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Research paper

Detection of novel astroviruses among rodents of Gabon, Central Africa

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ABSTRACT

Astroviruses (AstVs) are mostly responsible for mild to severe gastroenteritis infections in humans and animals. AstVs infect a wide range of host species, have a large genetic diversity with different circulating variants and are thus a high zoonotic risk for human populations. Among these host species, rodents are known to harbor several AstVs variants. Therefore, it is important to identify in rodent species which AstVs are circulating and evaluate their potential zoonotic risk for humans. In this context, this study aimed to screen the presence of AstVs in 267 rodents trapped in 2012 in Franceville and Makokou, two cities in Gabon. RNA extracted from grinded intestines were used for the screening of AstVs by amplification of a conserved region of the RNA dependent RNA polymerase. Results report the identification of AstVs in 12 individuals (4.6% rate), belonging to three different species including *Rattus rattus*, *Mus musculus* and *Hybomys univittatus*. These findings report the first identification of AstVs in *R. rattus* and *H. univittatus*. The phylogenetic analyses indicate host specificity of rodents AstVs. The absence of rodent AstVs within the human AstV clade suggests a low rate of interspecies transmission of these viruses and consequently a low zoonotic risk.

1. Introduction

Astroviruses (AstVs) (family *Astroviridae*) are small non-enveloped positive single stranded RNA viruses with an icosahedral capsid characterized by a star-like appearance when observed by electron microscopy (Madeley and Cosgrove, 1975). This family is divided into two genera *Mamastrovirus* and *Avastrovirus* which infect mammals and birds, respectively (Mendez and Arias, 2007). AstVs are characterized by a genome of approximately 6.8 to 7.9 kb in length with a poly-A tail and flanked at their 5′ and 3′ ends by untranslated regions (UTR) (Monroe et al., 1993). The genome codes three open reading frames (ORFs), ORF1a, ORF1b and ORF2 encoding the protease, the RNA-dependent RNA polymerase (RdRp) and the viral capsid protein, respectively (De Benedictis et al., 2011; Willcocks and Carter, 1993). AstVs have been identified in a large diversity of mammals such as humans, bovines, swine, bats, felines, rodents, as well as other mammal species (for review see (De Benedictis et al., 2011)) and their implication in zoonotic transfers has been recently highlighted to have occurred several times (Bosch et al., 2014). Human AstVs, divided into eight serotypes, are one of the main causative agents of mild to severe gastroenteritis in children (Bosch et al., 2014). Indeed, new AstVs, divergent from classic human AstVs and phylogenetically close to those infecting other animals, have been identified in humans such as the AstV-Human Mink Ovine group (named AstV-HMO) (Kapoor et al., 2009). These findings have raised several questions about the zoonotic origin of these AstVs, as animals may act as reservoirs for known human AstVs or be a source of new AstVs for humans (Rougeron et al., 2016). We currently need to improve our knowledge about the distribution and the diversity of AstVs, and identify animal reservoirs harboring AstVs that could be involved in zoonotic transfers.

Rodents represent an extremely diverse group of mammal species involved in zoonoses, with 2277 rodent species including 217 species that are reservoirs harboring 66 zoonosis caused by viruses, bacteria, fungi, helminthes and protozoa (Han et al., 2015). Among the viruses detected in rodents, AstVs were described for the first time in 1985 in nude mice with diarrhea (Kjeldsberg and Hem, 1985). Subsequently, several rodent AstVs have been described infecting laboratory, urban and wild rodents (Chu et al., 2010; Farkas et al., 2012). Since the discovery of diverse AstVs in rodents, researchers have suggested that a large diversity of AstVs could infect rodents (Chu et al., 2010). This has led to an increased interest about AstVs circulation in rodents that could be potentially transmitted to human populations.

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Table 1
Rate of detection of the AstV RdRp gene sequence in rodents collected in Franceville and Makokou, in Gabon. N represents the number of samples screened in this study for AstV and n (%) the number and the percentage of positive samples to AstV.

Rodent species	Franceville		Makokou	
	N	n (%)	N	n (%)
Rattus rattus	75	9 (12%)	20	1 (5%)
Mus musculus	–	–	28	1 (3.57%)
Mus monticola	–	–	1	–
Mus nannomys	4	–	9	–
Mus sp	–	–	3	–
Hylomyscus sp	–	–	25	–
Lophuromys sp	3	–	1	–
Praomys sp	13	–	44	–
Cricetomys emini	–	–	4	–
Deomys ferrugineus	6	–	–	–
Grammomys poensis	–	–	1	–
Hybomys univittatus	–	–	4	1 (25%)
Lemniscomys striatus	14	–	–	–
Malacomys longipes	–	–	3	–
Stochomys longicaudatus	4	–	–	–
Total	119	9 (7.56%)	143	3 (2.09%)

In this context, rodents were collected for the detection of AstVs in a global project interested in the detection of rodent-associated viral agents. A total of 262 rodents, trapped in 2012 in two localities, Franceville and Makokou, in Gabon, have been molecularly screened for AstVs.

2. Material and methods

2.1. Study area and samples collection

A total of 262 rodents (Table 1) were trapped in 2012 in two urban towns in Gabon, Makokou (n = 143) and Franceville (n = 119). Makokou (N 0°34′25″; E 12°51′51″) has a forest ecosystem while Franceville (S 1°37′59″; E 13°35′00″) has a mix of forest and savanna ecosystems. Rodents were trapped using Tomahawk and Sherman traps as described previously in (Duplantier, 1989), the traps being set inside and around human dwellings, in each city. After the euthanasia of each individual, identification of the rodent genus (and when possible species) was identified morphologically, and samples of different organs were collected (liver, spleen, kidney, lung, heart, intestine and brain), frozen and transported to the Centre International de Recherches Médicales de Franceville. Finally, the collected samples were stored at −80 °C until needed for molecular analyses.

Of the 262 rodents trapped, 119 rodents were trapped in Franceville that corresponded to at least seven species: *Rattus rattus* (n = 75), *Lemnicomys striatus* (n = 14), *Praomys sp.* (n = 13), *Deomys ferrugineus* (n = 6), *Mus nannomys* (n = 4), *Stochomys longicaudalis* (n = 4) and *Lophuromys sp.* (n = 3). In Makokou, 143 rodents were trapped that corresponded to a greater rodent species diversity with at least 12 species identified: *Praomys sp.* (n = 44), *Mus musculus* (n = 28), *Hylomyscus sp.* (n = 25), *R. rattus* (n = 20), *M. nannomys* (n = 9), *Cricetomys emini* (n = 4), *Hybomys univittatus* (n = 4), *Mus sp.* (n = 3), *Malacomys longipes* (n = 3), *Lophuromys sp.* (n = 1), *Grammomys poensis* (n = 1) and *Mus monticola* (n = 1). Four rodent species were observed in both cities, namely *R. rattus*, *M. nannomys*, *Lophuromys* sp. and *Praomys sp*.

2.2. Ethical approval

The study was conducted outside of protected areas in Gabon. Rodent trapping and sampling were conducted with the approvals of the Wildlife and Hunting Department of the Gabonese Ministry of Water and Forestry (N°003/MEFE-PA/SG/DGEF/DCF and N°0021/MEFE-PA/SG/DGEF/DCF). All the capture events, animal handling, euthanasia and transfer of samples across country borders were performed in accordance with the guidelines of the American Society of Mammalogists (http://www.mammalsociety.org/committees/animal-care-and-use).

2.3. RNA extraction and RNA-dependent RNA polymerase gene amplification by RT-PCR

Approximately 100 mg of intestine of each individual was grinded in 500 µl of cold phosphate-buffer saline as previously described by Rougeron et al., 2016 (Rougeron et al., 2016). RNA was then extracted from 100 µl of the suspension with 300 µl of lysis buffer after incubation at room temperature for ten minutes, using the EZ1 RNA tissue Mini kit (Qiagen, Hilden, DE), following the procedure provided by the manufacturer. AstVs screening was performed by amplifying (approximately) the 422 bp fragment of a conserved region of the RNA-dependent RNA polymerase (RdRp) gene through a hemi-nested reverse-transcription (RT)-PCR using degenerate primers situated at 3583 to 4001 nucleotides in the Human AstV1 Dresden strain (AY720892). Positive amplicons were sent to a dedicated laboratory for sequencing to obtain the sequences in both directions (Seqlab GmbH, Germany).

2.4. DNA extraction and rodent host species confirmation

The determination of host species was performed only for AstVs positive samples. DNA was extracted from grinded liver suspended in cold phosphate buffer saline using the DNeasy blood and tissue kit (Qiagen) according the manufacturer's procedure. A fragment of the mitochondrial DNA 16s was amplified as previously described (Boessenkool et al., 2012). Sequencing of positive samples was performed by Eurofins MWG.

2.5. Phylogenetic analyses

To genetically determine whether AstVs of this study were close to human or any known AstV, the sequences obtained in this study were compared to a dataset of complete RdRp sequences of all representative strains available in Genbank using the basic local alignment tool (BLAST). Then multiple alignments of sequences were performed using the ClustalW algorithm implemented in MEGA7 software package (Kumar et al., 2016). Phylogenetic trees were constructed by Maximum likelihood (freely available at www.phylogeny.fr bioinformatics platform (Dereeper et al., 2008)) using the GTR of branch support (Anisimova and Gascuel, 2006) and 1000 bootstrap replicates.

All sequences from this study have been deposited in Genbank under accession numbers MF741176 to MF741187 for AstVs and MK098130 to MK098141 for host species.

3. Results

Overall, 12 of the 262 rodents tested were positive for the presence of the AstV RdRp gene (overall mean detection rate of 4.58%). We observed a higher detection rate in Franceville with nine positive samples (mean detection rate of 7.6%) in comparison to Makokou with three positive samples (mean detection rate of 1.2%). Based on 16S PCR sequencing, the AstV positive rodent samples corresponded to three different rodent species. Specifically, the nine positive samples detected in Franceville all belonged to the species *R. rattus*, whereas the three positive samples from Makokou belonged to three different rodent species: *Hybomys univittatus*, *Mus Musculus* and *Rattus rattus* (Table 1). This is the first report describing *R. rattus* and *H. univittatus* infected by AstVs.

To genetically characterize AstVs circulating among these rodent species of Gabon, the 386 bp fragment of the RdRp of each positive sample was compared to all representative sequences of AstVs strains

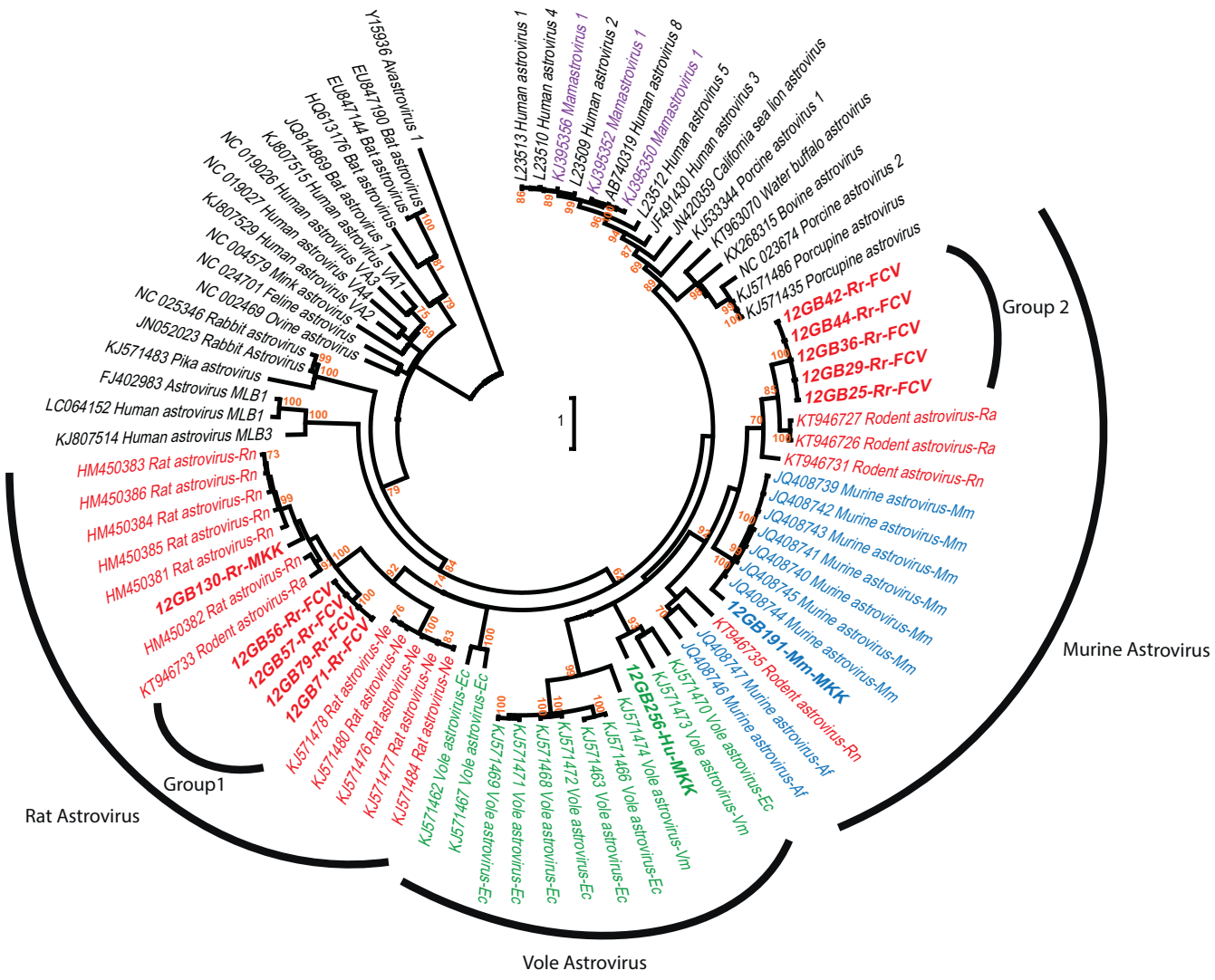

Fig. 1. Maximum-likelihood phylogenetic tree of AstV strains based on the analysis of approximately 386 nucleotides of the RdRp representing almost all groups of *Mamastrovirus* genus using Genbank accession numbers. Only boostraps values ≥70% were indicated above the branches. The scale bar indicates the number of substitution per sites. Sequences obtained in this study are indicated in bold. The rodent's species are indicated under the following abbreviations: Af for *Apodemus flavicollis*, Ec for *Eothenomys cachinus*, Mm for *M. musculus*, Ne for *Niviventer eha*, Ra for *Rattus adamanensis*, Rn for *R. norvegicus*, Rr for *R. rattus* and Vm for *Volemys milliciens*, Hu for *H. univittatus*. Red color is for AstVs detected in rats, green for voles and blue for mice, those detected in this study are indicated in bold. The purple color represents AstVs detected in humans in Gabon. Sample sites are also indicated under MKK for Makokou and FCV for Franceville. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

available in Genbank using BLAST. Phylogenetic analyses showed that the 12 AstV sequences obtained in this study fell into the *Mamastrovirus* genus and clustered within the murine, rat or vole AstVs (Fig. 1). Even though all rodents were trapped in or around houses, no human AstVs were detected. AstV sequences obtained in this study were highly divergent compared to the other mammalian AstVs (from pika, rabbit, feline, ovine, mink, bat, etc…), sharing 50% to 68.5% amino acid identity.

The phylogenetic tree showed that the AstV detected in the *H. univittatus* clustered within the vole AstVs clade detected in China (Hu et al., 2014), between the AstVs of *Eothenomys cachinus* (accession number KJ571473) and of *Volemys millicens* (accession number KJ571470) species. This AstV sequence shared 80.4% and 82.6% amino acid identity with these two species of vole, respectively. The AstV RdRp sequences obtained in the rodent species *M. musculus* from Makokou clustered within the laboratory mice AstVs group from Hungary and USA (Farkas et al., 2012), and shared from 88% to 89.1% amino acid identity. Finally, the ten AstVs sequences detected in *R. rattus* (one positive AstV from Makokou, and 9 from Franceville), clustered with diverse rodent AstVs. The AstVs sequences of *R. rattus* detected in Franceville were highly divergent (64.1% amino acid identity). These sequences could be separated into two groups such as Group 1 (four sequences) and Group 2 (five sequences). The sequences of Group 1 clustered within the Rat AstV, composed of AstVs infecting rodents of the *Rattus* genus such as *R. adamanensis* and *R. norvegicus* (78 to 79.1% amino acid identity) (Fig. 1). In this group, *R. rattus* AstVs were specifically close to AstVs detected in *R. adamanensis* that is considered as a novel astrovirus genotype species (To et al., 2017) (92.4% amino acid identity). Finally, the only AstV sequence of *R. rattus* detected in Makokou, belonged to the Rat AstV group, close to AstVs detected in *R. norvegicus* in China (Chu et al., 2010) (85.1 and 88% of nucleotide identity). Whereas sequences of Group 2 clustered within a Murine AstV group composed of multiple *Muridae* species (Fig. 1).

4. Discussion

Since the detection of AstVs in children (with a prevalence of 6.3%) in Gabon (Lekana-Douki et al., 2015), the only data available

concerning their circulation is from bats (with a prevalence of 4.57%). In order to improve our knowledge about AstV circulation and diversity in wild animals in Gabon, 262 rodents collected in two different cities in 2012 (Franceville and Makokou) were screened for AstVs. Overall 12 (2.36%) rodent specimens tested positive for AstVs, including nine from Franceville all from the species *R. rattus* and three from Makokou corresponding to three different species *R. rattus*, *M. musculus* and *H. univittatus*. AstV detection rates obtained in Franceville and Makokou were 7.56% and 2.09%, respectively. The absence of detection of AstV sequences in the other rodent species in our study could be explained by the low sample size obtained during trapping. Indeed, the trapping of rodent species in other cities of the Gabon, and a greater sample size may give a better estimation of the detection rate. Even though, AstVs have been already identified in different rats, mice and vole species (Chu et al., 2010), this is the first report of AstVs circulating in *R. rattus* and *H. univittatus*. Rodents of *Mus musculus* species were not obtained in Franceville in this study contrary to the study of the lymphocytic choriomeningitis virus (N'Dilimabaka et al., 2015). This may be explained by the shorter duration of trapping (one year versus three in Ndilimabaka et al. study).

The phylogenetic analyses based on the RdRp partial gene showed that the diversity of AstVs circulating among rodents in Gabon tend to be species specific. Indeed, the sequence of AstV detected in *H. univittatus* clustered with those of two other vole species, namely *E. cachinus* and *V. milicens*, with 80.4 and 82.6% amino acid identity. These findings suggest the potential existence of a novel AstV clade circulating specifically in this rodent host species. Concerning the AstV detected in *M. musculus*, the sequence clustered with AstVs infecting *M. musculus* laboratory mice. With an amino acid identity varying between 88 and 89.1%, this could suggest the existence of a new variant in *M. musculus*. Further studies need to be conducted in the future, by generating the full genomes of these AstV, in order to evaluate these two observations.

Phylogenetic analyses also revealed that AstVs sequences that were detected in *R. rattus* in Franceville could be separated into two different groups (Fig. 1). Each group is composed of AstVs discovered in *R. rattus* hosts trapped in the same area. The two groups are separated from one another by several kilometers, which could be explained by several factors related to *R. rattus*' lifestyle and ecology (Corbet, 1977). Indeed, *R. rattus* lives in social groups with a small home range (100 m^2 approximately) and have a polygynous mating system. The fecal-oral AstVs transmission route means an AstV strain could be maintained within different rodent populations generating after several virus life cycles different genetically distinct clades.

Recently, novel AstV genotype species have been characterized in different wild and urban rodent species in China (Chu et al., 2010). Phylogentic analyses revealed that AstVs of Group 1 and Group 2 were related to AstVs detected in *R. adamanensis* and/or *R. norvegicus* in China. Currently, more information on the complete capsid gene is needed to determine whether the AstVs groups 1 and 2 are novel genotype species.

5. Conclusions

In conclusion, the identification of such divergent AstVs increases our current knowledge about their diversity in rodents. Among the 12 AstVs detected in rodents from Gabon, we identified probable new variants that need to be confirmed through the sequencing of their full genomes. Moreover, the species-specificity of rodent AstV detected in our study suggests a low rate of interspecies transmission and thus a low zoonotic potential risk. Overall, these results are valuable for a general knowledge of these AstVs circulation in rodent species and to evaluate their potential transmission to human populations.

Declaration of interest

None.

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