Journal of Virology

A Novel Group of Avian Astroviruses in Wild Aquatic Birds

Daniel K. W. Chu, Connie Y. H. Leung, Harsha K. K. Perera, Erica M. Ng, Martin Gilbert, Priscilla H. Joyner, Alessandro Grioni, Gary Ades, Yi Guan, Joseph S. M. Peiris and Leo L. M. Poon

J. Virol. **2012, 86(24):13772. DOI: 10.1128/JVI.02105-12. Published Ahead of Print 3 October 2012.**

JVI Journals.ASM.org

A Novel Group of Avian Astroviruses in Wild Aquatic Birds

Daniel K. W. Chu,[a] Connie Y. H. Leung,[a] Harsha K. K. Perera,[a] Erica M. Ng,[a] Martin Gilbert,[b] Priscilla H. Joyner,[b] Alessandro Grioni,[c] Gary Ades,[c] Yi Guan,[a] Joseph S. M. Peiris,[a,d] and Leo L. M. Poon[a]

State Key Laboratory of Emerging Infectious Diseases, Centre for Influenza Research and School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, People's Republic of China[a]; Wildlife Conservation Society, Phnom Penh, Cambodia[b]; Fauna Conservation Department, Kadoorie Farm and Botanic Garden, Hong Kong SAR, People's Republic of China[c]; and HKU-Pasteur Research Centre, People's Republic of China[d]

Using a pan-astrovirus reverse transcription-PCR assay, a great diversity of novel avastroviruses was detected from wild bird and poultry samples. Two groups of astroviruses detected from wild birds are genetically related or highly similar to previously known viruses in poultry. Most interestingly, a novel group of astroviruses was detected in wild aquatic birds. Our results also reveal that different groups of astroviruses might have difference host ranges. This study has expanded our understanding regarding avastrovirus ecology.

Avian astroviruses (AstV) are classified within the genus *Avastrovirus* and are known to cause infection in poultry leading to economic losses to farms and affecting food production worldwide. These viruses have been associated with avian diseases, including enteritis in turkeys, chickens, and guinea fowl, mild growth depression and nephritis in chickens, and hepatitis in ducklings. The disease severity ranges from subclinical infection in apparently healthy adult birds (8, 14, 15) to heavy losses of ducklings in farms (11). Currently, at least six genetically distinct astroviruses have been identified in poultry (11, 17, 22). They are avian nephritis virus (ANV) in chicken, chicken astrovirus (CAstV), turkey astrovirus type 1 (TAstV1), turkey astrovirus type 2 (TAstV2), duck astrovirus (DuAstV) (formerly named duck hepatitis virus 2), and duck hepatitis virus 3 (DuHV3). Among these viruses, turkey astroviuses (TAstV) from turkey and avian nephritis virus (ANV) from chickens are the two virus strains most widely studied and surveys indicate that these viruses are widely distributed worldwide. Little is known about the ecology of astroviruses in wild birds and the possible associations between astsroviruses found in wild bird and avian poultry populations. In 2011, Kofstad and Jonassen reported the detection of novel astroviruses in pigeons caught in Oslo, Norway (16). The diversity and ecology of astroviruses in other wild avian species and populations, however, have not been explored, and such information would help us to better understand the origins, evolution, and epidemiology of these viruses in poultry.

Interspecies transmissions of avian astroviruses in poultry are not rare events. Incidents of these have included the detection of ANV in various poultry birds, including pigeons (24), guinea fowl (3), ducks (2), and turkeys (9, 18). TAstV2-like viruses were also detected in guinea fowl (7). These findings reveal the capability of some astroviruses for interspecies transmission. Infection of avian astroviruses in these hosts has not always been associated with diseases (18), but the significance to the astrovirus ecology of the interspecies transmission of astrovirus between these avian species requires further investigation.

To examine the diversity of astroviruses in wild birds and avian poultry, we studied (i) fecal samples of wild birds collected in Mai Po marshes, Hong Kong, (ii) cloacal swabs samples of wild birds collected in Cambodia and in Hong Kong, and (iii) cloacal swabs from poultry in Hong Kong and Sri Lanka. The Mai Po marshes in Hong Kong are a wetland habitat of international importance, especially for wild waterfowls. We studied avian populations, including migratory aquatic birds from northern latitudes that gather in Mai Po, particularly during the non-breeding season, in this area during the winter season. Here, we report the detection of astrovirus in our specimens. Phylogenetic analysis revealed a previously unrecognized diversity of novel astroviruses in wild birds.

Fresh and well-separated droppings of wild birds were sampled using sterile swabs at the Mai Po marshes in Hong Kong from October 2010 to January 2011. Cloacal swabs were collected by the Wildlife Conservation Society (WCS) from wild birds being sold in markets around the Tonle Sap Basin, Cambodia, in the year 2008 and from wild birds handled in the Wild Animal Rescue Centre of Kadoorie Farm and Botanic Garden (KFBG) Hong Kong in the years 2009 to 2011. In addition, cloacal swabs were collected during 2011 both from chickens in wet markets in Hong Kong and from chickens, quails, ducks, and geese from poultry farms in Sri Lanka.

RNA was extracted from bird dropping samples and swab samples kept in viral transport medium using a viral RNA extraction kit (Qiagen) following the protocol provided by the manufacturer. The extracted RNA was screened for astroviruses using a previously described pan-astrovirus reverse transcription-PCR assay targeting the RdRp gene (5). All PCR amplicons with the expected product size (422 bp) were subjected to DNA sequencing for confirmation. The host origins of selected wild bird droppings were identified by a previously described DNA "bar-coding" technique which employs a PCR assay targeting the avian mitochondrial DNA (mtDNA) COX1 gene followed by DNA sequencing as described before (4). Representative novel avian astroviruses were selected for additional genetic analyses. The RNA extract of the selected samples was subjected to first-strand cDNA synthesis using a 3′ rapid amplification of cDNA ends (RACE) system and kit (Invitrogen) followed by PCR amplification of the 3′-half genome

Received 10 August 2012 **Accepted** 22 September 2012
Published ahead of print 3 October 2012
Address correspondence to Joseph S. M. Peiris, malik@hku.hk, or Leo L. M. Poon, llmpoon@hku.hk.

doi:10.1128/JVI.02105-12

TABLE 1 Bird dropping samples collected at Mai Po that were reverse transcription-PCR positive for astrovirus

Species[a]	No. of AstV-positive samples (% of total)
Black-faced Spoonbill (*Platalea minor*)	1 (2)
Common Greenshank (*Tringa nebularia*)	1 (2)
Common Teal (*Anas crecca*)	5 (11)
Eurasian Wigeon (*Anas Penelope*)	8 (17)
Great Cormorant (*Phalacrocorax carbo*)	0
Gray Heron (*Ardea cinerea*)	0
Night Heron (*Nycticorax nycticorax*)	0
Northern Pintail (*Anas acuta*)	11 (23)
Northern Shoveler (*Anas clypeata*)	7 (15)
Unknown	14 (30)
Total	47 (100)

[a] Avian species were determined by DNA finger printing.

using gene-specific primers targeting the RdRp gene and targeting the poly(A) tail. Attempts at using 5′ RACE systems to deduce addition viral sequences at the 5′-end region were all unsuccessful.

Sequence alignment of the genes of interest was done using TranslatorX (1), which deduced the alignment based on translated amino acid sequences using the MUSCLE algorithm (10). Phylogenetic analysis was performed using PhyML (12) with the best-fit nucleic acid substitution model estimated by jModelTest (20). Pairwise amino acid sequence identities were deduced by BioEdit (13).

Astrovirus was detected in 47 of a total of 658 (positive rate = 7.1%) wild aquatic bird dropping samples collected in Mai Po marshes. Although the clinical status of the individual birds sampled via the droppings was not known, there were no overt outbreaks of disease recorded among wild birds at this site during the sampling period. Positive samples were detected from all sampling trips performed biweekly in a 3-month period, and the positive rates of each sampling occasion ranged from 2.8% to 14.7%. All the astrovirus-positive fecal samples were subjected to DNA bar coding to identify the host species. Seventy percent of these samples were PCR positive in this bar-coding assay, and this successful rate was similar to those previously reported by us (4, 5). These typable samples were from northern pintail (*Anas acuta*, $n = 11$), northern shoveler (*A. clypeata*, $n = 7$), common teal (*A. crecca*, $n = 5$), Eurasian wigeon (*A. penelope*, $n = 8$), common greenshank (*Tringa nebularia*, $n = 1$), and black-faced spoonbill (*Platalea minor*, $n = 1$) (Table 1). A randomly selected subset of astrovirus-negative samples was subjected to DNA bar coding for comparison ($n = 87$), and the diversity of bird species was broadly similar to that of astrovirus-positive ones (data not shown). Of the cloacal swabs collected in Cambodia, astrovirus was detected from 2.4% (3/123) of pond herons (*Ardeola* spp.) and from 3% (1/33) of lesser whistling ducks (*Dendrocygna javanica*) but not from ruddy-breasted crake (*Porzana fusca*, $n = 80$) (Table 2). None of

TABLE 2 Detection of astroviruses in cloacal swabs collected from different regions

Group and region	Bird species	No. of samples	No. (%) of AstV-positive samples
Wild birds			
Cambodia	Pond Heron (*Ardeola* spp.)	123	3 (2.4)
	Lesser Whistling Duck (*Dendrocygna javanica*)	33	1 (3.0)
	Ruddy-breasted Crake (*Porzana fusca*)	80	0
Hong Kong	Bulbul (*Pycnonotus* spp.)	17	0
	Buzzard (*Buteo* spp.)	11	0
	Rock Dove (*Columba livia*)	16	2 (12.5)
	Spotted Dove (*Spilopelia chinensis*)	16	1 (6.3)
	Goshawk		
	Crested Goshawk (*Accipiter trivirgatus*)	7	0
	Other Goshawk spp.	7	0
	Night Heron (*Nycticorax nycticorax*)	7	0
	Black Kite (*Milvus migrans lineatus*)	37	0
	Asian Koel (*Eudynamys scolopacea*)	5	0
	Magpie and Magpie-Robin (*Copsychus* sp., *Pica* sp., and *Urocissa* sp.)	8	0
	Collared Scops Owl (*Otus lettia*)	9	0
	Eurasian Eagle Owl (*Bubo bubo*)	10	0
	Common Scops Owl (*Otus* spp.)	7	0
	Black-collared Starling (*Sturnus nigricollis*)	7	0
	Barn Swallow (*Hirundo rustica*)	5	0
	House Swift (*Apus nipalensis*)	5	0
Domestic poultry			
Sri Lanka	Chickens (*Gallus gallus*)	282	27 (9.6)
	Quails (*Coturnix* sp.)	14	0
	Ducks (*Anas platyrhynchos*)	54	0
	Geese (*Anser anser*)	5	0
Hong Kong	Chickens (*Gallus gallus*)	109	11 (10.1)
Total		874	45 (5.1)

these aquatic bird species found positive for astrovirus in Hong Kong and Cambodia had previously been reported as hosts for astrovirus infection.

The majority of rescued birds sampled by cloacal swabs at KFBG in Hong Kong were resident nonmigratory wild birds. Astroviruses were detected from 12.5% of feral pigeons (*Columba livia*, 2/16) and from 6.3% of spotted doves (*Spilopelia chinensis*, 1/16) but not from the other species, although the number of samples collected from some species was very small (Table 2).

Astrovirus was detected in 10.1% (11/109) of cloacal swabs of chickens collected in Hong Kong and in 9.6% (27/282) of chickens collected in Sri Lanka. No positives were detected in cloacal swabs from quails, ducks, and geese collected in Sri Lanka, although the sample sizes from these species were smaller (Table 2).

Phylogenetic analysis of the partial RdRp sequence amplified by our detection assay was done in comparison with other previously known astrovirus sequences retrieved from GenBank. These avian viral sequences can be phylogenetically divided into 3 major groups (Fig. 1). No evidence of astrovirus coinfection was detected in the studied sample. We further selected genetically distinct viral sequences as indicated by the phylogenetical tree for further analyses. The average amino acid sequence identities of RdRp genes compared within group and between groups are shown (Fig. 2A). All astroviruses detected from wild aquatic birds were novel viruses except for one group of viruses from northern pintails and Eurasian wigeons that were closely related to DuAstV (or DuHV2) and one virus detected from a common teal falling within the virus group of ANV. Interestingly, multiple novel astroviruses were identified from each of the four common wild duck species (northern pintail, northern shoveler, common teal and Eurasian wigeon; Fig. 1, highlighted in green, red, blue, and brown, respectively) in Mai Po marshes within our 3-month sampling period. Some of the sequences detected from different avian hosts were found to be genetically similar (e.g., MPJ0580/common teal and MPJ0554/northern shoveler; Fig. 1). Three genetically distinct viruses (RdRp gene identities $< 68\%$) were detected from samples collected from pond herons in Cambodia. Moreover, novel viruses were detected from both a black-faced spoonbill and a common greenshank in Hong Kong and from a lesser whistling duck in Cambodia. These findings reveal a previously unrecognized and large diversity of avastroviruses in wild aquatic birds.

All group 1 avian astroviruses were detected from hosts under the superorder of *Galloanserae* (Fig. 1). Five (TAstV1, TAstV2, DuAstV, DuHV3, and CAstV) of six previously known avian astroviruses are in this group. This group of viruses can be further divided into 3 subgroups. Subgroup 1.1 includes only one previously known member, TAstV1. Remarkably, viruses closely related to TAstV1 were repeatedly detected from our chicken samples collected from a poultry farm in Sri Lanka (see below). In subgroup 1.2, previously known viruses are DuHV3 and TAstV2. An astrovirus closely related to TAstV2 recently identified in guinea fowl (7) formed a sister clade with TAstV2 in this subgroup. Novel astroviruses found here included one virus from a lesser whistling duck (KH08-0856) and a group of viruses from northern shovelers (MPJ0597 and MPJ1355). In addition, a group of wild duck viruses genetically related to DuHV3 and TAstV2 were detected in our samples (e.g., MPJ1334 and MPJ1470). In subgroup 1.3, previously known viruses are DuAstV and CAstV (subtypes 1 and 2). CAstV1 strains were detected in chickens in Hong Kong and Sri Lanka and were genetically very similar. Novel viruses in this subgroup identified in this study include viruses from northern pintails (e.g., MPJ1345), Eurasian wigeons (e.g., MPJ1292 and MPJ0779), common teals (e.g., MPK514 and MPJ0580), and northern shovelers (e.g., MPJ0554). This analysis showed that DuAstV is closely related to viruses in northern pintails (MPJ1345 group) and Eurasian wigeons (MPJ1292 group), with RdRp gene sequence identities ranging from 83.4% to 92.9% (data not shown).

Group 2 avian astroviruses detected from our wild bird samples were all collected from birds of the orders of *Charadriiformes*, *Pelecaniformes*, and *Columbiformes* (Fig. 1). Previously known members in this group are avian nephritis virus (ANV), which was detected primarily from chickens, and pigeon astroviruses reported by Kofstad and Jonassen in 2011 (16). Novel viruses in this group include 3 genetically distinct viruses (RdRp gene identities 57.1% to 67.2%) from pond herons (KH08-1279, KH08-1314, and KH08-1285), a virus from a common greenshank (MPJ0918), a virus from a black-faced spoonbill (MPJ0829), and viruses from rock doves (KG119 and KG788) and from a spotted dove (KG703). Viruses detected from doves in Hong Kong are genetically closely related to previously known pigeon astroviruses found in Norway. This group of astroviruses from pigeons and doves is phylogenetically related to ANV. A number of ANV-like viruses were detected in chickens in Sri Lanka and Hong Kong. Interestingly, a virus detected from a common teal (MPJ0570) in our study is grouped into the clade for ANV.

Group 3 avian viruses form a novel group of viruses with no previously known member. The hosts of this novel group of astroviruses have exclusively been detected from 4 common wild duck species (*Anas* spp.) found in Hong Kong (i.e., MPJ0127, MPJ1561, MPJ1332, MPJ1402, MPJ0552, and MPJ07559). These group 3 astrovirus sequences formed 6 distinct clades in our phylogenetic analysis. The RdRp gene sequence identities of these 6 distinct clades of viruses range from 0.643 to 0.761. Unlike the group 1 and 2 avian astroviruses, group 3 astroviruses from each species fell into distinct clades.

The 3′-half genomes of 6 novel avian astroviruses were sequenced from representative samples. Complete capsid genes were predicted from these sequences, and sizes of the genes ranged from 1,941 nucleotides (nt) to 2,049 nt, which are similar to the gene sizes determined for other astroviruses. Phylogenetic analyses of the 5′ conserved region of these capsid genes agreed with those deduced from the RdRp sequence analyses, and three major groups of avian astroviruses were observed (Fig. 3). The average capsid protein amino acid sequence identities compared within groups and between groups are shown (Fig. 2B). It should be noted that the sequence identity of group 3 astroviruses is higher than those observed for group 1 and group 2 astroviruses. This is due to the rather small number of samples used in the analysis ($n = 3$). Repeated attempts at deducing ORF2 sequences from other representative group 3 astroviruses, however, have so far been unsuccessful.

From our surveillance of astroviruses in poultry, ANV and CAstV were detected from chicken samples collected in Hong Kong and in Sri Lanka, while TAstV1 was detected from 3 cloacal swab samples collected from apparently healthy chickens in poultry in Sri Lanka (Table 2). This is the first report of the detection of TAstV1-like virus in chickens. The chicken farm where these chicken samples were collected did not house turkeys. The source

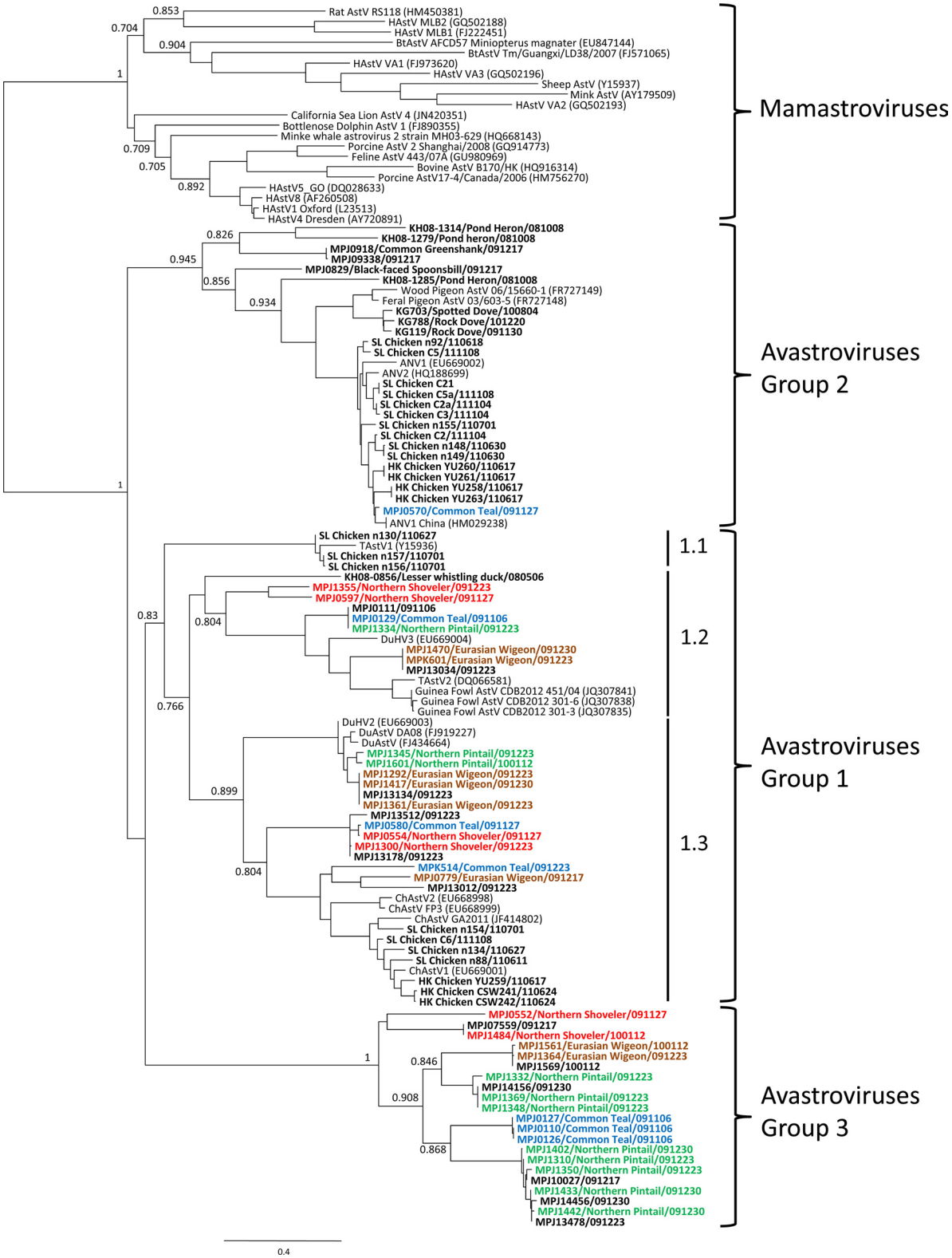

FIG 1 Phylogenetic analysis on RdRp genes of astroviruses using PhyML. Avastroviruses can be divided into 3 major groups. Astroviruses detected from this study ($n = 92$), excluding 14 viral sequences that yielded poor sequencing reads, were included in the analysis (highlighted in bold type). Viruses detected from northern pintail, northern shoveler, common teal, and Eurasian wigeon are highlighted in green, red, blue, and brown, respectively. The sampling site (for wild birds, KG = KFBG, Hong Kong, KH = Cambodia, and MPJ or MPK = Mai Po, Hong Kong; for poultry, HK = Hong Kong and SL = Sri Lanka), bird species (if available), and sampling time (indicated as last two digits of year, month, and day [YYMMDD]) of each sample is shown. Approximate likelihood ratio test (aLRT) values of major branches with values > 0.7 are indicated. GenBank accession numbers of retrieved genes are indicated in parentheses.

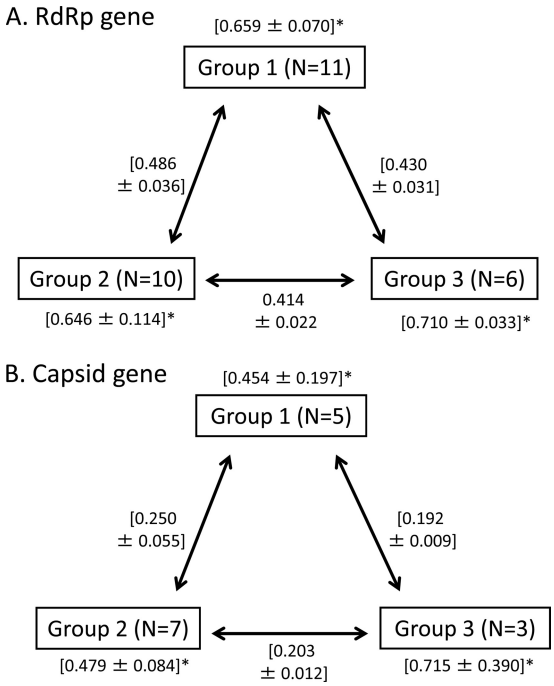

FIG 2 Mean amino acid sequence identities of representative viruses within and between the three major groups of avastroviruses were estimated. Standard deviations of the values are also indicated. The number of representative sequences used in each group is indicated in parentheses. An asterisk indicates that the intragroup sequence identity was found to be significantly higher than the relevant intergroup sequence identities ($P < 0.0005$, Student's t test). Viruses were selected for the analysis as follows. (A) Group 1, TAstV1, KH08-0856/lesser whistling duck, MPJ0597/northern shoveler, DuHV3, MPK601/Eurasian wigeon, TAstV2, DuAstV, MPJ0554/northern shoveler, MPK514/common teal, ChAstV2, and ChAstV1; group 2, MPJ0918/*Tringa nebularia*, KH08-1314/pond heron, KH08-1279/pond heron, MPJ0829/*Platalea minor*, KH08-1285/pond heron, wood pigeon astrovirus strain 06/15660-1, feral pigeon astrovirus strain 03/603-5, KG703/spotted dove, ANV1, and ANV2; group 3, MPJ0552/northern shoveler, MPJ1484/northern shoveler, MPJ1561/Eurasian wigeon, MPJ1332/northern pintail, MPJ0126/common teal, and MPJ1350/northern pintail. (B) Group 1, TAstV, chicken AstV GA2011, duck AstV DA08, TAstV3, and TAstV2; group 2, KH08-1279/pond heron, ANV China, ANV1, ANV2, KG119/rock dove, wood pigeon astrovirus 06/15660-1, and feral pigeon astrovirus 03/603-5; group 3, MPJ1332/northern pintail/capsid, MPJ1442/northern pintail/capsid, and MPJ1433/northern pintail.

from which chickens acquired infection of these viruses was unknown. No novel astroviruses were detected from the poultry samples tested. Nonetheless, results from surveillances conducted in other geographical regions, together with our observations, suggested that chickens are susceptible to avian astroviruses of diverse genetic backgrounds.

In this study, we detected astrovirus in 7.1% of fecal dropping samples from apparently healthy populations of wild aquatic birds in Hong Kong and in 1.7% of cloacal swab samples from wild birds sampled both in Cambodia and in Hong Kong, suggesting that infection with diverse astroviruses is common in wild bird populations. This study demonstrated a wide genetic divergence of novel avian astroviruses in different species of wild birds, a finding which significantly increases our understanding of the genetic diversity of astroviruses in avian hosts. Satellite tracking studies have shown that the migratory birds travel from Hong Kong to the north of China and to northeast Siberia along the Asia-Australia flyway (http://www.werc.usgs.gov/Project.aspx?ProjectID=37). Subsequent surveillance should be encouraged to further explore the ecology of astroviruses in wild birds in different countries, especially in areas along the bird migratory routes, as previous studies of avian influenza virus and coronaviruses have shown that migratory birds are able to carry viruses across widely disparate geographical locations (6, 23).

The discovery of diverse astroviruses in wild birds in this study enabled us to deduce the evolutionary relationships of astroviruses in poultry, and in avian hosts as a whole, more precisely. We observed that TAstV1 and TAstV2 clustered in different subgroups in the phylogenetic analysis, lending support to the conclusion that these viruses differ both genetically and serologically (17, 21). However, we observed close genetic relationships between TAstV2 and TAstV3, ANV1 and ANV2, and CAstV1 and CAstV2 (Fig. 3). Hence, the classification of these previously known avian astroviruses may need to be reconsidered. We also detected multiple astroviruses circulating in a single avian host species within a short period of time. Cocirculation of viruses provides ample chances for recombination to occur between viruses, a phenomenon which is well known for astroviruses (19). Our analyses of RdRp genes and capsid genes from the novel avastroviruses in wild birds revealed no evidence of recombination between these viruses. However, we observed that the RdRp gene of a recently published guinea fowl astrovirus (7) has a close genetic relationship to TAstV2 in subgroup 1.2, while the corresponding capsid gene was found to have a close genetic relationship with CAstV in subgroup 1.3. This observation agrees with the hypothesis that the guinea fowl virus emerged from a recombination event (7).

Based on our sequences, group 3 avian astroviruses appear to show a more stringent species specificity. This novel group of viruses was not detected in poultry by this or previous surveillance studies. In contrast, we repeatedly detected the same or very similar group 1 and group 2 viruses from multiple host species. Cross-host species infections of avastroviruses in poultry have been documented before (2, 3, 7, 9, 18, 24). Our findings of these events reconfirm the ability of some astroviruses to infect new hosts. For example, ANV and astroviruses in wild doves and pigeons are phylogenetically closely related. Notably, ANV has also recently been detected in pigeons (24). Moreover, we have detected an ANV-like virus from a common teal. It is not clear whether wild common teals acquired the virus in regions where ANV is endemic or whether this infection was acquired from another species. The role of migratory wild ducks in maintaining and spreading ANV and the significance of this for poultry farms need to be evaluated further. Apart from this observation, astroviruses that are genetically related to astroviruses found in ducks, chickens, and turkeys were also detected in our wild waterfowl samples, suggesting there were multiple interspecies transmissions between wild bird and domestic poultry populations. Future astrovirus surveillance in both wild birds and poultry might help to address this issue. In particular, the possible role of migratory wild ducks in maintaining avian astroviruses and the significance of this for poultry farms need to be studied. Given the frequency of the detection of astroviruses in migratory wild bird species, it is important to consider their interactions with other viruses, such as the avian influenza viruses, which are common in these species.

More than 20 novel viruses were discovered in this study, en-

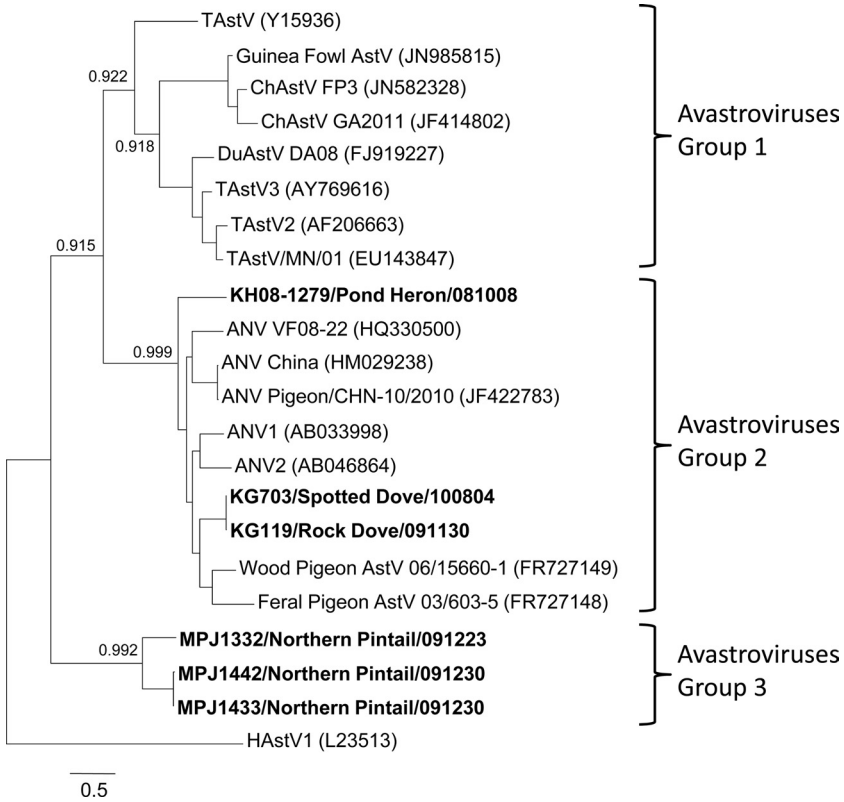

FIG 3 Phylogenetic analysis of the 5′ region of capsid genes (1,458 bp) of avastroviruses using PhyML. The capsid gene of human astrovirus was used as an outgroup. aLRT values of major branches with values > 0.7 are indicated. Due to the lack of sequence homology of the 3′ region of capsid genes (~1,000 bp), this was removed. The findings determined for the three major groups of avastroviruses shown in the analysis of RdRp genes were supported by this analysis of capsid genes. Novel viruses detected from wild birds in this study are indicated in bold type. GenBank accession numbers of retrieved genes are indicated in parentheses.

hancing our understanding of the diversity of astroviruses in wild birds. Nonetheless, based on the limited sample sizes and the geographical areas involved, it is likely that we have explored only the tip of the iceberg of avastrovirus diversity in nature. Future surveillance for avian astroviruses in wild birds will very likely elucidate further the diversity of avastroviruses and their ecological relationships to astroviruses in poultry.

Nucleotide sequence accession numbers. All novel virus gene sequences generated in this study were deposited in GenBank under accession numbers JX985647 to JX985730.

ACKNOWLEDGMENTS

We thank the surveillance team of the Centre of Influenza Research, School of Public Health, The University of Hong Kong, for sample collection. We thank colleagues at the Wild Animal Rescue Centre, Kadoorie Farm and Botanic Garden, in Hong Kong for collecting wild bird samples for this study. We also thank the Agriculture, Fisheries & Conservation Department of the Hong Kong SAR government for support regarding the use of wild rescued birds in this study.

This project was supported by the National Institutes of Health (NIAID contracts HHSN266200700005C and HHSN2662007 00007C) and EMPERIE (grant no. EU FP7 223498).

REFERENCES

1. **Abascal F, Zardoya R, Telford MJ.** 2010. TranslatorX: multiple alignment of nucleotide sequences guided by amino acid translations. Nucleic Acids Res. **38**:W7–W13.
2. **Biðin M, et al.** 2011. Detection and characterization of avian nephritis virus in ducklings. Avian Pathol. **40**:173–177.
3. **Canelli E, et al.** 2012. Astroviruses as causative agents of poultry enteritis: genetic characterization and longitudinal studies on field conditions. Avian Dis. **56**:173–182.
4. **Cheung PP, et al.** 2009. Identifying the species-origin of faecal droppings used for avian influenza virus surveillance in wild-birds. J. Clin. Virol. **46**:90–93.
5. **Chu DKW, Poon LLM, Guan Y, Peiris JSM.** 2008. Novel astroviruses in insectivorous bats. J. Virol. **82**:9107–9114.
6. **Chu DKW, et al.** 2011. Avian coronavirus in wild aquatic birds. J. Virol. **85**:12815–12820.
7. **De Battisti C, et al.** 2012. Genetic characterization of astroviruses detected in guinea fowl (Numida meleagris) reveals a distinct genotype and suggests cross-species transmission between turkey and guinea fowl. Arch. Virol. **157**:1329–1337.
8. **De Benedictis P, Schultz-Cherry S, Burnham A, Cattoli G.** 2011. Astrovirus infections in humans and animals—molecular biology, genetic diversity, and interspecies transmissions. Infect. Genet. Evol. **11**:1529–1544.
9. **de Wit JJ, et al.** 2011. Detection and characterization of a new astrovirus in chicken and turkeys with enteric and locomotion disorders. Avian Pathol. **40**:453–461.
10. **Edgar RC.** 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics **5**:113. doi: 10.1186/1471-2105-5-113.
11. **Fu Y, et al.** 2009. Complete sequence of a duck astrovirus associated with fatal hepatitis in ducklings. J. Gen. Virol. **90**:1104–1108.
12. **Guindon S, et al.** 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. **59**:307–321.

13. **Hall TA.** 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. **41**:95–98.
14. **Jindal N, Patnayak DP, Chander Y, Ziegler AF, Goyal SM.** 2011. Comparison of capsid gene sequences of turkey astrovirus-2 from poult-enteritis-syndrome-affected and apparently healthy turkeys. Arch. Virol. **156**:969–977.
15. **Jindal N, Patnayak DP, Chander Y, Ziegler AF, Goyal SM.** 2010. Detection and molecular characterization of enteric viruses from poult enteritis syndrome in turkeys. Poult. Sci. **89**:217–226.
16. **Kofstad T, Jonassen CM.** 2011. Screening of feral and wood pigeons for viruses harbouring a conserved mobile viral element: characterization of novel astroviruses and picornaviruses. PLoS One **6**:e25964. doi:10.1371/journal.pone.0025964.
17. **Pantin-Jackwood MJ, et al.** 2011. Molecular characterization of avian astroviruses. Arch. Virol. **156**:235–244.
18. **Pantin-Jackwood MJ, Spackman E, Woolcock PR.** 2006. Molecular characterization and typing of chicken and turkey astroviruses circulating in the United States: implications for diagnostics. Avian Dis. **50**:397–404.
19. **Pantin-Jackwood MJ, Spackman E, Woolcock PR.** 2006. Phylogenetic analysis of Turkey astroviruses reveals evidence of recombination. Virus Genes **32**:187–192.
20. **Posada D.** 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. **25**:1253–1256.
21. **Strain E, Kelley LA, Schultz-Cherry S, Muse SV, Koci MD.** 2008. Genomic analysis of closely related astroviruses. J. Virol. **82**:5099–5103.
22. **Todd D, et al.** 2009. Identification of chicken enterovirus-like viruses, duck hepatitis virus type 2 and duck hepatitis virus type 3 as astroviruses. Avian Pathol. **38**:21–30.
23. **Wang G, et al.** 2008. H5N1 avian influenza re-emergence of Lake Qinghai: phylogenetic and antigenic analyses of the newly isolated viruses and roles of migratory birds in virus circulation. J. Gen. Virol. **89**:697–702.
24. **Zhao W, et al.** 2011. Detection of astrovirus infection in pigeons (Columbia livia) during an outbreak of diarrhoea. Avian Pathol. **40**:361–365.