

# Detection and phylogenetic characterization of astroviruses in insectivorous bats from Central-Southern Italy

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## Abstract

In recent years, bats have been found to harbour many viruses, raising several questions about their role as reservoirs and potential disseminators of zoonotic viruses. We investigated the presence of six virus families in bats in three regions of Central-Southern Italy. Astroviruses were identified in seven of 13 bat species. Sequence analysis revealed marked genetic heterogeneity among the astroviruses identified, with nucleotide identity ranging between 60.26% and 87.62%. Astrovirus diversity was not associated with the bat species, the geographic areas or the bat colony, suggesting the circulation of several astrovirus strains in Italian ecosystems. Genetic diversification and interspecies transmission appear common in bat astroviruses and could provide, potentially, the bases for transmission to humans and other mammals. Yet overemphasizing this risk might have detrimental consequences for bat conservation and preservation of the important ecosystem services bats provide.

## KEYWORDS

astrovirus, insectivorous bats, phylogenetic analysis

## 1 | INTRODUCTION

As the majority of human emerging infectious diseases are zoonotic, many efforts have been made to identify the animal species that may act as potential reservoir of zoonotic viruses (Olival et al., 2017). Bats (*Chiroptera*) are regarded as potential reservoirs or source of zoonotic infections, as they sum up a number of traits, including (a) an outstanding variety of species (Tsang, Cirranello, Bates, & Simmons, 2016); (b) gregarious habits (with colonies that often comprise hundreds or thousands of individuals); (c) resistance to viral infections, as they often harbour viruses without clinical signs (Zhang et al., 2013); and iv) high mobility, in some cases represented by long-distance migratory movements (e.g. Flaquer et al., 2009).

To date, over 60 distinct viruses have been detected in bats, suggesting that these mammals can act as frequent viral hosts (Brook & Dobson, 2015; Fischer et al., 2016; Lelli et al., 2015; Liang et al., 2017; Smith & Wang, 2013). Identified viruses belong to the following families: *Adenoviridae*, *Astroviridae*, *Bunyaviridae*, *Caliciviridae*, *Circoviridae*, *Coronaviridae*, *Filoviridae*, *Flaviviridae*, *Hepeviridae*,

*Herpesviridae*, *Orthomyxoviridae*, *Papillomaviridae*, *Paramyxoviridae*, *Parvoviridae*, *Picornaviridae*, *Polyomaviridae*, *Poxviridae*, *Reoviridae*, *Retroviridae*, *Rhabdoviridae*, *Totiviridae* (Chen, Liu, Yang, & Jin, 2014). Virological investigations worldwide have identified in bats zoonotic pathogens like Lyssavirus (Family *Rhabdoviridae*), Nipah and Hendra viruses (*Paramyxoviridae*), Ebola and Marburg viruses (*Filoviridae*), beta coronaviruses, including SARS-like CoV and MERS-like CoV, astroviruses, orthoreoviruses (Calisher, Childs, Field, Holmes, & Schountz, 2006; Fischer, Pinho Dos Reis, & Balkema-Buschmann, 2017; Lelli, Moreno, et al., 2013; Lelli, Papetti, et al., 2013), Melaka virus (Chua et al., 2011). Spillover events from bats to human can happen directly or indirectly via intermediate hosts (domestic or wildlife animals). It should be stressed, however, that direct bat-to-human transmission of viruses causing highly pathogenic disease has been demonstrated only for rabies viruses and related lyssaviruses, and for Nipah and Melaka viruses, whilst for other viruses (e.g. SARS-CoV), transmission has been only supposed (Calisher et al., 2006; Chua et al., 2011; Guan et al., 2003). Evaluating this potential

risk is crucial in Western countries. Several bat species avoid human settlements and have little chance to enter into contact with humans (Russo & Ancillotto, 2015), and other species are at risk of extinction (e.g. Conenna, Rocha, Russo, & Cabeza, 2016; O'Shea, Cryan, Hayman, Plowright, & Streicker, 2016), making the risks negligible. However, increasing anthropization of the environment is altering the ecosystems, disrupting usual habitats and/or creating novel ecological niches that may overlap with human activities. It should be considered, however, that bats provide important ecosystem services (Aizpurua et al., 2018; Ancillotto et al., 2017; Boyles, Cryan, McCracken, & Kunz, 2011; Kunz, Braun de Torrez, Bauer, Lobova, & Fleming, 2011). Overemphasizing the potential risks posed by bats to humans may generate unjustified alarmism, a fact that has raised considerable concern in conservationists (López-Baucells, Rocha, & Fernández-Llamazares, 2017), as also highlighted by the EUROBATS agreement on the conservation of bat populations in Europe (working group on "Communication, Bat Conservation and Public Health"). Under this perspective, surveillance may still be important to avoid or mitigate potential conflicts and eventually improve bat conservation policies.

In Italy, a few studies have been carried out to assess the presence of viruses in bats (Lelli, Moreno, et al., 2013; Lelli, Papetti, et al., 2013) but only in the Northern regions. These studies detected orthoreoviruses and coronaviruses in various bat species. However, there are no data for the Central-Southern Italian regions, which are characterized by a warmer, drier climate and a different biogeography.

In this study, we screened 13 bat species living in these geographic areas. The bat species were selected ad hoc to represent a range of environmental and behavioural differences. Our data set covered species that form large colonies in caves and show high fidelity to their roosting sites (e.g. *Miniopterus schreibersii*); species that roost in trees in small groups and switch roosts frequently (e.g. *Barbastella barbastellus*); and synanthropic species that are more likely to enter into contact with humans (e.g. *Pipistrellus kuhlii*). The bats were screened for a large panel of viruses, including rabies viruses, coronaviruses, reoviruses, caliciviruses, astroviruses and enteroviruses.

## 2 | MATERIALS AND METHODS

### 2.1 | Sample collection

We overall sampled 147 individuals from three regions of Central-Southern Italy. Most bats we screened were caught on emergence from their roosts with harp traps or at drinking sites with mist nets. In such cases, bats were promptly removed from the trap or the net and their forearm length and body mass were measured, respectively, with a caliper to the nearest 1 mm and a digital scale to the nearest 0.1 g. Sex was assessed by inspecting genitalia (Racey, 1988), and wings were trans-illuminated to distinguish juveniles from adults (Anthony, 1988). In females, we ascertained pregnancy by palpation (Racey, 1988), and lactation by the presence of

### Impacts

- Identification of astroviruses in four bat species never found positive before.
- Identification of astrovirus in bats living in Italy.
- Strains of astrovirus identified revealed a high degree of genetic diversity, not related to bat species.
- Mechanisms other than host specificity may drive virus genetic diversification.

enlarged nipples surrounded by a hairless skin area and by extruding milk with a gentle finger pressure on the nipple base. Bats were immediately released after processing. A few samples came from animals hosted at rehabilitation centres. Saliva was sampled from each bat with a dry sterile swab, which was placed in viral transport medium (Chu, Poon, Guan, & Peiris, 2008), and transported in a cool box to the laboratory for the analysis. Bat droppings expelled during manipulation of specimens were collected, stored in sterile vials and preserved in cool boxes during transportation.

Sampling was carried out in Abruzzo, Lazio and Campania regions. Species identification was carried out by a bat taxonomist, and the bat species included *Barbastella barbastellus*, *Eptesicus serotinus*, *Miniopterus schreibersii*, *Myotis capaccinii*, *Myotis emarginatus*, *Myotis myotis*, *Myotis mystacinus*, *Myotis nattereri*, *Nyctalus leisleri*, *Pipistrellus kuhlii*, *Pipistrellus pipistrellus*, *Plecotus auritus* and *Rhinolophus euryale*.

### 2.2 | Nucleic acid extraction

Nucleic acids were extracted with the MagMax automated system (Applied Biosystems, Monza, Italy). Oro-pharyngeal swabs were suspended in 0.4 ml of phosphate-buffered saline (PBS) and incubated at room temperature for 30 min under shaking. After incubation (0.3 ml), samples were loaded in the MagMAX extraction sample plate. Faecal samples were suspended in 1 ml PBS, vigorously vortexed for 3 min and centrifuged at 17,949 g for 3 min, and faecal suspension was loaded on the MagMax plate. RNA was extracted from all the samples following the manufacturer's instructions, and nucleic acids were eluted in 90 µl elution buffer containing 40 µl Rnase Inhibitor (Promega, Milan, Italy) and immediately analysed by real-time RT-PCR/RT-PCR or stored at -80°C until use.

### 2.3 | Real-time RT-PCR for the detection of rabies virus and MERS-CoV

The presence of rabies virus and MERS-coronavirus (MERS-CoV) was investigated by real-time RT-PCR using two commercial kits, that is Rabies virus Real Time RT-PCR kit (Shanghai ZJ Bio-Tech Co., Ltd) and MERS-CoV Real Time RT-PCR kit (Shanghai ZJ Bio-Tech Co.,

Ltd). The reaction was carried out following the manufacturer's instructions. All the tests were performed with a 7500 Fast Real time PCR system (Applied Biosystems, Monza, Italy). Positive and negative controls were included in the kits.

## 2.4 | Identification of other viruses by end-point RT-PCR

The primers employed for detection of the different viruses are indicated in Table 1. All the assays were carried out with the Flexid Mastercycler NexusX2 (Eppendorf) using the SuperScript™One-Step RT-PCR kit (Life Technologies Italia). Coronaviruses (CoVs) were searched as described by Drosten et al. (2003) (Table 1). For reovirus detection, the nucleic acids were preventively denatured (2 µl viral extract with 1.4 µl of DMSO at 97°C for 5 min) and then reverse-transcribed and amplified using a nested protocol, as described previously (Leary et al., 2002; Table 1). For the detection of calicivirus RNA, a set of calicivirus universal primers was used, targeting conserved motives of the RNA-dependent RNA polymerase (RdRp; Jiang et al., 1999; Zintz et al., 2005; Table 1). Detection of astroviruses (AstVs) was performed with a nested protocol using a set of conserved primer targeting the RdRp region (Chu et al., 2008) using 1 µl of RNA (Table 1). Screening for enteroviruses was carried out using a nested protocol (Iturriza-Gomara, Megson, & Gray, 2006). The primer details and sequence are listed in Table 1. Positive controls for the various PCR assays included human (Mamastrovirus species 1) and canine astrovirus (Mamastrovirus species 5), bovine and canine CoVs and canine reovirus strains. Members of the *Norovirus*

(genogroups II and IV) and *Vesivirus* genus (feline and canine strains) were used as positive control for the calicivirus PCR. Human enterovirus A (EV71), bovine enterovirus E and swine enterovirus G were used as positive controls for enterovirus PCR.

All the PCR products were analysed by Tape Station 2200, an automated platform for electrophoresis, (Agilent Technologies), using the D1000 screentape system.

## 2.5 | Sequence and phylogenetic analysis

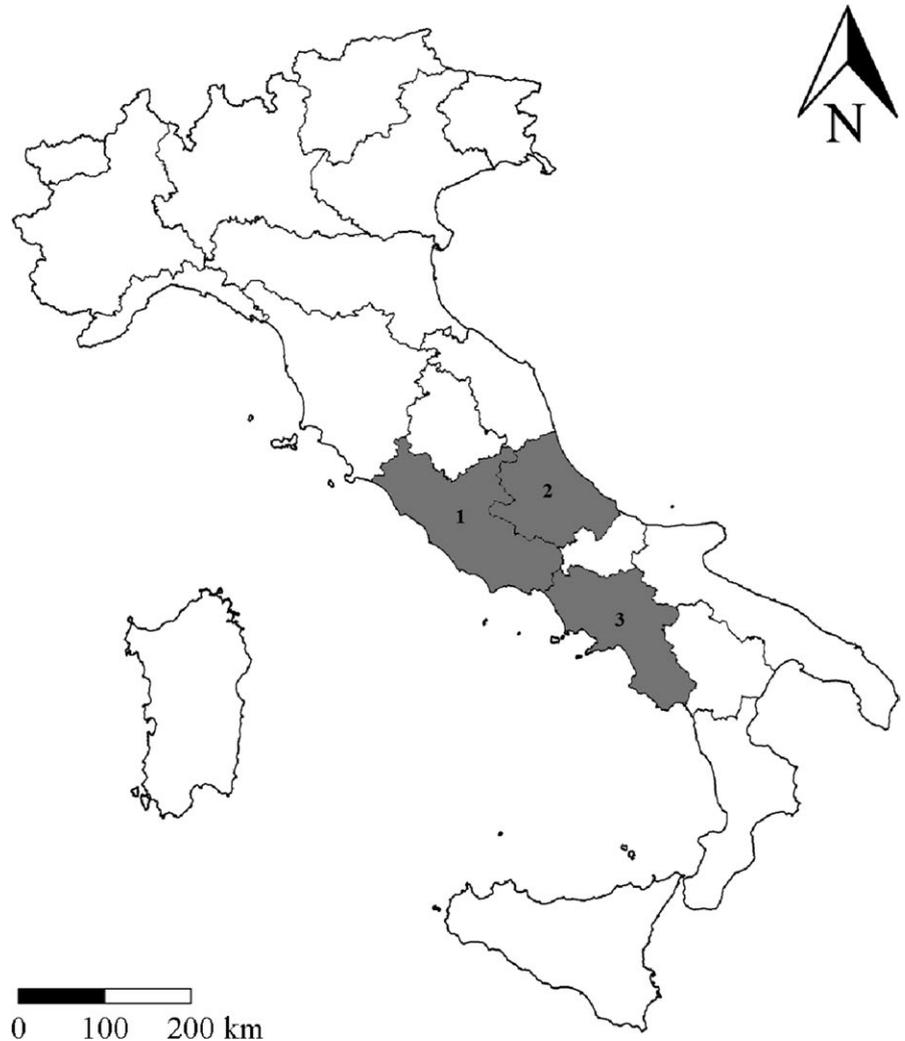
Amplicons of the PCR-positive samples were sequenced as previously described (Amoroso et al., 2013). The nucleotide sequence similarity searches were performed using the BLAST server (<http://www.ncbi.nlm.nih.gov/genbank/index.html>). Nucleotide sequences of AstVs were aligned using the program Clustal W (Larkin et al., 2007) with reference sequences of *Mamastrovirus* (MAstV), using an avian astrovirus (AvAstV) JF414802 as outgroup (Supporting Information Table S1). Phylogenetic analyses were carried out by Mr Bayes (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) program implemented in the software package Geneious v. 9.1.8 (Biomatters, New Zealand). Bayesian inference was performed using four "chains" run over one million generations (with the first 2000 trees discarded as "burn-in") and supplying statistical support with subsampling over 200 replicates. jModelTest (Posada, 2008) was used to identify the most appropriate model of evolution. The identified program settings for all partitions, under the Akaike information criteria, included six character states (general time-reversible model), a proportion of invariable sites and a gamma

Virus	Primer	Sequences	Reference
Coronavirus	IN-2	GGGTGGGACTATCCTAAGTGTGA	Drosten et al. (2003)
	IN-4	TAACACACAAACACCATCATCA	
Reovirus	L1RV5	GCATCCATT GTAAATGACGAGTCTG	Leary et al. (2002)
	L1RV6	CTTGAGATTAGCTCTAGCATCTTCTG	
	L1RV7	GCTAGGCCGATATCGGGAATGCAG	
	L1RV8	GTCTCACTATTACCTTACCAGCAG	
Calicivirus	P289	TGACAATGTAATCATCACCATA	Jiang et al. (1999)
	P290	GATTACTCCAAGTGGGACTCC	Zintz et al. (2005)
	186	TGACGATTTTCATCATCMCCRTA	
	187	GATTACTCCASSTGGGAYTCMAC	
Astrovirus	FOR 1	GARTTYGATTGGRCKTAY	Chu et al. (2008)
	FOR 2	GARTTYGATTGGRCKAGGTAYGA	
	REV	GGYTTKACCCACATNCCRAA	
	FOR N1	CGKTAYGATGGKACKATHCC	
	FOR N2	AGGTAYGATGGKACKATHCC	
Enterovirus	EV1	CGGCCCTGAATGCGGC	Iturriza-Gomara et al. (2006)
	EV2	CACCGGATGGCCAATCCA	
	EVD2	CCCCTGAATGCGGCTAAT	
	EVU2	ATTGTCACCATAAGCAGCCA	

**TABLE 1** Primers used for the detection of viruses

**TABLE 2** Presence of astrovirus in the bat species monitored in this study. Only the PCR-positive samples confirmed upon sequence analysis were considered in the table

Bat species	Geographic area of capture	Number of animals	AstV Positive
<i>Barbastellabarbastellus</i>	Abruzzo	14	1
<i>Eptesicusserotinus</i>	Lazio	1	1
<i>Miniopterusschreibersii</i>	Campania	78	10
<i>Myotiscapaccinii</i>	Campania	14	3
<i>Myotisemarginatus</i>	Campania	5	1
<i>Myotismyotis</i>	Abruzzo	2	1
<i>Myotismystacinus</i>	Abruzzo	5	0
<i>Myotisnattereri</i>	Abruzzo	2	0
<i>Nyctalusleisleri</i>	Abruzzo	2	0
<i>Pipistrelluskuhlii</i>	Lazio	1	1
<i>Pipistrelluspipistrellus</i>	Abruzzo	3	0
<i>Plecotusauritus</i>	Abruzzo	10	0
<i>Rhinolophuseuryale</i>	Campania	10	0



**FIGURE 1** Map of the sampling sites and geographic location of astrovirus positive samples. 1 = *Eptesicus serotinus*, *Pipistrellus kuhlii*; 2 = *Myotis myotis/blythii*, *Barbastella barbastellus*; 3 = *Miniopterus schreibersii*, *Myotis emarginatus*, *Myotis capaccinii*. Regions in which captures occurred are indicated in grey

distribution of rate variation across sites (GTR+I+G). The Sequences of the AstV strains identified in this study were deposited in the GenBank database with the accession numbers from MG004195 to MG004212.

### 3 | RESULTS

All the screened bats were negative to reoviruses, caliciviruses, enteroviruses, rabies viruses and MERS-CoV. When analysed for the

**TABLE 3** Interrogation by BLAST search of NCBI nucleotide database (December 2017) based on the ORF1b (RdRp) sequences generated in this study. The strain with the highest % nucleotide identity (% PI) and E value (E-v) found in the database (BM, best match) is shown for each bat sequence generated in this study

Italian bat strain	Accession	BM	Accession of the BM	E-v	%PI
<i>Miniopterusschreibersii</i> /84	MG004202	Bat/HKY/2005/AFCD246	EU847196	1.75e-57	80.9
<i>Myotis capaccinii</i> /100	MG004203	Bat/HKY/2005/AFCD246	EU847196	1.75e-57	80.9
<i>Miniopterusschreibersii</i> /2015	MG004197	Bat/CHN/2010/ <i>Miniopterusschreibersii</i> 3	JQ814858	3e-60	81.2
<i>Barbastellabarbastellus</i> /39	MG004201	Bat/CHN/2010/ <i>Miniopterusschreibersii</i> 6	JQ814861	2e-82	86.1
<i>Myotis emarginatus</i> /1915	MG004196	Bat/CHN/2010/ <i>Miniopterusschreibersii</i> 7	JQ814862	1e-78	85.1
<i>Miniopterusschreibersii</i> /74	MG004209	Bat/CHN/2010/ <i>Miniopterusschreibersii</i> 7	JQ814862	9e-56	81.3
<i>Pipistrellus kuhlii</i> /99	MG004204	Bat/CHN/2010/ <i>Miniopterusschreibersii</i> 8	JQ814863	1e-48	79.2
<i>Eptesicus serotinus</i> /01	MG004208	Bat/HKY/2006/AFCD303	EU847220	1.39e-46	81.1
<i>Miniopterusschreibersii</i> /86	MG004205	Bat/HKY/2005/AFCD208	EU847195	3e-45	78.3
<i>Myotis myotis blythii</i> /38	MG004200	Bat/HKY/2005/AFCD208	EU847195	2e-71	83.2
<i>Miniopterusschreibersii</i> /08	MG004195	Bat/HKY/2005/AFCD269	EU847197	1e-79	85.1
<i>Miniopterusschreibersii</i> /75	MG004210	Bat/HKY/2004/WCF16	EU847159	1e-54	80.2
<i>Miniopterusschreibersii</i> /99	MG004212	Bat/CHI/2007/Guangxi/LD110	FJ571120	8e-81	92.1
<i>Miniopterusschreibersii</i> /83	MG004211	Bat/CHI/2008/MG30/Hainan	KJ571418	5e-103	90.4
<i>Miniopterusschreibersii</i> /94	MG004206	Bat/HUN/2013/Bb1	KJ652321	1e-79	89.2
<i>Miniopterusschreibersii</i> /88	MG004207	Bat/MDG/2014/FMNH 228882	KY575650	2e-52	86.5
<i>Myotis capaccinii</i> /2315	MG004198	Bat/MDG/2014/SMG18853	KY575651	7e-67	84.1
<i>Myotis capaccinii</i> /28	MG004199	Bat/GAB/2009/09GB738Mi	KU51046i	4e-84	87.2

presence of CoV, 10 bat samples yielded faint amplicons of the expected size (453 bp). Upon direct sequencing, the quality of the sequences was very low and the sequences were not usable, likely due to the low DNA concentration.

Astrovirus RNA was detected in 33/147 samples (22.45%), and, upon direct sequencing, 18/33 samples (54.54%) contained AstV RdRp sequences, for a total confirmed prevalence of 18/147 (12.24%).

A total of 33/147 bats (22.45%) tested positive for AstVs, yielding a 422-bp amplicon from the viral RdRp. Upon sequencing, only 18 sequences were of good quality and were further analysed. The sequence-confirmed prevalence in our samples was as high as 12.24% (18/147). Astrovirus RNA was identified in 10 of 78 *M. schreibersii* (12.82%), in three of 14 *M. capaccinii* (21.42%), in one of 14 *B. barbastellus* (7.14%) and in an additional four bat species (Table 2). Geographic location of sampling sites from which positive samples were taken is indicated in Figure 1. Upon interrogation (November 2017) of GenBank sequence database (Table 3), different patterns of recognition (best nucleotide matches) were displayed by the 18 Italian AstV strains (Table 3). Only two strains (*Miniopterusschreibersii*/84, GenBank Accession Number MG004202 and *Myotis capaccinii*/100, MG004203) showed 100% nucleotide (nt) sequence identity to each other, although they were identified in two different bat species (*Miniopterus schreibersii* and *Myotis capaccinii*). The two strains exhibited the highest (80.9%) nt identity to a strain (EU847196) detected in Hong Kong 2005 from a *Miniopterus magnate* (Table 3).

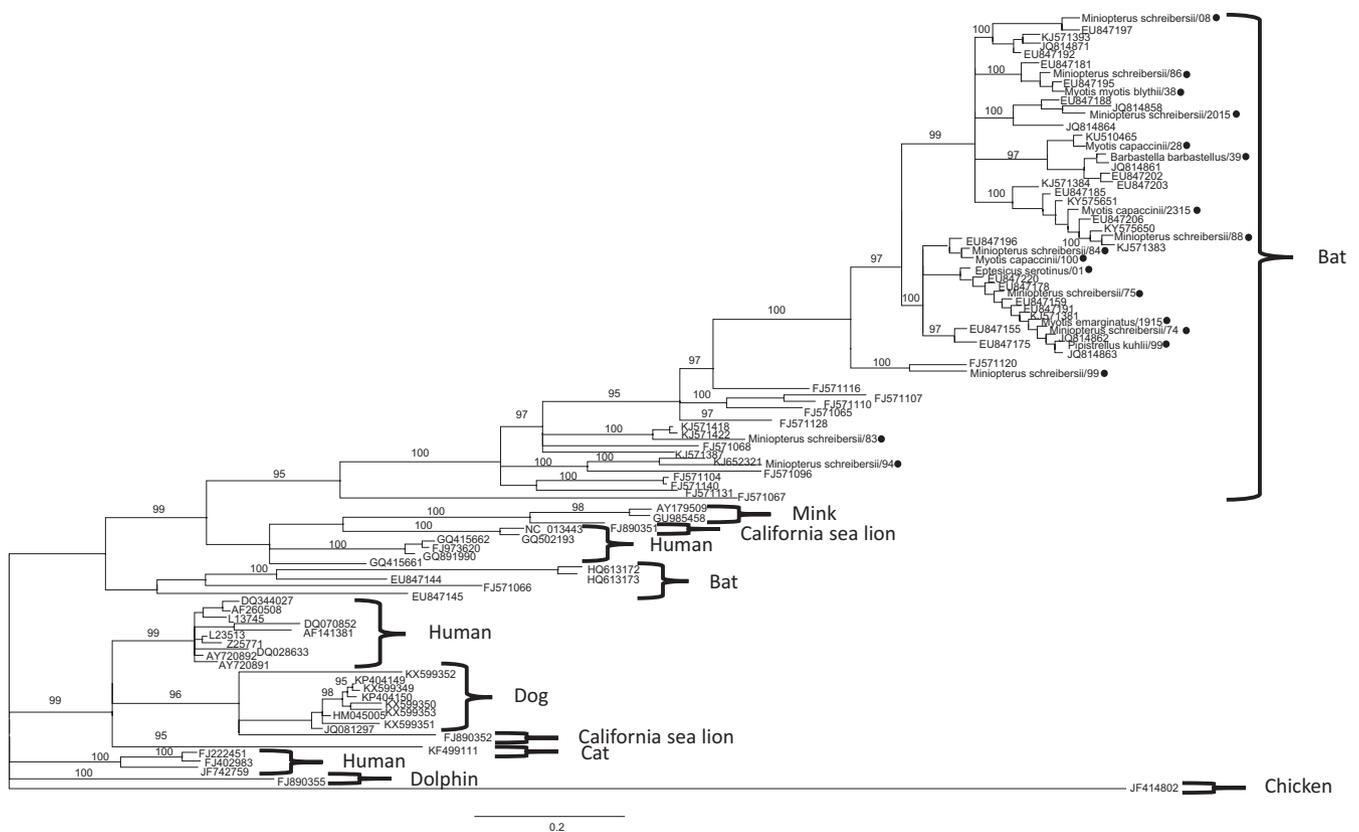
Five AstV strains from different bat species (*Miniopterusschreibersii*/2015, MG004197, *Barbastellabarbastellus*/39, MG004201,

*Myotis emarginatus*/1915, MG004196, *Miniopterusschreibersii*/74, MG004209 and *Pipistrellus kuhlii*/99, MG004204) showed 79.2–86.1% nt identity to AstV strains (JQ814858, JQ814861, JQ814862, JQ814863, respectively) identified in China from *Miniopterusschreibersii* in 2010 (Table 3). Five AstV strains (*Miniopterusschreibersii*/75, MG004210, *Eptesicus serotinus*/01, MG004208, *Miniopterusschreibersii*/86, MG004205, *Myotis myotis blythii*/38, MG004200 and *Miniopterusschreibersii*/08, MG004195) displayed 78.3–85.1% nt identity to AstV strains (EU847220, EU847195, EU847197 and EU847159, respectively) detected in Hong Kong from *Miniopterus pusillus* and *Miniopterus magnate* in 2004–2006 (Table 3).

Two AstV strains identified in *M. Schreibersii* (*Miniopterusschreibersii*/83, MG004211 and *Miniopterusschreibersii*/99, MG004212) showed nt identity as high as 90.4–92.1% to AstV strains (FJ571120 and KJ571418) identified in Hong Kong from two different bat species, *Taphozous melanopogon* and *Hipposideros larvatus*, respectively, in 2007–2008 (Table 3).

One AstV strain from *M. Schreibersii* (*Miniopterusschreibersii*/94, MG004206) showed the highest (89.2%) nt identity to an AstV strain (KJ652321) identified in Hungary from *Myotis emarginatus* in 2013 (Table 3).

Two AstV strains from different bat species (*Miniopterusschreibersii*/88, MG004207 and *Myotis capaccinii*/2315, MG004198) showed 84.1–86.5% nt identity to AstV strains (KY575650 and KY575651) identified in Madagascar from *Miniopterus griveaudi* in 2014 (Table 3).



**FIGURE 2** Phylogenetic tree based on a 333 nucleotide (nt) fragment of ORF1b (RdRp) of astrovirus detected in the present study or retrieved from the GenBank database. Bayesian evolutionary analyses were carried out using four “chains” run over one million generations (with the first 2,000 trees discarded as burn-in) with the generalized time-reversible (GTR), gamma variation model and supplying statistical support with subsampling over 200 replicates. Posterior probability values >95 are reported on the tree branches. The scale bar indicates the number of nt substitutions per site. Black circles indicate the AstV sequences detected in this study

One AstV strain (*Myotis capaccinii*/28, MG004199) revealed 87.2% nt identity to an AstV strain (KU510465) identified in Gabon from *Miniopterus inflatus* in 2009 (Table 3).

Upon sequence comparison, a marked genetic heterogeneity was revealed among the 18 AstV strains characterized in this study, with the nt identity ranging from 63.26% to 100% to each other. The AstV sequences were also aligned with representatives strains retrieved from GenBank database and subjected to phylogenetic analysis in the partial ORF1b (RdRp) region. The topology of the phylogenetic tree was supported by a Bayesian posterior probability approach using both a generalized time-reversible (GTR) and a gamma variation model and supplying statistical support with subsampling over 200 replicates. Upon phylogenetic analysis, the eighteen strains identified in this study segregated into a distinctive monophyletic group intermingled with AstV strains identified in bats from China, Hong Kong, Hungary, Madagascar and Gabon (Figure 2) (Supporting Information Table S1) and supported by bootstrap values  $\geq 95$ .

## 4 | DISCUSSION

In this study, we investigated the presence of various human viral pathogens in 14 different species of bats captured in Central and

Southern Italy. None of the samples was positive to rabies virus that is the most serious concern for the potential human transmission. From an epidemiologic point of view, bat-associated rabies cases are rare, with the incidence rates in Canada and the United States being as low as 2.2–6.7 human cases per billion persons/year over a 57-year period (Velasco-Villa et al., 2017). However, there is still a potential risk that needs attention, especially for bat specialists and rehabilitators, who handle bats and are often exposed to bites. Our study, in spite of the relatively small number of sampled animals, confirms that this risk is negligible in Italian territories.

Consensus diagnostic molecular assays are useful to detect novel viral species or genetically different viral strains, but they are usually not highly sensitive. Using broadly reactive consensus primers, CoV RNA was detected in 10/147 (6.8%) samples. These results could not be confirmed with sequence analysis. However, the samples also tested negative by a quantitative assay specific for MERS-CoV, thus ruling out the presence of this virus in the sampled population.

Using consensus primers universal for the *Astroviridae* family, AstV RNA was detected in 33/147 samples (22.45%) and, upon direct sequencing, 18/33 samples (54.54%) contained AstV RdRp sequences, for a total confirmed prevalence of 18/147 (12.24%). The presence of AstV in bats was first reported in 2008 (Chu et al., 2008), and it has been subsequently confirmed in various investigations

in Europe and Asia (Drexler et al., 2011; Fischer et al., 2016, 2017; Kemenesi et al., 2014; Zhu et al., 2009). The RT-PCR prevalence (22.45%) observed in our study was higher than that observed in Hungary (6.94%) (Kemenesi et al., 2014) and similar to that found in Germany (25.8%; Fischer et al., 2016). Studies in China identified AstV in 46% of the tested bats (Chu et al., 2008; Xiao et al., 2011). The prevalence was also found to greatly vary by bat species (Fischer et al., 2017). As an example, a study carried out in China revealed the presence of Astrovirus in 83.3% of the samples of *Myotis pylosus* analysed, whilst the prevalence was found much lower for other species (Chu et al., 2008). To detect and characterize the AstV strains, we amplified and sequenced a fragment of 422 bp of the RdRp gene, which represents the most conserved region of the AstV genome. Upon sequence analysis, we observed a remarkable genetic diversity among the various bat AstV strains detected in Italy. Such a high degree of variation was observed also within species and in the same geographic area or colony. These findings do not confirm the bat species specificity of AstV, proposed by other authors (Fischer et al., 2016). Intriguingly, two AstV strains were virtually identical (100% nt) to each other in the fragment of the RdRp region, but they were identified from two different bat species, *M. schreibersii* and *M. capaccinii*.

So far, AstVs in bats have been searched for and identified in Asia, Africa and in some European countries (Germany, Czech Republic and Hungary; Fischer et al., 2016; Kemenesi et al., 2014). Our study extended the available information in terms of geographic distribution and also of bat species harbouring AstVs, as we could identify AstV in four novel bat species (*B. barbastellus*, *M. capaccinii*, *P. kuhlii*, *M. emarginatus*).

Astrovirus infection is associated with gastro-enteritis in most animal species, and humans AstVs are regarded as a common cause of viral diarrhoea in children (Mendez, Aguirre-Crespo, Zavala, & Arias, 2007; Xiao et al., 2017). Avian AstVs have also been associated with extra-intestinal diseases, such as nephritis in chicken (Imada et al., 2000) and hepatitis in ducks (Todd et al., 2009). Recently, AstVs have been also detected in the nervous tissues of minks with shaking disease (Blomstrom, Widen, Hammer, Belak, & Berg, 2010) and of bovines with neurological disorders (Bouzalas et al., 2014; Li et al., 2013). Neurological disease in immunocompromised human patients has been associated with AstV infection (Brown et al., 2015; Fremond et al., 2015; Lum et al., 2016; Quan et al., 2010). In our research, all the animals positive to AstV appeared healthy, as also reported in previous studies in bats (Fischer et al., 2017). Astroviruses could therefore simply be nonpathogenic members of the bat virome. However, more information is needed on bat-borne immune response to state whether these viruses are really nonpathogenic for bats.

Bats could play an important role in transmitting such viruses to humans, as AstV transmission usually follows an oral-faecal route. Contamination of food or drinking water could for example occur by bat droppings. On this regard, it is however important to underline that the probability that human ingest food and water contaminated by AstV coming from human faeces looks

much higher—see, for instance, the high percentage (28.70%) of human AstVs recently found in mussels harvested in the Gulf of Naples, Italy (Fusco et al., 2017). Interspecies transmission of AstV has been documented on more occasions (De Battisti et al., 2012; De Benedictis, Schultz-Cherry, Burnham, & Cattoli, 2011; Mihalov-Kovacs et al., 2017; Nagai et al., 2015). Also, novel human AstVs (MLB1, MLB2, VA1, HMO-C, HMO-B, HMO-A, VA-2) have been identified that are genetically unrelated to “classical” human AstVs (Banyai, Meleg, Moschidou, & Martella, 2010; Finkbeiner, Holtz, et al., 2009; Finkbeiner, Le, Holtz, Storch, & Wang, 2009; Finkbeiner, Li, et al., 2009; Kapoor et al., 2009) and closer to animal AstVs. The origin of animal-like human AstVs has not been deciphered yet.

The potential zoonotic risks associated with bats have attracted the attention of researchers, mostly after the discovery of SARS-like and MERS-like CoVs (two coronaviruses highly pathogenic for humans) in European bat species, although the zoonotic risks posed by bat viruses, likely very limited, should be assessed more properly (Kohl & Kurth, 2014), in large structured studies.

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## CONFLICT OF INTEREST

None.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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