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Full Length Research Paper

Yaoundé-like virus in resident wild bird, Ghana

Williams, Richard A. J.[1,2]*, Vázquez, Ana[3], Asante, Ivy[4], Bonney, Kofi[4], Odoom, Shirley[4], Puplampu, Naiki[4], Ampofo, William[4], Sánchez-Seco, María Paz[3], Tenorio, Antonio[3] and Peterson, A. Townsend[1]

[1]University of Kansas Biodiversity Institute, 1345 Jayhawk Blvd, Lawrence, Kansas, USA.
[2]Departamento de Zoología y Antropología Física, Complutense Universidad, Madrid 28040, Spain.
[3]Centro Nacional de Microbiología, Institute of Health Carlos III, Majadahonda, Spain.
[4]Noguchi Memorial Institute for Medical Research, Accra, Ghana.

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Tissue and swab samples from 551 wild birds collected in Ghana (October-November 2007) were assayed for alphaviruses, flaviviruses, and influenza A viruses using polymerase chain (PCR) techniques. One pool sample tested positive for *Flavivirus* RNA; further testing revealed that the amplified sequence was Yaoundé virus (YAOV), or closely related to it. YAOV is an apparently rare *Flavivirus* closely related to medically important human pathogens Japanese Encephalitis virus and West Nile virus. It is known only from West Africa. This is the first detection from Ghana, and only the second detection from a bird. Samples were negative for alphaviruses and Influenza A virus.

Key words: Birds, Flavivirus, Ghana, West Nile virus, Yaoundé virus.

INTRODUCTION

Recent range expansion of bird-borne viruses, particularly highly pathogenic avian influenza H5N1 (HPAI-H5N1), and West Nile virus (WN), have caused significant mortality in humans, wildlife and livestock (Daszak et al., 2000). Both viruses, and related taxa, are associated with wild birds, and spill over from wild bird reservoirs to infect humans (Webster et al., 1992; Komar, 2003). Although, these bird-borne pathogen shifts have attracted considerable research and public health attention, the details of bird-virus host associations in many cases remain poorly known.

Host-virus dynamics may vary according to viral and avian species, for instance WN amplifies principally among passerine songbirds (Ezenwa, 2006; Komar et al., 2003), whereas JE amplifies principally among Ciconiiformes (herons and allies; Buescher et al., 1959; Endy et al., 2002; Jamgaonkar, 2003), and so forth. Certainly experimental infection of avian hosts demonstrates that both susceptibility to infection and the level and duration of viremia vary by species for WN (Komar et al., 2003) and St. Louis Encephalitis virus (McLean et al., 1985). Similarly, Anseriformes (ducks and geese) and Charadriiformes (shorebirds, gulls, and terns) are considered the primary reservoirs for avian influenza (AI) strains, notwithstanding the detection of more broadly across class Aves (Hesterberg et al., 2009).

No data are available on host range, prevalence, or habitat type for Flaviviruses or Influenza A viruses in wild birds in Ghana. Indeed, very little information is available for West Africa. Several Flaviviruses, notably WN, are present in West Africa (MacKenzie, 2002), and likely present in Ghana. A recent human survey found high WN seroprevalences, 30% (Wang, 2009), suggesting transmission in amplifying (avian) hosts. Investigation of AI strains in wild birds in sub-Saharan Africa has thus far concentrated on water birds, and several AI strains have been detected in West Africa (Gaidet et al., 2007), including HPAI-H5N1 (Enserink, 2006), which has been detected throughout West Africa, including Ghana (Williams et al., 2008).

Although, AI strains and Flaviviruses are clearly

*Corresponding author. E-mail: ricw.ku@gmail.com. Tel: +34 91 854 0360. Fax: +1 785 864 5335.

versatile as regards their range of possible hosts, some viruses appear to demonstrate some degree of non-random host association. These associations nevertheless remain untested and uncharacterized both over most virus distributions, and across most of host diversity.

As a result, in October and November 2007, we conducted broad–scale virus surveillance among land birds, in two distinct habitat types, savannah-woodland and rain forest, to assess the distribution of avian Influenza A viruses and Flaviviruses, using PCR techniques. In addition, we tested all samples for the presence of another bird-associated group, Alphaviruses. To the best of our knowledge, this survey is the first broad-scale study of these virus groups in Ghanaian land birds.

MATERIALS AND METHODS

We sampled and tested 551 free ranging birds and 12 backyard chickens for *Alphavirus*, *Flavivirus*, and influenza A virus infections using PCR techniques. Sampling was conducted in Ghana, at Ankasa Conservation Area (*N* = 305 individuals, 70 species), Western Region, and Gbele Resource Reserve (*N* = 259, 73 species), Upper West Region, in October-November 2007. Birds were obtained by mist netting and selective harvesting with shotguns; all birds were apparently healthy at the time of collection. Buccal and cloacal swabs were collected for up to 30 individuals per species, and mixed tissue samples (brain, gonad, intestine, kidney and lung) for up to 10 individuals per species. Samples were frozen immediately in liquid nitrogen, and voucher specimens were prepared for all samples and deposited at the University of Kansas Biodiversity Institute. 95.5% of individuals sampled were resident, 2.9% were Palearctic migrants, and the remaining 1.6% were species with populations that include internal African migrants.

Viral RNA was extracted from homogenized tissue pools using RNEasy minikit (QIAGEN, Valencia, CA, USA), and from swab pools prepared in VTM (containing 2.5% veal infusion broth, 0.5% BSA, 100 µg/ml gentamicin sulfate and 2 µg/ml Fungizone) using QIAamp Viral RNA Mini Kit (QIAGEN). Tissues were homogenized using TissueRuptor (QIAGEN). Molecular RNA detection techniques were used to detect genomic material from alphaviruses (Sánchez-Seco et al., 2001), flaviviruses (Pierre et al., 1994; Sánchez-Seco et al., 2005) and influenza viruses (CDC, 2009; Spackman et al., 2002). A flavivirus was detected from one pool of tissues from seven birds using the nested RT-PCR technique, although other tests resulted negative. The 100 nt amplification product was detected by electrophoresis, and purified using QIAquick PCR Purification Kit (QIAGEN). Sequencing reactions were performed with ABI Prism BigDye Terminator Cycle Sequencing v.3.1 Ready Reaction (Applied Biosystems, Foster City, CA, USA), and analyzed using an ABI PRISM model 3730 automated sequencer (Applied Biosystems). Assembly of consensus sequences and translation into amino acid sequences was performed with EditSeq (DNASTAR Inc., Madison, WI, USA). Phylogenetic analyses were developed relative to 13 *Flavivirus* sequences; all aligned using ClustalX (http://www.clustal.org/). Phylogenetic trees were constructed by Bayesian analysis, using MrBayes v. 3.1.2 (http://mrbayes.csit.fsu.edu/), with 10,000,000 cycles for the Markov chain Monte Carlo algorithm, and specifying TBEV (GenBank Acc No: NC_001672) as outgroup. Bayesian posterior probabilities were calculated from the consensus of 10,000 trees.

RESULTS AND DISCUSSION

One tissue pool of seven resident wild birds captured among the two sites was positive for *Flavivirus* NS5 sequence. The positive pool contained one *Alethe diademata*, two individuals of *Andropadus virens*, one *Oriolus brachyrhynchus*, and one *Tauraco macrorhynchus* from Ankasa, and one each of *Francolinus bicalcaratus* and *Petronia dentata* from Gbele. Attempts to determine viral host identity by testing samples from seven individuals from the *Flavivirus* positive pool individually, using the generic RT-nested PCR (Sánchez-Seco, 2005), were negative, presumably hampered by multiple failures of the cold chain for the diagnostic samples. Triturated tissue (and associated swab storage buffer) from individuals in the positive pool were inoculated into C6/36 (*Aedes albopictus* cells) and Vero (African green monkey cells) monolayer cells; no cytopathic effects were detected after three blind passages. Supernatant samples drawn from all passages of cell cultures were negative using the generic RT-nested PCR (Sánchez-Seco, 2005). Further attempts to amplify a more informative sequence using nine generic RT-nested PCR protocols (Kuno et al., 1998, and ISCIII designed primers) were unsuccessful.

The putative YAOV sequence (GenBank accession number HQ290163) showed 92% sequence identity with a published YAOV strain (EU074036), with 8 nt differences (Figure 1), none producing amino acid replacements. Phylogenetic analyses (data not shown) confirmed the close relation of our sequence and YAOV (93% posterior probability). The virus we detected is thus YAOV, or one that is quite closely related.

Yaoundé virus (YAOV) is a mosquito-borne *Flavivirus* within the *Japanese encephalitis virus* (JEV) serocomplex which includes human pathogens such as JEV and *West Nile virus* (WNV; Kuno et al., 1998). YAOV has been isolated from one bird (*Bycanistes sharpii*), 2 mammal species (*Praomys* sp. and *Cavia porcellus*), and 10 mosquito species from four sub-Saharan African nations (Cameroon, Central African Republic, Congo, and Senegal; Adam and Digoutte, 2005). The virus was first isolated from a *Culex nebulosus* pool collected from degraded semi-deciduous forest near Nkolbisson, Cameroon, in 1968 (Digoutte, 1992). Although, YAOV has not been detected in humans (Adam and Digoutte, 2005), many other JEV group taxa have been.

To our knowledge, an active JEV group infection has not previously been detected directly in Ghana (that is, through the detection of viral RNA, viral culture, etc.). However, WNV presence has been inferred from a human serologic study that found an IgG seroprevalence of 27.9% in adults, and IgM seroprevalence of 2.4% in children <6 years old (Wang et al., 2009), the latter result indicates recent infection, presumably within Ghana. Given the close serologic relationships among JEV serocomplex viruses, it is possible that Yaoundé virus

Strain	0’				50’
YAOV: EU074036	AAAACGTGAG	AAGAAGCCAG	GGGAATTCGG	AAAGGCAAAA	GGAAGCCGAG
Ave-Ghana: HQ290163	----------	-----A----	----------	---A------	--------G-
JEV: M18370	----A-A---	--------T-	-A--G--T--	---A--T---	------A-G-
WNVI: DQ211652	---GA-A---	--A--A--C-	-A--G-----	------C--G	------A---

	51’				100’
YAOV: EU074036	CCATCTGGTA	CATGTGGCTC	GGAGCCCGAT	TCTTGGAGTT	TGAAGCCCTT
Ave-Ghana: HQ290163	----T-----	----------	-----T----	-TC-------	---G------
JEV: M18370	----T----T	---------T	-----A--G-	ATC-A-----	------TT-G
WNVI: DQ211652	----T----T	----------	-----T--C-	-TC-------	C--G--T--G

Figure 1. Nucleotide -level comparison of sequenced fragment and *Yaoundé virus* (YAOV) sequences available on GenBank (catalog numbers provided). Comparisons with published sequences of known flaviviruses were performed by searches with the FASTA program in the EMBL database (www.ebi.ac.uk/embl) to identify the detected agent and study the level of homology. Here we show a nucleotide level comparison of sequenced fragment and *Yaoundé virus* (YAOV) available on GenBank (catalog numbers provided). Sequences from *Japanese Encephalitis virus* (JEV; 22 nt differences) and *West Nile virus* lineage 1 (WNV1; 21 nt differences), taxa closely related to YAOV are included to illustrate the close relationship between our sequence and YAOV. Dash indicates coincident nucleotide representation.

could be the source of WNV-reactive antibodies.

In conclusion, we detected one Flavivirus in a survey of asymptomatic wild birds in Ghana. The sequence showed a close relationship with YAOV. No alphaviruses or Influenza A viruses were recovered, suggesting that circulation of these virus groups is low, or zero, at these sites at the time of sampling.

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