has been described. Some NoVs and SaVs found in animals are related to human strains (Mattison et al., 2007; Martella et al., 2008; Mombo et al., 2014). Despite that, there is no evidence of animal-related NoVs and SaVs detected in humans (Mombo et al., 2019; Bohou Kombila et al., 2023).

Astroviruses (AstVs) belong to the genera *Avastrovirus* (*AAstVs*) for AstVs infecting birds and *Mamastrovirus* (*MAstV*) for those infecting mammals within the family *Astroviridae*. Since the detection of human astroviruses of the groups HAstV-VA/HMO and HAstV-MLB, which are genetically close to animal AstVs but divergent from classical human astroviruses (HAstV-1 to 8), the zoonotic origin of AstVs has been suggested (Finkbeiner et al., 2008; Kapoor et al., 2009).

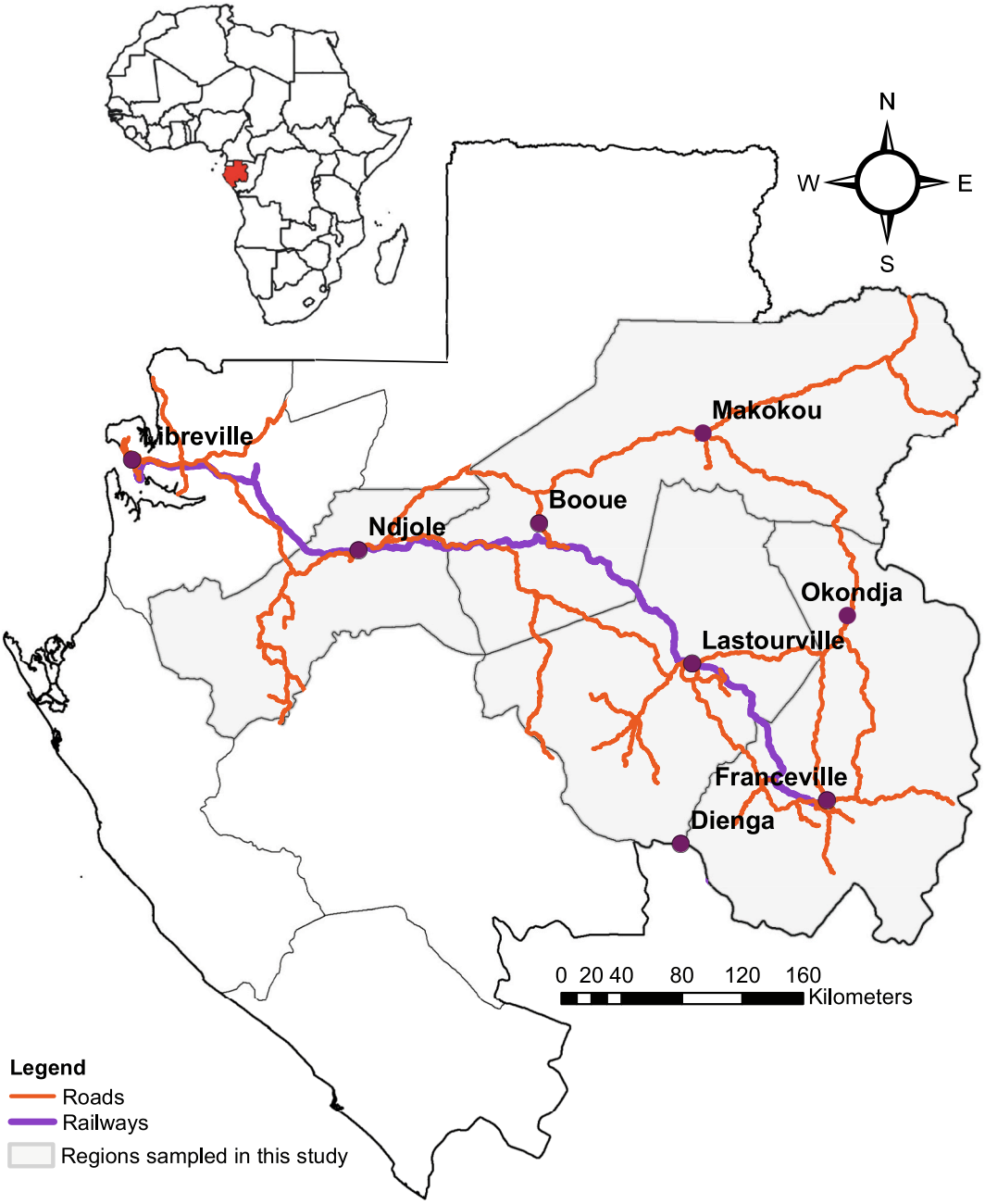

Fig. 1. Location of rodent sampling sites in Gabon. On this map, only the main roads (orange) in the sample area have been shown.

NoVs, SaVs and AstVs are among the most common causes of foodborne outbreaks of non-bacterial diarrhea and viral acute gastroenteritis therefore are interest for public health (Segone, 2020, Ibrahim et al., Ibrahim et al., 2022, Kowada et al., 2018, Finkbeiner et al., 2009). In Gabon, AstVs have been widely studied in animals including rodents (Mombo et al., 2019). By contrast, there are no data regarding the diversity and the circulation of CaVs in rodents specifically NoVs and SaVs in this country. Thus, this study aimed to screen caliciviruses and astroviruses in rodents from various locations from Gabon to characterize their diversity and to determine whether they can harbor strains capable of infecting humans.

2. Materials and methods

2.1. Ethical statement

Rodent trapping campaigns were carried out following authorization by the Commission Scientifique d'Examen and the application for an Exploration Licence by Gabon (N°AR005/20/MESRSTT/CENAREST/CG/CST/CSAR). The capture, handling and euthanasia of animals and the transfer of samples from one country to another, were carried out in accordance with the guidelines of the American Society of Mammalogists (http://www.mammalsociety.org/committees/animal-care-and-use) and in strict compliance with the recommendations of the National Ethics Committee of Gabon (authorization N°PROT/0020/2013I/S G/CNE).

2.2. Study areas and capture

Between October 2021 and June 2022, sampling of small terrestrial rodents was carried out in several rural areas in Gabon (Hereafter referred as Dienga, Okondja, Lastourville, Booué and Ndjolé, Fig. 1). Inside each of these areas, several sampling sessions were performed in different villages (Table 1). Two types of traps were used: Sherman traps and Tomahawk traps (Granjon and Duplantier, 2009). The traps were set in houses and in the forest (Dienga and Ndjolé only) for three consecutive nights. Rodents caught were sacrificed by cervical dislocation, then measured, sexed and dissected. Tissues (brain, lung, intestine, liver) and blood were collected for the detection of pathogens circulating among small mammals. Samples were then and stored in liquid nitrogen until storage in −80 °C freezer for further molecular analyses at CIRMF.

2.3. Rodent species identification

Rodent species were identified by morphometric (Granjon and Duplantier, 2009) and genetic tools (partial *cytochrome b* Sanger sequencing) (Nicolas et al., 2012) to avoid any ambiguity in the identification. Due to the existence of cryptic species in Central African rodents, care was taken to identify most, if not all, of the small mammals captured by molecular analyses, using kidney or phalanx samples.

2.4. RNA extraction and reverse transcription

Of the 717 small mammals captured and for costs limitations a subsample of 245 rodents was chosen to test for the presence of calicivirus and astrovirus. For this purpose, we chose to keep all the rodents captured in Dienga because of the cohabitation between native and invasive species, with a view to determining the exchange of enteric viruses between these two groups of rodents. Finally, we randomly sampled a subset of *Rattus rattus* in the other localities. Approximately 100 mg of intestine of each individual was grinded in 500 µl of cold phosphate- saline buffer (PBS), as previously described by Rougeron et al., 2016 (Rougeron et al., 2016). RNA was extracted from the suspension using the RNA isolation kit Nuclespin® RNA, Macherey-Nagel (Düren, Germany), according to the manufacturer's instructions. The extracted RNA was then reverse-transcribed into cDNA using the Superscript IV Reverse Transcriptase (Invitrogen, Illkirch, France) in a final volume of 20 µL. Specifically, 10 µL of RNA in a mix consisting of 1 µL dNTPs, 1 µL of random hexamer and 1 µL of DNase-free water, was incubated at 65 °C for 5 min followed by 1 min on ice. A second mixture consisting of 4 µL of 5× Superscript IV Buffer, 1 µL of DTT (100 mM), 0.5 µL of RNAse Out, 0.5 µL of enzyme and 1 µL of DNAse-free water, was added to the previous mixture. The reverse-transcription program was set to 23 °C for 10 min, 50–55 °C for 10 min, and 80 °C for 10 min.

Table 1
Small mammal species sampled in five locality rural habitats and forest of Gabon.

Localities	Number sites	Number house	Trap nights	Total Captures
Booué	5	42	1314	99
Dienga	1	94	1143	149
Lastourville	5	37	886	122
Ndjolé	12	44	1344	165
Okondja	4	44	714	182
Total	27	261	5401	717

2.5. Calicivirus and astrovirus detection

Caliciviruses and astroviruses were screened respectively by PCR and nested PCR using Platinum Taq DNA Polymerase (Invitrogen) according to the manufacturer's instructions with primers targeting a fragment of (319–331) bp of the RdRp of CalVs and a fragment of 422 bp of the RdRp of AstVs (Table 2). For CalCVs the PCR mix was performed in a final volume of 25 µl, consisting of 5 µL of cDNA, 2.5 µL of 10 X reaction buffer, 0.75 µL of MgCl2 (50 mM), 0.5 µL of dNTPs, 1 µL of bovine serum albumin (BSA) (1 µg/µL), 1 µL of each primer (10 µM), and 0.1 µL of Platinum Taq Enzyme. Similarly, for the detection of AstVs, both rounds of the nested PCR, were performed with Platinum Taq DNA polymerase using the same components and volumes used for CaClVs with the exception of BSA (not included) in the second round. Amplification programs for AstVs and CaVs were set up as previously described (Jiang et al., 1999; Mombo et al., 2019), without the reverse-transcription step for the first round. The PCR products were visualized on a 1.5% agarose gel after electrophoresis and the amplicons were sent to Eurofins Genomics (Ebersberg, Germany) for Sanger sequencing in both directions.

2.6. Phylogenetic analyses

Phylogenetic analyses were performed on the RdRp gene sequences obtained in this study (Genbank accession numbers PP516503 to PP516520) with those of AstVs referenced on Genbank. Sequences were aligned using the ClustalW algorithm implemented in MEGA11 software package. Phylogenetic trees were constructed by Maximum likelihood (freely available at http://phylogeny.lirmm.fr) (Dereeper et al., 2008) using Kimura 2-parameter + Gamma distribution (K2 + G) estimed with MEGA11 (Anisimova and Gascuel, 2006) and 100 bootstrap were used to test for the robustness of each node in the tree.

2.7. Statistic analyses and locality mapping

Statistical analyses were performed using R software (v.3.2.1). A chi-square test was performed to determine the influence of sex and age on AstV infection. Maps were generated using ArcGIS 10.3 software (Environmental Systems Research Institute).

3. Results

3.1. Rodent trapping

A trapping effort of 5401 trap.nights allowed the capture of 717 individuals (Table 1). The 245 rodents analysed belong to eleven species

Table 2
Sequences of primers used for the detection of *Astroviruses* and *Caliciviruses*.

Target Virus Name	Gene	Primer Name	Primer Sequence (50–30)		References
Astrovirus	RdRp	FWD1	GARTTYGATTGGRCKCGKTAYGA		
		FWD2	GARTTYGATTGGRCKAGGTAYGA	422 bp	(Chu et al., 2008)
		RVS1	GGYTTKACCCACATNCCRAA		
		FWD3	CGKTAYGATGGKACKATHCC		
		FWD4	AGGTAYGATGGKACKATHCC		
Calicivirus	RdRp	P289	TGACAATGTAATCATCACCATA	319–331 bp	(Jiang et al., 1999)
		P290	GATTACTCCAAGTGGGACTCCAC		

already described in Gabon (Mangombi et al., 2021; Duplantier, 1982; Duplantier, 1989; Nicolas et al., 2012). The species *Rattus rattus* was found in all localities: Booué 24.2% (24/99), Dienga 65.8% (98/149), Lastourville 18.8% (23/122), Ndjolé 23% (23/113) and Okondja 15.9% (29/182). In contrast, the 48 native rodents captured in this study came from two localities: Dienga ($N = 46$) and Lastourville ($N = 2$). The native rodents captured in Dienga were: *Hybomys univittatus* ($N = 6$), *Mus minutoides* ($N = 3$), *Hylomyscus aeta* ($N = 1$), *Lophuromys roseveari* ($N = 15$), *Malacomys longipes* (N = 2), *Mylomys* cf. *dybowskii* (N = 1), *Oenomys hypoxanthus* (N = 2), *Praomys petteri* (N = 1), *Praomys jacksoni* ($N = 9$) and *Lemniscomys striatus* (N = 6). In Lastourville, two native rodents: *Praomys jacksoni* (N = 1) and *Lemniscomys striatus* (N = 1) were found (Table 3).

3.2. Caliciviruses and astroviruses detection

A total of 245 intestine samples were tested for the presence RNA of CalCVs and AstVs. None of the samples were tested positive for CalCVs, while AstVs were detected in 18 samples (detection rate of 7.3%). According the rodent species, *R. rattus* species was the one species tested positive and found in every locality with detection rates according the localities as 21.7% (5/23) in Lastourville, 16.0% (4/25) in Booué, 13.8% (4/29) in Okondja and 13.0% (3/23) in Ndjolé. No factor significantly influenced astrovirus infection: males with 9.8% (12/122) had an infection rate close to that of females 4.9% (6/123; $\chi^2 = 1.5434$, *p*-value = 0.2141). Similarly, adult *Rattus rattus* had an AstV infection rate of 7.7% (10/130) similar to the 7.0% (8/115) found in juveniles (χ^2 = 8.6037e-31, p-value = 1).

3.3. Phylogenetic analyses

In order to describe the genetic diversity of AstV strains detected in *R. rattus* in this study, the partial RdRp sequences were compared to reference sequences retrieved from Genbank. Our results showed that all the sequences of this study clustered with other sequences of AstVs detected in rodents (Fig. 2). Specifically, phylogenetic analyses showed that some sequences are specific to Gabon. Indeed, five sequences including three from rats trapped in Lastourville (RLT-103, RLT-114, RLT-118), two from Booué (RB-078, RB-082) clustered with AstVs previously described in rats from Franceville (Genbank accession numbers MF741182, MF741183 and MF741185) (Mombo et al., 2019). The sequences shared nucleotide identities ranging from 89% to 100%. In addition, an AstV sequence found at Dienga (RD-083) was phylogenetically divergent (89% nucleotide identities) from the AstV sequences grouped within this group. Five other sequences (ROK-023, RB-095, RB-098, NDJ-149 and NDJ-164) from rats trapped in Okondja, Booué and Ndjolé, clustered with a *R. rattus*-related AstV (MF741187) previously detected in Makokou. These sequences formed the second clade of rodent-borne AstVs sequences from Gabon that shared 90 to 99% nucleotide identities. Besides, the phylogenetic analyses also showed that some sequences are grouped with AstVs identified outside Gabon. Indeed, three sequences (RD-011, RLT-110 and RLT-112) clustered with sequences of AstVs detected in *Rattus rattus* in Kenya and in Reunion free tailed bats (Joffrin et al., 2021). Despite, the sequences were divergent showing nucleotide identities ranged from 85% to 100% (Fig. 2). Moreover, two sequences, one from Ndjolé (NDJ-061) and the other from Okondja (ROK-136), were grouped with AstV sequences found in China in *Rattus tanesumia*, *Rattus norvegicus*, *Rattus losea* (Genbank accession numbers OP482212, KY370002 and HM450382). These sequences showed 88–100% nucleotide identity). Finally, the last two sequences (ROK-041 and ROK-080) also clustered with sequences of AstVs detected in China in various species of *Rattus* genus such as *R. norvegicus*, *R. tanezumi* but also from the insectivorous bat species *Myotis fimbriatus*. The sequences shared 89% to 93% nucleotides identities with sequences of AstVs detected in the other *Rattus* species (Fig. 2).

4. Discussion

Caliciviruses (CaVs, such as noroviruses and sapoviruses) and astroviruses (AstVs) are common causes of diarrhea in human populations (Finkbeiner et al., 2009). These viruses infect various vertebrate species including rodents. Rodents, especially in domestic and peridomestic contexts, could then disseminate these viruses and humans

Table 3
Results of screening for *Astroviruses* in the various locations sampled.

Rodent species	Booué	Dienga	Lastourville	Ndjolé	Okondja	Total
	Astrovirus					
	Positive/Tested					
Rattus rattus	**4/24 (16.7%)**	**2/98 (2%)**	**5/23 (21.7%)**	**3/23 (13%)**	**4/29 (13.8%)**	**18/197 (9.1%)**
Praomys jacksonni	–	0/9 (0%)	0/1 (0%)	–	–	0/10 (0%)
Praomys peteri	0/1 (0%)	–	–	–	–	0/1 (0%)
Mus minutoides	–	0/3 (0%)	–	–	–	0/3 (0%)
Hybomys univitattus	–	0/6 (0%)	–	–	–	0/6 (0%)
Hylomyscus aeta	–	0/1 (0%)	–	–	–	0/1 (0%)
Lophuromys roseveari	–	0/15 (0%)	–	–	–	0/15 (0%)
Lemniscomys striatus	–	0/6 (0%)	0/1 (0%)	–	–	0/7 (0%)
Mylomys dybowskii	–	0/1 (0%)	–	–	–	0/1 (0%)
Malacomys longipes	–	0/2 (0%)	–	–	–	0/2 (0%)
Oenomys hypoxanthus	–	0/2 (0%)	–	–	–	0/2 (0%)
Total	**4/25 (16.0%)**	**2/143 (1.4%)**	**5/25 (20%)**	**3/23 (13%)**	**4/29 (13.8%)**	**18/245 (7.3%)**

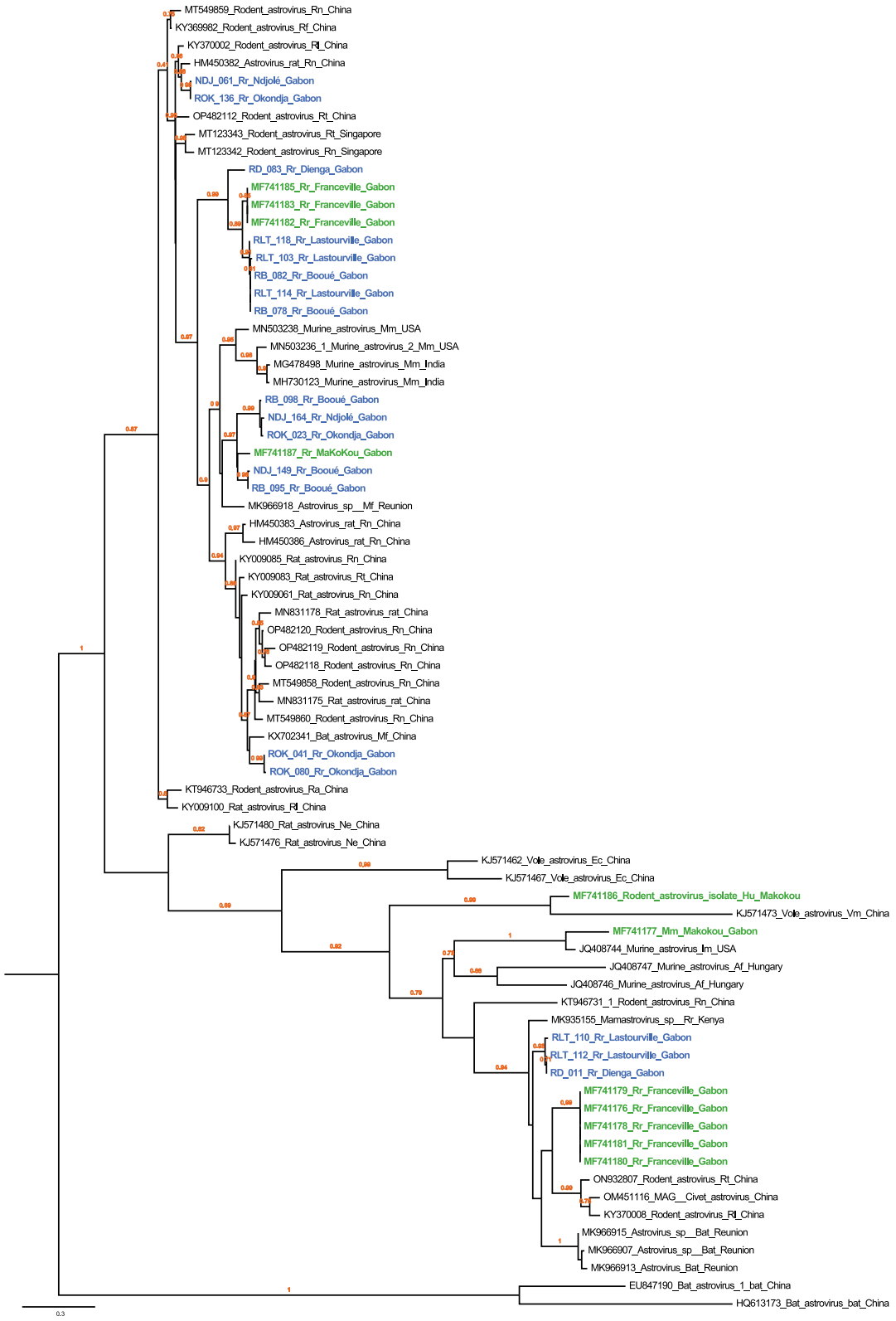

Fig. 2. Phylogenetic tree of rodent astroviruses (AstVs) based on approximately 360 bp of a fragment of the RNA-dependent RNA polymerase (RdRp) gene. Only rodent astroviruses were included since none of sequences fell into rodent AstVs. Only bootstrap values ≥70% were indicated at the nodes. The phylogenetic tree was constructed using Maximum Likelihood, (K2 + G) estimated, 100 bootstrap. Bat Astroviruses sequences (Genbank accession numbers EU847190 and HQ613173) were used as the outgroup. The rodent's and other animals species belonging to Rodent astroviruses groups are indicated using the following abbreviations: Af for *Apodemus flavicollis*, Ec for *Eothenomys cachinus*, Hb for *Hystrix brachyurus* Hu for *Hybomys univittatus*, Mf for *Myotis fimbriatus*, Mm for *Mus musculus*, Ne for *Niviventer eha*, Ra for *R. adamanensis*, Rn for *R. norvegicus*, Rf for *R. flavipectus*, Re for *R. exulans*, Rl for *R. losea*, Rt for *R. tanezumi*, Rr for *R. rattus* and Vm for *Volemys milliciens*. The blue color corresponds to the AstV detected in this study and the green color to the AstVs previously detected in Gabon. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

could be infected directly or indirectly via a zoonotic transmission (Milton et al., 2020). Little is known about the diversity and spillover potential of these viruses carried by rodents on a large scale in Gabon, particularly caliciviruses. In the 245 intestine samples from eleven rodent species trapped in forest and in human houses in five Gabonese localities and screened for the presence of CaVs and AstVs, only AstVs were detected in *R. rattus*. None of the samples tested were positive for CaVs. Among CaVs noroviruses are the one only detected in rodents. However most of the studies have focused on noroviruses due to their significance in public health, and to serve of as model to understand human infection (Manuel et al., 2018; Barclay et al., 2014). Rodent noroviruses were first identified in laboratory mice in 2003 and subsequently in other laboratory and also wild mice (Farkas et al., 2012; Karst et al., 2003). In addition to laboratory mice, NoVs have also been detected in wild rodent such as *R. rattus* and *Apodemus* spp., and recently in *R. norvegicus* (Tsunesumi et al., 2012; Farkas et al., 2012). All NoVs detected were specific to rodents, except in *R. norvegicus* in which human-related strains were also detected (Niendorf et al., 2021). The use of specific primers targeting NoV RNA in previous studies may have favored detection. Thus, the absence of detection in this study could be explained not only by the use of a more generic system, but also by the low number of samples tested.

In our study, 18 rodents (7.3%) were found positive for AstVs, which is a higher detection rate than in previous studies where AstVs were detected in rodents captured in Franceville and Makokou, two urban cities in Gabon (4.6%) (Mombo et al., 2019). In the previous study, AstVs were detected in three species *Hybomys univittatus*, *Mus musculus* and *R. rattus* while only *R. rattus* was found to be positive in our study. For the *R. rattus* species, the prevalence seemed to be relatively the same, i.e. 10.5% in the previous study and 9.1% in the present one. The absence of AstVs in other species could be explained by the small sample sizes obtained during trapping, as well as the fact that no other rodent species were captured in all localities except Dienga. Yet, our findings showed that the prevalence of astroviruses in Dienga was significantly lower than in other localities. The low prevalence of AstV in Dienga could be explain by several factors. Firstly, the sample size taken in this locality was larger than in the other sites. The discrepancy in sample size may have resulted in an underestimation of the true prevalence of infection. Furthermore, Dienga is a remote forested village with limited interactions with other localities. This characteristic could serve to limit the circulation of AstV in rodents in Dienga in comparison with other sites, which would also contribute to the low prevalence observed.

Concerning the age class, the results indicate that the prevalence of AstV between young and adult *R. rattus* does not differ. This suggests that age is not a significant factor in determining the prevalence of Astrovirus infection in *R. rattus* rodents. Nevertheless, studies in humans have demonstrated that astroviruses are responsible for gastroenteritis in young children and the elderly, with varying susceptibility depending on the age group (Korsman et al., 2014; Vu et al., 2017). Although astroviruses are known to affect children and the elderly in humans (Kurtz and Lee, 1978; Nguekeng Tsague et al., 2020), no study has demonstrated a difference in infection rates between the two age groups. It is unclear whether there is a difference in the rate of infection between young and adult mammals, particularly (Chu et al., 2010; To, K. K. W, et al., 2017). In light of the currently available data, it is not possible to draw any conclusions regarding the influence of age in astrovirus infection in rodents, further studies are then needed.

With regard to sex, although our results are not statistically significant, we nevertheless show that AstV appears to be more prevalent in males than females. This observation is in line with the fact male rats tend to persist longer in foraging areas than females, indicating a sex difference in foraging-related decision making startegies (Garcia et al., 2023).

Phylogenetic analyses based on the RdRp gene revealed that all sequences obtained in this study belong to the genus *Mamastrovirus* with host restriction, as these sequences fell into the large clade of rodent astroviruses. Moreover in this big clade, all *R. rattus* AstVs sequences always clustered with other rat AstVs. The phylogenetic analyses also revealed that some sequences were specific to Gabon. Firstly, some AstV sequences from rats captured in Lastourville and Booué were genetically close to AstVs previously described in *R. rattus* from Franceville (Mombo et al., 2019). Secondly, some other sequences from Booué, Ndjolé, and Okondja clustered with AstV sequences previously described in *R. rattus* captured in Makokou (Mombo et al., 2019). The phylogenetic groups correspond to the distribution of communities along the single line passing through Booué, Lastourville and Franceville, as well as one of the land routes that includes Ndjolé, Booué, Makokou and Okondja. These routes are crucial for transporting food and supplies to the country's various cities and communities. Therefore, these transport routes could play a significant role in the spread of AstVs, by facilitating the proliferation of rodents. This is consistent with studies conducted in Senegal, which suggest that road transport facilitates the spread of black rats between different areas, and even the dissemination of the pathogens they may harbor (Lombard, 2017; Lucaccioni et al., 2016). In Gabon, during the double epidemic of chikungunya and dengue fever between 2007 and 2010, the railroads may have contributed to the spread of chikungunya and dengue viruses, by enabling infected mosquitoes to spread more rapidly. Cases have appeared in communities located along the railway line over time (Nkoghe et al., 2012). Additionally, an AstV sequence from Dienga (RD-083) was highly divergent sharing 89% nucleotide identities with AstVs specific to Gabon from the AstV sequences grouped together in this group. This suggest that this could be a new genotype However, a complete analysis of the full genome and capsid is necessary to draw a conclusion.

Phylogenetic analyses also revealed that three sequences (RLT-110, RD-011 and RLT-112) grouped with AstVs of *R. rattus* identified in Kenya which formed a cluster phylogenetically close to AstVs of Reunion fruit bats. The detection of the AstVs strains in different host species (bats and rodents) but phylogenetically close indicates potential exchanges. In Reunion, for example, rodents are seen in the vicinity of caves where bat guanos have been tested positive (Joffrin et al., 2021). Rodents and bats also interact near fruit trees and dwellings, consequently such interactions could favor the exchange of AstVs. These findings raise questions about the nomenclature of AstVs, which are typically named after the host in which they were first detected, which therefore minimize the possibility of passive transmissions of the virus or external contamination. Consequently, Kerstin et al. (2016) recommended carrying out successful virus isolations in these species as evidence of productive infection (Fischer et al., 2016).

Finally, phylogenetic analyses showed that two sequences cluster with a groups of AstVs detected in diverse host species within the genus *Rattus* such as *R. rattus*, *R. norvegicus*, *R. tanezumi*, and *R. losea* from China (Wu et al., 2018). This finding suggests the intra-species transmission of AstVs within the *Rattus* genus that coevolved with their host. Wu et al. (2018) also suggested that frequent host shifts during the viral evolutionary history, which may have created opportunities for the emergence of new viruses that can adapt to new hosts (Wu et al., 2018).

5. Conclusions

This study did not detect CalVs in rodents and a high host restriction for AstVs, which were detected only in black rats *R. rattus*. The dissemination of AstVs could be facilitated by railroads and roads, a hypothesis that could be tested by screening samples from localities outside the main network of circulation. The detection of rodent AstVs phylogenetically close to those infecting bats suggests events of interspecies transmission. This finding challenges the current nomenclature of AstVs, which mainly focuses on the host species. Finally, AstVs may have evolved within the genus *Rattus*, allowing an adaptation to each host species. However, further studies based on the complete genome could bring more information regarding the evolution of rodent AstVs in rodent in Gabon.

CRediT authorship contribution statement

Clark Mbou-Boutambe: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Illich Manfred Mombo:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Virginie Rougeron:** Writing – review & editing, Resources. **Fanny Degrugillier:** Investigation. **Philippe Gauthier:** Investigation. **Boris Makanga:** Investigation. **Barthélemy Ngoubangoye:** Investigation. **Eric M. Leroy:** Investigation. **Franck Prugnolle:** Validation, Resources, Project administration, Funding acquisition. **Larson Boundenga:** Writing – review & editing, Validation, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author. The partial genome/coding sequences of study viruses can be found in the National Center for Biotechnology Information Database/ GenBank under the accession numbers PP516503 to PP516520. Access to the fasta files can be provided upon request.

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