# Chapter 8
# Astrovirus Infections in Animal Mammalian Species

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**Abstract** The first reports of astroviruses in animals date back to the end of the 1970s, when infections in mammals such as lambs and calves suffering from diarrhea were reported for the first time. Since then, several mammalian species have been shown to be susceptible to astroviruses which appear to be genetically diverse and to have acquired host-specificity. To date, astroviruses have been detected in 16 different orders or species of mammals in addition to humans, and signs of infection range from unapparent infection or very mild disease to diarrhea, lethargy, and anorexia, mainly observed in young individuals. This chapter describes those astroviruses detected in nonhuman mammalian species worldwide, as well as their molecular and phenotypic characteristics and their role in diseases. The capacity of these viruses to cross-species barriers and their subsequent adaptation to novel hosts is also highlighted.

## Introduction

Following the first description of astroviruses in human stool in 1975 [1, 27], astrovirus-like particles were described and reported in domesticated animals. The first reports derived from infections in mammals such as lambs and calves

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suffering from diarrhea [40, 48]. Indeed, between the end of the 1970s and the first half of the 1980s several animal species were found positive for astrovirus and many novel animal astroviruses were described probably as a consequence of the description of the discovery of astrovirus infection in humans few years before. Over the next 20 years, there was a hiatus of information about the occurrence of novel astroviruses or the clinical manifestations associated with known astroviruses in mammalian species. The lack of a clear association of astrovirus detection in clinical specimens with signs of disease in animals, together with the limited sensitivity and availability of laboratory tests (i.e., electron microscopy and virus isolation) contributed to this gap in information. The advent of the genomic and metagenomic era in the recent period appears to have revitalized investigations in this field and there is now new interest in the circulation of astroviruses in animal and human populations, with an increasing number of reports concerning new host species infected and novel astroviruses described in the last ten years.

## Astrovirus Infections in Domestic Ruminants (Ovine and Bovine)

### *Ovine Astrovirus*

Ovine astrovirus (OAstV) replicates in the absorptive epithelial cells of the small intestine and induces mild diarrhea in gnotobiotic lambs [13, 41]. The genome of OastV isolated in 1977 has been completely sequenced in more recent years [22], although information on its capsid protein sequence were published a few years before [21]. Results from these papers indicated that human, feline, and porcine astroviruses were most closely related to each other than to OAstV. However, as with most of the known astroviruses, OAstV harbors a RNA motif in the 3′-end of the genome common to distinct and genetically different virus families (i.e., *Coronaviruses* and *Picornaviruses*) and is supposed to derive from a recombination event between these viruses [20]. Genetic sequencing of astroviruses recently led to the detection of viruses in human stool that are genetically closely related to OAstV and mink astrovirus (MiAstV), and has revealed the possibility that these viruses have a common ancestor, as previously suggested also for the HAstVs, feline astroviruses (FAstVs), and porcine astroviruses (PAstVs) [11, 20, 23, 35]. Despite the detailed genetic studies undertaken on the genome of this virus, no further clinical or epidemiological reports indicating its occurrence and circulation in animals have appeared in the last 35 years. As a consequence, the epidemiology as well as the genetic and antigenic diversity of OAstV is poorly defined and only the complete genome sequence of the original isolate is available in the public genetic database (GenBank, http://www.ncbi.nlm.nih.gov).

## Bovine Astrovirus

One year after the detection of astrovirus in lambs, the first isolate of bovine astrovirus (BAstV) was reported in England [48]. Astrovirus was considered to be avirulent as experimentally infected gnotobiotic calves remained clinically normal, although pathological studies on infected calves were not performed at that time. In Florida in1984, a bovine enteric virus antigenically related to the UK strain of BAstV was shown to cause infection and cytopathology of the M cells of the dome epithelium covering Peyer's patches of the ileum of gnotobiotic calves. Similar to the England strain, these animals remained clinically normal during the course of the infection and only a change in the color of the feces from brown to yellow was noticed [49]. In 1985, a further isolate of BAstV was obtained from a colostrum-deprived calf which developed diarrhea [50]. Serotyping of the strains from England and USA based on neutralization and immunofluorescence assays indicated that these viruses were antigenically related although likely belonging to different serotypes. The antigenic diversity detected in the earlier studies is also reflected in the more recently described genetic diversity for BAstV. Tse et al. [44] detected sequences from two different BAstVs coinfecting 2 out of 5 positive specimens [44]. The phylogenetic analyses support classification of BAstVs and the newly discovered astroviruses in roe deer (CCAstV) under the proposed "genocluster GI" of the *Mamastrovirus* [4, 38, 44].

Originally, a serologic study conducted on cattle in the United States led to the detection of a 30% seropositivity for astrovirus [49] but the limited clinical significance initially attributed to BAstV infection in cattle probably explains why there has been a lack of further reports for more than 25 years, until 2011 [44]. Although a previous study suggested that BAstV could be excreted by 60–100% of calves on farms [5], a much lower rate of isolation (2.6%) was found by analyzing 1,060 field samples of diarrhea in calves aged from 3 days to 3 weeks of age [50]. A very similar detection rate (2.4%) was revealed by analyzing 209 rectal swabs collected from asymptomatic adult cattle by RT-PCR [44]. It was originally concluded that in natural conditions, BAstV did not seem to be directly associated with a severe diarrheic disease in calves [5, 44, 48, 49] and few controversial data are available on the prevalence of this infection. For this reason, it has recently been recommended that a large-scale surveillance study for BAstV should be performed in commercially farmed cattle to re-evaluate the circulation and the epidemiology of this virus.

## Astrovirus Infections in Other Ruminants

Soon after the reports of astrovirus infections in lambs and calves, astrovirus-like particles were detected by electron microscopy in the diarrheic feces of red deer reared in captivity in the UK [45]. Attempts to propagate the viruses by serial

passages on bovine embryonic kidney cells failed but seroconversion was detected in convalescent sera collected 2 weeks after virus shedding. Following this report, no more cases of astrovirus infections in red deer have been published and the role of these viruses in the enteric outbreak described in the UK remains uncertain. However, an outbreak of enteric disease recently occurred in a dense wild population of European roe deer (*Capreolus capreolus*) in Denmark at the beginning of 2010. Virological investigations led to the identification and characterization of novel astroviruses (CcAstV) in the absence of evidence of other viral, bacterial, or parasitic pathogens [38]. In the original study, two CcAstV detected in two distinct samples revealed a relatively low amino acid sequence identity (84.9%) in the region encoded by the RdRP gene. As the genetic distance between the two CcAstV is at least as large as that between the eight human astrovirus serotypes, the authors concluded that these novel astroviruses may constitute two different serotypes [38]. The viruses responsible for the infection was shown to genetically belong to the genus *Mamastrovirus* and are related to BAstV, suggesting that this virus could cross the species barrier to infect both cattle and roe deer [38, 44].

## Astrovirus Infections in Carnivores

### *Feline Astrovirus*

After the first clinical report in 1981, astrovirus-like particles have been described in the feces of domestic cats in Australia, England, Germany, New Zealand, and the USA [15, 17, 18, 29, 36]. The first report originating from the USA described the occurrence of severe and prolonged enteric signs with green and watery diarrhea for about 2 weeks, poor body condition and dehydration in a female 4-month-old kitten [18]. Typical astrovirus-like particles were detected in feces by electron microscopy but virus isolation attempts in primary feline kidney cells and fetal rhesus monkey kidney cell line failed. A few years later, a naturally acquired astrovirus infection was described in a 1-year-old domestic cat in England that was presented to the veterinary clinic with a history of intermittent vomiting and diarrhea [15]. No parasites or significant bacterial pathogens were detected in the feces but the presence of a large number of astrovirus-like particles was documented. Fecal filtrates containing astrovirus-like particles were administered to 14-week-old specific pathogen-free kittens causing two episodes of pyrexia and mild diarrhea [15]. Inoculated SPF kittens had intermittent excretion of virus between day 4 and day 6 postinfection. Viruses from feces of SPF kittens were isolated on feline embryo cells incubated in the presence of 10–50 µg of trypsin. Under experimental conditions, events of pyrexia coincided with episodes of virus shedding and seroconversion was observed in the experimentally infected animals by immunofluorescence assay. No cross reactivity between feline and human astroviruses was observed by immune electron microscopy [15]. Based on the report from England, astrovirus-like infection in kittens appeared to be associated to clinical disease. However in a 16-month study

conducted in Australia, the occurrence of this viral infection was not related to the age of cats and to the presence of diarrhea [29]. Interestingly, in this study, a human serum was found to react against a cat astrovirus by immune electron microscopy, implying similarity in antigenic regions between human and FeAstV [29]. A later report from New Zealand described the presence of an astrovirus-like particle in the diarrheic feces of two young kittens [36]. Since 1993, no further reports of astrovirus infections in cats have been reported and the role of these viruses in feline enteritis and their epidemiology remains obscure. Furthermore, to date, only partial sequences of the ORF1b and of the ORF2 of these viruses have been deposited in public databases and feline astrovirus diversity has not yet been explored. Interestingly, a study on both astrovirus genome sequences and host species evolution has led to the hypothesis of a likely cross-species transmission from pigs to cats and further from cats to humans, possibly involving intermediate species [25].

In 2009, a novel astrovirus was identified in captive cheetahs (*Acinonyx jubatus*) showing clinical signs of gastroenteric disease, such as lethargy, anorexia, watery diarrhea, and regurgitation [2]. The virus was shown to be related to the FeAstV in ORF2 (88.6%, 97.1% nucleotide and amino acid identity, respectively). Currently, it is unclear whether the cheetah astrovirus crossed the species barrier from domestic cats or if it was established in cheetahs independently.

## Canine Astrovirus

Astrovirus-like particles have been reported in dogs with and without diarrhea since 1980 [28, 46, 47]. However, these reports were based on electron microscopy descriptions only, thus the correct and complete identification of the small virus particles detected was not possible. The occurrence of astrovirus infection in dogs was only recently confirmed by RT-PCR detection and further genetic characterization by sequencing [43, 53]. In 2011, a canine astrovirus (CaAstV) has also been successfully isolated in cell culture for the first time [30]. To date, CaAstV or astrovirus-like particles in dogs have been reported in the USA, Germany, Austria, Italy, China, and France, suggesting the worldwide circulation of these viruses [14, 28, 43, 46, 47, 53]. The clinical significance of CaAstV infections in dogs requires further investigation; however, CaAstV was detected from symptomatic puppies occasionally suffering from diarrhea in association with other enteric viruses, such as rotaviruses [43] and corona- and/or parvo-like viruses [30, 47]. The clinical sign most commonly associated with the presence of astrovirus in puppies is a self-limiting watery diarrhea. However, based on RT-PCR virus shedding can persist long after the termination of the clinical signs [30]. A recent study on the prevalence of CaAstV in Shanghai, China, suggested that 12% (22/183) of the puppies showing clinical signs of diarrhea were positive for astrovirus by RT-PCR as compared to none of the 138 healthy controls [53]. In a recent study conducted in Italy, 24.5% of 110 stool samples collected from symptomatic dogs and 9.3% of 75 from asymptomatic animals surveyed tested positive for the

presence of CaAstV RNA [30]. In the same study, serologic assay indicated that 59% of 54 dogs aged >3 months surveyed in Italy presented specific CaAstV antibodies [30]. A similar prevalence based on viral RNA detection was found in France where 20.9% (66/316) of the puppies in 42% (14/33) in breeding kennels surveyed were CaAstV-positive by RT-PCR [14]. In the same report, the authors noted that puppies less than 7 week-old were especially susceptible to CaAstV infection [14]. These data indicate that CaAstV is ubiquitous in the canine populations in several parts of the world.

Genetic analysis of ORF2 and partial ORF1b identified the virus as a member of the genus *Mamastrovirus*, genocluster GI comprising "classic" human, porcine, and FeAstVs but genetically well distinguishable from the other *Mamastroviruses* [4, 43]. Among available sequences, the closest similarity was found with HAstV-7 and HAstV-1 (22% and 59.4% nucleotide similarity for ORF2 and partial ORF1b sequences, respectively). Thus, genospecies GI.E has been further proposed as being a new species [4]. Based on the genetic data reported from Italy and China, the genetic variability of CaAstV also appears to be relevant within the same country and perhaps indicative of the existence of multiple antigenic types [30, 43, 53].

Replication in vitro of CaAstV was successfully replicated on the MDCK cell line supplemented with 10 μg/ml of trypsin after three passages. Enlargement and detachment of the cells and the appearance of fine cytoplasmatic granules were the main features of the cytopathic effect [30].
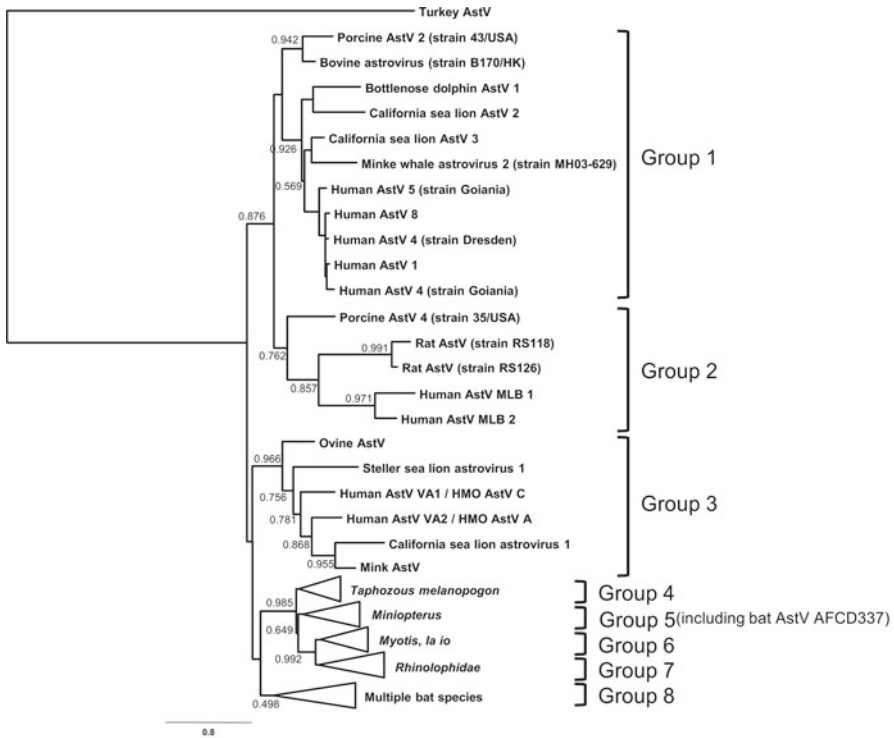
## Mink Astrovirus

A preweaning syndrome has been well described in mink farms in Denmark since 1954 [42]. This syndrome usually affects minks 1–4 weeks of age and mink farmers refer to it as "sticky," "greasy," or "wet" kits, due to the hypersecretion from apocrine glands in the dorsal neck region resulting in soiling of the neck and back [39], together with diarrhea and dehydration at various degrees [16]. The postmortem lesions described in kits dying from this syndrome are catarrhal enteritis with villous atrophy and desquamation of epithelial cells at the tip of intestinal villi [16, 19]. Many causative factors have been associated with the occurrence of this syndrome, including diet, bacterial, and viral pathogens [10]. In 2002, astrovirus infection was epidemiologically linked to the preweaning syndrome in minks, although its definitive etiology remained obscure [10]. In 2003, the astrovirus associated with this syndrome was entirely sequenced and characterized as a novel astrovirus with less than 67% similarity at the nucleotide level with the closest related OAstV, thus called MiAstV [32, 33]. Interestingly, sequence analysis of MiAstVs from geographically distinct Swedish and Danish farms indicated that they were conserved genetically, although this observation was mainly based on a short fragment of the ORF1b and geographical clusters were revealed [32, 33]. This appears to be in contrast with the data collected for astroviruses infecting

other species, such as swine, bats or turkeys, indicating a high degree of genetic variability [6, 26, 34, 52]. More recently, a novel astrovirus has been discovered in brain tissue of mink developing the so-called shaking mink syndrome (SMS), a transmissible neurological syndrome described for the first time in farmed minks of Denmark, followed by detection in both Sweden and Finland. The main clinical signs are shaking, staggering gait, and ataxia, with no evident gross lesions present postmortem. Nonsuppurative encephalomyelitis is a common lesion revealed by histopathology [3, 12]. Blomstrom et al. [3] have identified a unique astrovirus associated with SMS. The astrovirus associated with SMS and found in the brain tissue of minks was very similar to MiAstV in the ORF1a and ORF1b regions (88% and 98% homology, respectively) and a lower identity for ORF2 (67% and 59% homology for nucleotide and amino acid sequences, respectively) [3]. Interestingly, in 2007, a lethal case of human encephalitis occurred in a boy with X-linked agammaglobulinemia and an astrovirus phylogenetically distinct from HAstVs but related to MiAstV was detected in the brain tissue. Although the definitive source of infection remained unknown, it should be noted that the patient's residence was located in proximity to a mink farm [35].

## Astrovirus Infections in Sea Mammals and Other Species

Five genetically distinct astroviruses have recently been found in the fecal samples of three species of marine mammal: a California sea lion (*Zalophus californianus*) (CslAstV-1, CslAstV-2, CslAstV-3); a Steller's sea lion (*Eumetopias jubatus*) (SslAstV); and a bottlenose dolphin (*Tursiops truncates*) (BdAstV, 37). As for astrovirus infections in other species, the clinical significance of infection in sea mammals is unclear. In two cases (California sea lion and Steller sea lion) astroviruses were detected in young animals. The free-ranging California sea lion tested positive for astrovirus suffered from diarrhea but also had salmonella [37]. Genetic analyses of ORF1b and ORF2 indicate that these viruses do not cluster in a monophyletic group but across the *Mamastrovirus* tree, as confirmed by the proposal of reclassification submitted to the ICTV. Recombination analyses suggested that a recombination event may have occurred between a human strain (HAstV-4) and a CslAstV-2, possibly resulting in CslAstV-3 [37].

At the time of writing, the most recently discovered mammalian astrovirus infection is in rabbits. A recent investigation conducted in Italy on farmed rabbits suffering from enterocolitis revealed a novel astrovirus distantly related to other mammalian astroviruses, based on the ORF2 encoding for the capsid protein [31]. According to this study, astroviruses were detected in the intestinal content or feces of both symptomatic and asymptomatic rabbits. Considering the clinical relevance in rabbit farming of enteritis complex syndrome, this finding certainly deserves further investigations to assess the real impact and role of astrovirus infections in this species.

**Fig. 8.1** Phylogenetic analysis of the RdRp gene (422 nt) of mamastroviruses using PhyML with alrt (approximate likelihood ratio test) values indicated. Eight groups of mamastroviruses are denoted. A turkey astrovirus was included as an outgroup in this analysis

## Astroviruses in Rats

Fecal samples from *Rattus norvegicus* (brown rats, $n=371$) and *Rattus rattus* (black rats, $n=70$) captured in an urban area of Hong Kong were tested for astroviruses using broad-range primers designed to be conserved across all available astroviruses genetic sequences. While none of the *R. rattus* ($n=70$) tested were positive for astroviruses, 6 (1.6%) of 371 *R. norvegicus* specimens had astroviruses. Attempts at culturing these viruses in BHK, Caco-2, Vero or HEK293 cells, with or without exogenously added trypsin was unsuccessful.

Phylogenetic analysis of partial genome sequences of the RdRp gene of these rat astroviruses revealed that they cluster within a separate group distinct from all other astroviruses and form two clades represented by AstVRS118 and AstV RS126, respectively. The rat astroviruses have a sister clade relationship to human astroviruses MLB1 and MLB2 (Fig. 8.1). The phylogenetic analysis suggests that the rat and human MLB1 and 2 viruses appear to share a common ancestor virus, the mean date of the most recent common ancestor virus was estimated to have
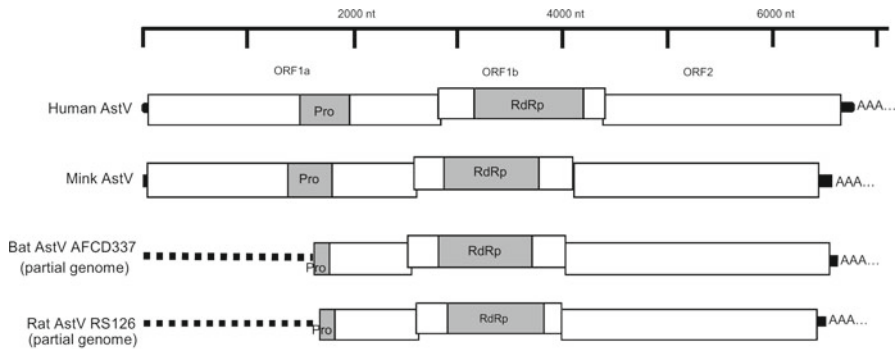
**Fig. 8.2** Schematic comparing the organization of typical human, mink, bat, and rat astrovirus genomes. The partial genome structure of the novel bat and rat astroviruses showed a typical astrovirus genome organization

occurred around 1054 A.D. (95% highest posterior densities 837–1827 A.D.) *R. norvegicus* is known to be a peri-domestic rodent species of human settlements for centuries and it is plausible that human AstV MLB and these rat astroviruses arose from a common ancestor centuries ago. The analysis of dN/dS ratios of ORF1b sequences does not show increased positive selection for either rat astrovirus or MLB viruses suggesting that each has been well adapted in the respective hosts over long periods of time [9].

Two-thirds of the genome of these two representative viruses was genetically sequenced, including part of ORF1a and the complete ORF1b and ORF2 genes and the 3′ UTR. A serine protease and RdRp gene can be identified in the ORF1a and ORF1b, respectively, from both rat astrovirus sequences. A heptameric slippery sequence (AAAAAAC) can be identified in the putative ORF1a/1b junction. In rat astrovirus, as with bat astrovirus AFCD337 and MLB virus, the conserved nucleotide sequence of a stem-loop II-like motif which is commonly found in many other astroviruses could not be found (Fig. 8.2).

Rat astroviruses may potentially provide a physiologically relevant small mammal model of astrovirus infections that may help understanding the pathogenesis of astrovirus infections. Similarly, CaAstVs have also been cultured in vitro [30] and could provide an alternative animal model.

## Astroviruses in Chiroptera

Following the detection of diverse group 1 and group 2 coronaviruses, including the putative precursors of SARS coronavirus, in insectivorous bats, there has been increased interest in the virus ecology of these species. Astroviruses were initially detected by consensus PCR methods in rectal swabs of apparently healthy *Miniopterus magnater* and *Miniopterus pusillus* bats captured in Hong Kong.

From 116 *M. magnater* and 33 *M. pusillus* bats captured and tested at different times of the year, the overall detection rates of astroviruses was 54% and 55% in fresh fecal droppings or rectal swabs, respectively, but lower (7%) in oro-pharyngeal swabs. All of the bats with positive oro-pharyngeal swabs were also found to be positive in their rectal swabs. The astrovirus positive rates remained roughly comparable at each of the four visits that spanned the winter, spring, and summer seasons [8].

Astroviruses detected in positive specimens collected from *M. magnater* failed to replicate in vitro in primary lung fibroblast or primary kidney fibroblast derived from the same host species or in Caco-2 cells, with or without exogenously added trypsin.

Approximately three-fourths of the genome of a bat astrovirus (AFCD337) detected in the rectal swab of a *M. pusillus* bat has been sequenced, including part of the ORF1a, and complete ORF1b, ORF2 and the 3′ untranslated region and poly-A tail at the 3′end of the genome (Fig. 8.2). These studies show that bat astroviruses are distinct from other known mammalian astroviruses (Fig. 8.1). In the ORF1b and ORF2 regions, batAstV AFCD337 has <55% and <27% amino acid similarity, respectively, to other known mammalian astroviruses. Characteristic features of BatAstV AFCD337 include a protease motif in ORF1a, an astrovirus "slippery sequence" (AAAAAC) at the junction between ORF1a and ORF1b which is required for inducing a ribosomal shift event, a RdRp motif in ORF1b and a conserved sequence at the end of ORF1b [8]. A conserved stem-loop structure that is predicted at the 3′-end of the genomic RNA seen in human, ovine, porcine, and turkey astroviruses was not found in BatAst AFCD337 [8, 20, 22].

The putative ORF2 of AFCD337 has a size of 2,553 nt and bat viruses appear to have the largest astrovirus capsid gene known to date. As with other astroviruses, the N-terminal half of ORF2 which is believed to be the core assembly domain of the viral capsid [24] is more conserved. Compared to an overall amino acid similarity of <27% for the ORF2 region overall, the amino acid sequence similarities of the N-terminal half of ORF2 to HAstV-1 Oxford, OAstV, and MAstV being 36.3%, 45%, and 39.5%, respectively. Phylogenetic analysis of ORF1a (partial), ORF1b, and ORF2 confirms that BatAst AFCD337 is a novel astrovirus (Fig. 8.1).

Representative insectivorous bats of families Rhinolophidae (12 species tested), Vespertilionidae (12 species), Emballonuridae (*Taphazous melnopogon*), Megadermatidae (*Megaderma lyra*), and Hipposideridae (2 species) and fruit bats of the family Pteropodidae (*Rousettus leschenaultia*) captured or collected in southern China have been tested [8, 51, 52]. The insectivorous bats had relatively high detection rates of astroviruses. In contrast, the only fruit bat species tested (*R. leschenaultia)* had low rates of astroviruses detected. While one astrovirus detected from *R. leschenaultia* in China clustered phylogenetically with viruses from Rhinolophidae [51], one other virus from this species was phylogenetically distinct from all astroviruses detected from insectivorous bats (unpublished data).

The RdRp gene (422 nt) of these bat astroviruses detected so far has been sequenced to compare the evolutionary diversity of these viruses in comparison

to other mammalian astroviruses. All bat astroviruses clustered within the genus Mamastrovirus. Within this genus, eight monophyletic groups can be identified. Group 1 contains the typical human astroviruses and those from porcine, bovine, and sea mammal origin. Group 2 contains human atypical AstV MLB1 and MLB2, rat, and a porcine astrovirus. Group 3 comprises mink, ovine, and atypical human astroviruses VA1/2/3 (also alternatively termed HMO astroviruses A/B/C). Groups 4, 5, 6, and 7 were monophyletic clusters of viruses from different families of bats, viz., Taphozous, Miniopterus, Myotis, and Rhinolophidae, respectively. Group 8 has weak statistical support and has viruses from diverse bat species including those from Taphozous, Miniopterus, and Myotis bats (Fig. 8.1).

Two features of the ecology and phylogeny of bat astroviruses are unusual. The virus detection rates are high from apparently healthy animals and there is great genetic diversity even within bats of the same species at the same habitat at one sampling occasion (e.g., in *Miniopterus* bats within a single bat cave in Hong Kong). Astrovirus infections are typically acute short lasted infections and while virus detection rates >20% may be found in the context of outbreaks of infection, to find such (or even higher) detection rates repeatedly within a single species within one habitat is surprising. It is even more surprising to find high genetic diversity from animals within the same habitat at one sampling occasion, so that almost every animal has a genetically unique virus. The RdRp gene sequences of 50 viruses from one monophyletic group of astroviruses (from *Miniopterus* bats) are shown (Fig. 8.3). Pairwise genetic distances of the RdRp gene of these monophyletic viruses were estimated for all virus pairs and we found that 96.2% of these pair-wise distances were larger than or equal to 0.18 nucleotide substitution per site. In contrast, the pair-wise genetic distance analysis for all available typical human astroviruses worldwide was never larger than 0.17 nucleotide substitution per site. Thus >96% of all the pairs of bat astroviruses in this monophyletic group in a single habitat have diversity larger than that of typical human astroviruses considered globally.

In 50 astroviruses detected from this population of *M. magnater* bats, 45 viruses had genetic distances of at least 0.18 nucleotide substitutions per sites in the RdRp gene to their closest partner. Assuming an RNA virus mutation rate of $10^{-3}$ per year, 0.18 substitutions per site represents separation of >180 years for each virus pair found within a single habitat, i.e., the most recent common ancestor for any two viruses would be at least 90 years. The observation that almost each virus detected in Miniopterus bats even in one sampling occasion was genetically unique is reminiscent of the findings on bat coronaviruses in this same population [7] and raises the question whether astroviruses may persistently infect bats and whether bats are unusual in their virus–host interactions with these viruses. Longitudinal studies to re-sample tagged bats may be required to address these questions. An alternative explanation that these viruses found in bats are insect viruses ingested by these insectivorous bats is implausible given the close phylogeny with other mammalian viruses and also the clear clustering of viruses within bat families.
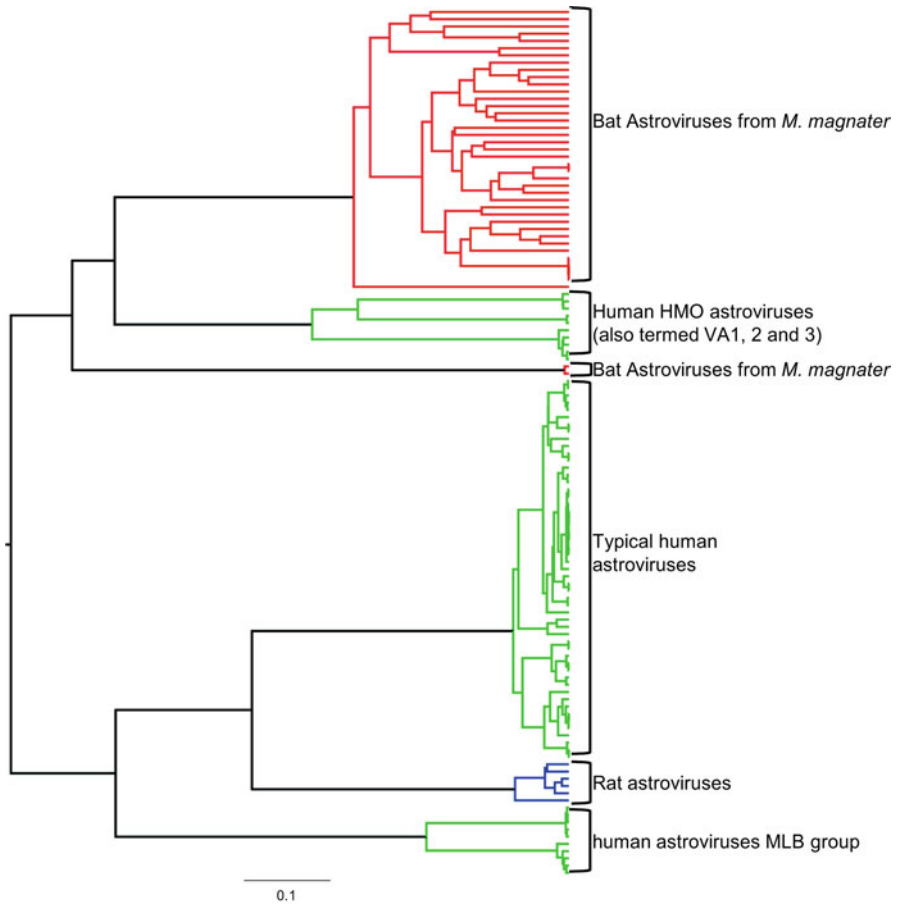
**Fig. 8.3** Comparison of the diversity of astrovirus genomes of astroviruses in *Miniopterus magnater* bats sampled in one habitat in Hong Kong with that of typical human astroviruses worldwide. The analysis was based on the RdRp genes (422 nt) and the phylogenetic tree was constructed using constant substitution rate. The genetic diversity of astroviruses detected in *M. magnater* bats captured in a single habitat was significantly greater than that of typical human astroviruses sampled all worldwide

## Nomenclature of Astroviruses

The International Committee for Taxonomy of Viruses has estimated within species and between species amino acid sequence diversity in the capsid gene of ≤0.312 and ≥0.378, respectively. The capsid gene is generally more genetically diverse than the RdRp gene. Thus, if these criteria are directly applied to astroviruses in bat populations, even within a single bat species, viruses from most individual bats within one bat species would qualify as a different virus species.

This issue would have to be considered with some flexibility, especially in regard to bat astroviruses. Given that different bat species from different regions of China group together in monophyletic clades, even though their within group diversity is large, may have to be considered as one species.

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