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Short-form paper

A novel group of avian astroviruses in wild aquatic birds

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Abstract

Using a pan-astrovirus RT-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.

Main text

Avian astroviruses 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. Severity varies 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 (DAstV) (formerly named as duck hepatitis virus 2) and duck hepatitis virus 3 (DHV3). Among these viruses, turkey astroviuses (TAstV) from turkey and avian nephritis virus (ANV) from chickens are the two viruses 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, has 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 were 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 inter-species transmission. Infection of avian astroviruses in these hosts has not always been associated with diseases (18), but the significance of the interspecies transmission of astrovirus between these avian species to the astrovirus ecology requires further investigation.

To examine the diversity of astroviruses in wild birds and avian poultry, we studied: 1) fecal samples of wild birds collected in Mai Po marshes, Hong Kong, 2) cloacal swabs samples of wild birds collected in Cambodia and in Hong Kong and 3) cloacal swabs from poultry in Hong Kong and Sri Lanka. The Mai Po marshes in Hong Kong are a wetland habit of international importance, especially for wild waterfowls. We studied avian populations in this area during the winter season, including migratory aquatic birds from northern latitudes that gather in Mai Po particularly during the non-breeding 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 Mai Po Marshes in Hong Kong from October 2010 to January 2011. Cloacal swabs were collected from wild birds being sold in markets around the Tonle Sap Basin, Cambodia by the Wildlife Conservation Society (WCS) 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 years 2009 to 2011. In addition, cloacal swabs were collected from chickens in wet markets in Hong Kong and from chickens, quails, ducks and geese from poultry farms in Sri Lanka both during 2011.

RNA was extracted from bird dropping samples and swab samples kept in viral transport medium using 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 RT-PCR assay targeting the RdRp gene (5). All PCR amplicons with 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 avian 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 3’RACE system for rapid amplification of cDNA ends kit (Invitrogen) followed by PCR amplification of the 3’ half genome using gene specific primers targeting the RdRp gene and targeting the poly-A tail. Attempts in 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 by 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 three-month period and the positive rates of each sampling occasion ranged from 2.8% to 14.7%. All the astrovirus positive faecal 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 that was broadly similar to that of astrovirus-positive ones (data not shown). From 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 these aquatic bird species found positive for astrovirus in Hong Kong and Cambodia had previously been reported as being hosts for astrovirus infection.

The majority of rescued birds sampled by cloacal swabs at KFBG in Hong Kong were resident non-migratory 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 is 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 chicken 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 (Figure 1). No evidence of astrovirus co-infection was detected in the studied sample. We further selected genetically distinct viral sequences as indicated by this phylogenetical tree for further analyses. The average amino acid sequence identities of RdRp genes compared within group and between groups were shown

(Figure 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 in Mai Po marshes within our three-month sampling period (northern pintail, northern shoveler, common teal and Eurasian wigeon; Figure 1, highlighted in green, red, blue and brown, respectively). Some of the sequences detected from different avian hosts were found to be genetically similar (e.g. MPJ0580/Common Teal and MPJ0554/Northern Shoveler; Figure 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 a black-faced spoonbill and a common greenshank both in Hong Kong, and 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* (Figure 1). Five out of six previously known avian astroviruses are in this group (TAstV1, TAstV2, DuAstV, DuHV3, CAstV). This group of viruses can be further divided into 3 sub-groups. 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 clades with TAstV2 in this subgroup. Novel astroviruses found here include one virus from a lesser whistling duck (KH08-0856), a group of viruses from northern shovelers (MPJ0597 and MPJ1355). In addition, a group of wild duck viruses which is genetically related to the DuHV3 and TAstV2 was 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 was 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 ranged from 83.4% and 92.9% (data not shown).

Group 2 avian astroviruses detected from our wild bird samples were all collected from birds under the orders of *Charadriiformes*, *Pelecaniformes* and *Columbiformes* (Figure. 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% – 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 are phylogenetically related to ANV. A number of ANV-like viruses were detected in chicken 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 are a novel group of viruses with no previously known member. The hosts of this novel group of astroviruses are exclusively 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 between these 6 distinct clades of viruses range from 0.643 - 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 1941 nt to 2049 nt, which are similar to 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 (Figure 3). The average capsid protein amino acid sequence identities compared within group and between groups are shown (Figure 2B). It should be noted that the sequence identity of group 3 astrovirus is higher than those observed from group 1 and group 2 astroviruses. This is due to the number of samples used in the analysis is rather small (N=3). Repeated attempts in deducing ORF2 sequences from other representative group 3 astroviruses, however, were unsuccessful so far.

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 detecting TAstV1-like virus in chicken. The chicken farm where these chicken samples were collected did not house turkeys. The source 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 faecal dropping samples from apparently healthy populations of wild aquatic birds in Hong Kong and in 1.7% of cloacal swab samples from wild birds both sampled 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 in travel from Hong Kong to 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 as well as serologically (17, 21). However, we observed close genetic relationships between TAstV2 and TAstV3, ANV1 and ANV2, and CAstV1 and CAstV2 (Figure 3). Hence, the classification of these previously known avian astroviruses may be needed to be reconsidered. We also detected multiple astroviruses circulating in a single avian host species within a short period of time. Co-circulation of viruses provides ample changes 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 close genetic relationship to TAstV2 in subgroup 1.2, while the capsid gene of that was found to have close genetic relationship with CAstV in subgroup 1.3. This observation agrees with the hypothesis that the guinea fowl virus could have 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 reiterate the ability of some astroviruses to infect in 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 ANV could be endemic in wild common teals, 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 to poultry farms needs 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 for in maintaining avian astroviruses and the significance of this to poultry farms needs to be studied. Given the frequency of the detection of astroviruses in migratory wild bird species, it will be important to consider their interactions with other viruses such as avian influenza viruses which are common in these species.

More than 20 novel viruses were discovered in this study, enhancing our understandings on the diversity of astroviruses in wild birds. Nonetheless, based on the limited sample sizes and the involved geographical areas, it is likely that we have only explored the tip of the iceberg of avastrovirus diversity in nature. Future surveillance for avian astroviruses in wild bird will very likely elucidate further the diversity of avastroviruses and their ecological relationships to astroviruses in poultry.

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Figure legends

Figure 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 bolded texts). Viruses detected from northern pintail, northern shoveler, common teal and Eurasian wigeon are highlighted in green, red, blue and brown, respectively. The sampling site (Wild birds: KG=KFBG, Hong Kong; KH=Cambodia and MPJ or MPK=Mai Po, Hong Kong; Poutlry: HK= Hong Kong; and SL=Sri Lanka), bird species (if available) and sampling time (YYMMDD) of each sample is shown. Approximate likelihood ratio test (aLRT) values of major branches with values > 0.7 were indicated. GenBank accession numbers of retrieved genes were indicated in brackets.

Figure 2. Mean amino acid sequence identities of representative viruses were estimated within and between the three major groups of avastroviruses. Standard deviations of the values were also indicated. The number of representative sequences used in each group was indicated in brackets. An asterisk indicates that the intra-group sequence identity was found to be significantly higher than the relevant inter-group sequence identities ($P < 0.0005$, Student' t-test). Viruses selected for the analysis are: 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.

Figure 3. Phylogenetic analysis on the 5' region of capsid genes (1458 bp) of avastroviruses using PhyML. Capsid gene of human astrovirus was used as an outgroup. aLRT values of major branches with values > 0.7 were indicated. Due to the lack of sequence homology of the 3' region of capsid genes (~1000 bp) this was removed. Three major groups of avastroviruses shown in analysis on RdRp genes were supported by this analysis on capsid genes. Novel viruses detected from wild birds in this study are presented in bold type. GenBank accession numbers of retrieved genes were indicated in brackets.

Table 1. Bird droppings collected at MaiPo samples that are RT-PCR positive for astrovirus.

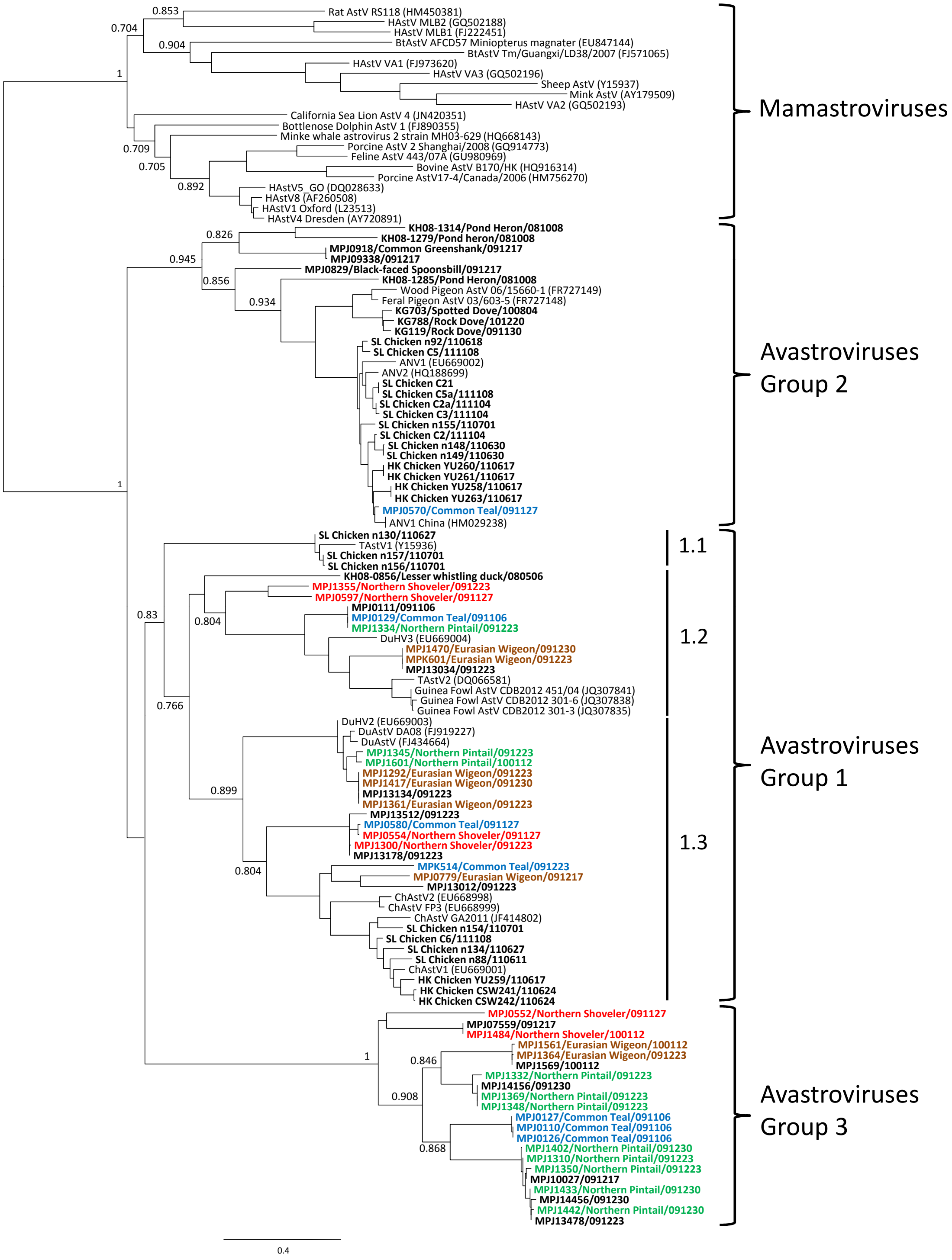
Species*	AstV pos (% of total)
Black-faced Spoonbill (*Platalea minor*)	1 (2%)
Common Greenshank (*Tringa nebularia*)	1 (2%)
Common Teal (*Anas crecca*)	5 (11%)
Eurasian Wigeon (*Anas Penelope*)	8 (17%)
Great Cormorant (*Phalacrocorax carbo*)	0
Grey Heron (*Ardea cinerea*)	0
Night Heron (*Nycticorax nycticorax*)	0
Northern Pintail (*Anas acuta*)	11 (23%)
Northern Shoveler (*Anas clypeata*)	7 (15%)
Unknown	14 (30%)
Total	47 (100%)

* Avian species were determined by DNA fingerprinting

Table 2. Detection of astroviruses in cloacal swabs collected from different regions

	Bird species	No. of samples	No. of AstV pos (%)
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%)

Figure 1



0.853
Rat AstV RS118 (HM450381)
HAstV MLB2 (GQ502188)
0.704
HAstV MLB1 (FJ222451)
0.904
BtAstV AFCD57 Miniopterus magnater (EU847144)
BtAstV Tm/Guangxi/LD38/2007 (FJ571065)
HAstV VA1 (FJ973620)
1
HAstV VA3 (GQ502196)
Sheep AstV (Y15937)
Mink AstV (AY179509)
HAstV VA2 (GQ502193)
California Sea Lion AstV 4 (JN420351)
Bottlenose Dolphin AstV 1 (FJ890355)
Minke whale astrovirus 2 strain MH03-629 (HQ668143)
0.709
Porcine AstV 2 Shanghai/2008 (GQ914773)
Feline AstV 443/07A (GU980969)
0.705
Bovine AstV B170/HK (HQ916314)
Porcine AstV17-4/Canada/2006 (HM756270)
0.892
HAstV5_GO (DQ028633)
HAstV8 (AF260508)
HAstV1 Oxford (L23513)
HAstV4 Dresden (AY720891)
KH08-1314/Pond Heron/081008
0.826
KH08-1279/Pond heron/081008
MPJ0918/Common Greenshank/091217
0.945
MPJ09338/091217
MPJ0829/Black-faced Spoonbill/091217
0.856
KH08-1285/Pond Heron/081008
Wood Pigeon AstV 06/15660-1 (FR727149)
0.934
Feral Pigeon AstV 03/603-5 (FR727148)
KG703/Spotted Dove/100804
KG788/Rock Dove/101220
KG119/Rock Dove/091130
SL Chicken n92/110618
SL Chicken C5/111108
ANV1 (EU669002)
ANV2 (HQ188699)
SL Chicken C21
SL Chicken C5a/111108
SL Chicken C2a/111104
SL Chicken C3/111104
SL Chicken n155/110701
SL Chicken C2/111104
SL Chicken n148/110630
SL Chicken n149/110630
HK Chicken YU260/110617
HK Chicken YU261/110617
1
HK Chicken YU258/110617
HK Chicken YU263/110617
MPJ0570/Common Teal/091127
ANV1 China (HM029238)
SL Chicken n130/110627
TAstV1 (Y15936)
SL Chicken n157/110701
SL Chicken n156/110701
KH08-0856/Lesser whistling duck/080506
MPJ1355/Northern Shoveler/091223
MPJ0597/Northern Shoveler/091127
0.83
MPJ0111/091106
0.804
MPJ0129/Common Teal/091106
MPJ1334/Northern Pintail/091223
DuHV3 (EU669004)
MPJ1470/Eurasian Wigeon/091230
MPK601/Eurasian Wigeon/091223
MPJ13034/091223
TAstV2 (DQ066581)
Guinea Fowl AstV CDB2012 451/04 (JQ307841)
0.766
Guinea Fowl AstV CDB2012 301-6 (JQ307838)
Guinea Fowl AstV CDB2012 301-3 (JQ307835)
DuHV2 (EU669003)
DuAstV DA08 (FJ919227)
DuAstV (FJ434664)
MPJ1345/Northern Pintail/091223
MPJ1601/Northern Pintail/100112
MPJ1292/Eurasian Wigeon/091223
MPJ1417/Eurasian Wigeon/091230
0.899
MPJ13134/091223
MPJ1361/Eurasian Wigeon/091223
MPJ13512/091223
MPJ0580/Common Teal/091127
MPJ0554/Northern Shoveler/091127
MPJ1300/Northern Shoveler/091223
MPJ13178/091223
0.804
MPK514/Common Teal/091223
MPJ0779/Eurasian Wigeon/091217
MPJ13012/091223
ChAstV2 (EU668998)
ChAstV FP3 (EU668999)
ChAstV GA2011 (JF414802)
SL Chicken n154/110701
SL Chicken C6/111108
SL Chicken n134/110627
SL Chicken n88/110611
ChAstV1 (EU669001)
HK Chicken YU259/110617
HK Chicken CSW241/110624
HK Chicken CSW242/110624
MPJ0552/Northern Shoveler/091127
MPJ07559/091217
MPJ1484/Northern Shoveler/100112
MPJ1561/Eurasian Wigeon/100112
1
MPJ1364/Eurasian Wigeon/091223
0.846
MPJ1569/100112
MPJ1332/Northern Pintail/091223
MPJ14156/091230
0.908
MPJ1369/Northern Pintail/091223
MPJ1348/Northern Pintail/091223
MPJ0127/Common Teal/091106
MPJ0110/Common Teal/091106
MPJ0126/Common Teal/091106
0.868
MPJ1402/Northern Pintail/091230
MPJ1310/Northern Pintail/091223
MPJ1350/Northern Pintail/091223
MPJ10027/091217
MPJ1433/Northern Pintail/091230
MPJ14456/091230
MPJ1442/Northern Pintail/091230
MPJ13478/091223
0.4
Mamastroviruses
Avastroviruses
Group 2
1.1
1.2
Avastroviruses
Group 1
1.3
Avastroviruses
Group 3

Figure 2

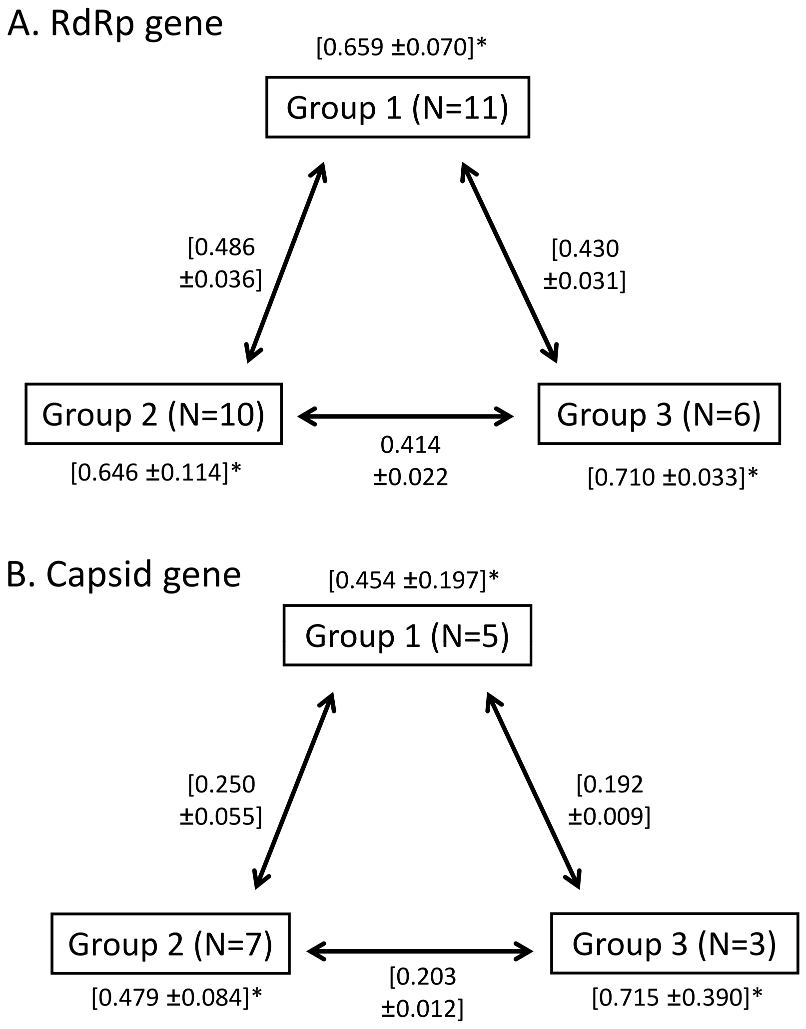
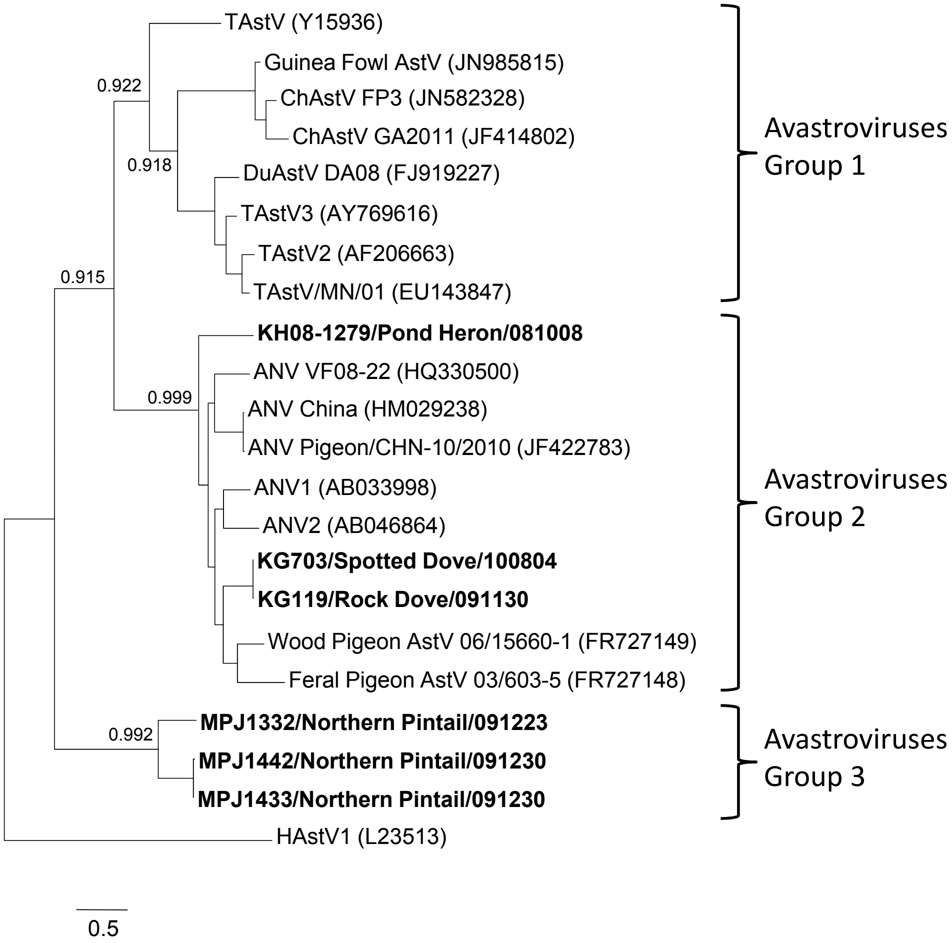

A. RdRp gene
[0.659 ±0.070]*
Group 1 (N=11)
[0.486 ±0.036]
[0.430 ±0.031]
Group 2 (N=10)
Group 3 (N=6)
0.414 ±0.022
[0.646 ±0.114]*
[0.710 ±0.033]*
B. Capsid gene
[0.454 ±0.197]*
Group 1 (N=5)
[0.250 ±0.055]
[0.192 ±0.009]
Group 2 (N=7)
Group 3 (N=3)
[0.479 ±0.084]*
[0.203 ±0.012]
[0.715 ±0.390]*

Figure 3.



TAstV (Y15936)
Guinea Fowl AstV (JN985815)
ChAstV FP3 (JN582328)
ChAstV GA2011 (JF414802)
DuAstV DA08 (FJ919227)
TAstV3 (AY769616)
TAstV2 (AF206663)
TAstV/MN/01 (EU143847)
KH08-1279/Pond Heron/081008
ANV VF08-22 (HQ330500)
ANV China (HM029238)
ANV Pigeon/CHN-10/2010 (JF422783)
ANV1 (AB033998)
ANV2 (AB046864)
KG703/Spotted Dove/100804
KG119/Rock Dove/091130
Wood Pigeon AstV 06/15660-1 (FR727149)
Feral Pigeon AstV 03/603-5 (FR727148)
MPJ1332/Northern Pintail/091223
MPJ1442/Northern Pintail/091230
MPJ1433/Northern Pintail/091230
HAstV1 (L23513)
0.922
0.918
0.915
0.999
0.992
0.5
Avastroviruses Group 1
Avastroviruses Group 2
Avastroviruses Group 3