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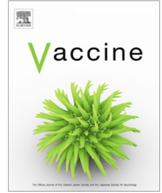

Brief report

Reverse spillover of avian viral vaccine strains from domesticated poultry to wild birds

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Transmission of viruses from the commercial poultry to wild birds is an emerging paradigm of livestock–wildlife interface. Here, we report the identification and isolation of vaccine strains of avian paramyxovirus serotype 1 (APMV1) and avian coronaviruses (ACoV) from different wild bird species across eight Egyptian governorates between January 2014 and December 2015. Surveillance of avian respiratory viruses in free-ranging wild birds (n = 297) identified three species that harboured or excreted APMV1 and ACoVs. Genetic characterization and phylogenetic analysis of recovered viruses revealed a close association with the most widely utilized vaccine strains in the country. These results highlight the potential spillover of vaccine-viruses probably due to extensive use of live-attenuated vaccines in the commercial poultry, and close interaction between domesticated and wild bird populations. Further exploring the full spectrum of vaccine-derived viral vaccine strains in wild birds might help to assess the emergence of future wild-birds origin viruses.

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1. Introduction

Continuous evolution and emergence of new antigenic and genetic variants of avian respiratory viruses represents the main risk to poultry populations and disease occurrence worldwide. Despite mass vaccination, avian influenza (AIV), avian paramyxovirus serotype 1 (APMV1) (i.e. Newcastle disease viruses, NDV) and avian coronaviruses (ACoV) (i.e. infectious bronchitis virus, IBV) are regarded as the most important avian viruses that are causing serious economic losses, trade restrictions, and food security issues in the poultry industries. Vaccinations are being applied in domestic poultry to achieve high standards of poultry production, control viral infections and to reduce economic losses [1]. The impacts of these vaccines on the interface between domestic and wild bird population are incompletely studied especially when intensive poultry farming are being practiced under poor biosecurity settings.

Wild birds have been proposed to play crucial roles in the spillover and in potentiating the virulence of avian viruses [2]. However, intensive vaccines application, contamination of the environment with infectious material through disposal or re-use of poultry litter and due to close contacts between wild birds and commercial poultry may lead to reversal of spillover (reverse spillover) from farmed poultry to wild birds [3]. Likewise, the transmission of virulent (e.g. escape mutant) viruses that have evolved either in response to vaccine pressure or through undetected viral contaminants within commercial vaccines are being transmitted to wild birds [2]. In high-tech commercial poultry farming, the possibility of spillover scenario into wild birds can be mitigated by the strict biosecurity measures. However, backyard poultry rearing meliorates these contacts especially in agriculture-enriched countries [4].

It is critically important to improve approaches for viral surveillance and epidemiology that can assist understanding the in-depth evolutionary aspects of viruses in wild birds. In the present study, considering all preliminary evidences on the presence of live vaccines in wild birds [5,6], we examined the hypothesis that APMV1 and ACoV vaccines may spill into wild birds. To this end, we performed virus surveillance in the Egyptian wild birds, and assessed the magnitude of ACoV and APMV1 prevalence in the country. The sequenced data from identified strains of APMV1 and ACoV from Egyptian wild birds were assessed along with the previously reported data in public domains on the vaccine-derived APMV1 and ACoV strains. Cumulative data indicates that ACoV and APMV1

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vaccine strains can be identified in wild birds that are housed in the vicinity of commercial poultry farms. These viral vaccine-strains isolated from wild birds have shown significant genetic similarities with the vaccine strains being applied in the commercial poultry farms suggesting the possible reverse spillover of these viruses to nearby wild life. These findings highlight the need to fully investigate the dynamics and spectrum of vaccine-derived viral strains in wild birds and that such virus detection would severely jeopardise the welfare of the wild birds and may in future leads to virus evolution with increased virulence, as has been proposed for avian influenza viruses [1,2].

2. Materials and methods

2.1. Samples collection and virus isolation

A total of two hundred and nighty seven (n = 297) oral and cloacal swabs were collected from randomly selected and apparently healthy wild birds from eight Egyptian governorates between January 2014 and December 2015 for active and passive surveillance of avian respiratory viruses including AIV, ACoV and APMV1 (Table 1). Wild birds were captured using a combination of hand-nets, drop-nets, mist-nets, and ground traps. Authorized veterinarians of the Central Laboratory for Evaluation of Veterinary Biologics, Egypt obtained swabs samples from live birds. Samples were propagated for three blind passages in the allantoic cavity of 9-days-old specific pathogen free (SPF) embryonated chicken eggs following the OIE standard procedures [7,8]. Pathotyping of APMV isolates were carried out by mean death time (MDT) and intracerebral pathogenicity index (ICPI).

2.2. RNA extraction, PCR amplification and nucleotide sequencing

All swabs were screened for ACoV and APMV1-specific real time and conventional RT-PCR before isolation and genomic characterization [9,10,11,12]. RNA extractions were performed using TRIzol LS (Life Technologies, Carlsbad, CA, USA), as per manufacturer instructions. One-step RT-PCR was performed to amplify the fusion (F) and spike (S1) genes of APMV1 and ACoV using the SuperScript1 III One-Step RT-PCR System with Platinum I *Taq* DNA Polymerase (Life Technologies, Carlsbad, CA, USA) and previously described primers [9,10]. Appropriate positive and negative controls were included, and positive samples were back-screened to exclude the possibility of laboratory- and/or cross-contamination. Amplicons were separated on a 1.2% agarose gel, and desired bands were excised and purified using the QuickClean II Gel Extraction Kit (GenScript, Piscataway, NJ, USA). Nucleotide sequencing and assembly were performed as described previously [13]. DNA sequencing was performed with BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Carlsbad, CA, USA) in ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA USA). Obtained sequences were submitted to GenBank using BankIt tool, and are available under accession numbers: KU251490.1, KU251491.1 and KY549653.

2.3. Evolution and phylogeny

To explore an overall differences in selection pressure on the F and S1 genes, especially on epitopes that defines the cross-neutralization and escape mutant, the occurrences of synonymous (dS) and non-synonymous (dN) substitutions were determined using SNAP web tool (available at https://www.hiv.lanl.gov/content/sequence/SNAP/SNAP.html) [14], which plots the cumulative and per-codon occurrence of each substitutions. Phylogenetic trees were constructed utilizing the obtained nucleotide sequences for F and S1 genes of APMV1 and ACoV, respectively based on Bayesian Inference within the program MrBayes version 3.1.2 [15]. Two independent Markov chain Monte Carlo were executed and sampled every 1000 generations using the default parameters of the priors' panel. The analysis was based on the GTR + I + G model, which allow significantly changed posterior probability estimates. To confirm the Bayesian tree topologies, phylogenetic relationship was also established with the MEGA version 6.0 software program using the maximum likelihood method with the Kimura two-parameter model [16]. The evolutionary distances were inferred using the pairwise distance method and expressed as the number of nucleotide substitutions per site giving a statistical significance of the tree topology by 1000 bootstrap resampling of the data [16].

3. Results

3.1. Prevalence of APMV1 and ACoV in wild birds

A total of 297 samples were processed from wild birds-dense, and APMV1 and ACoV-endemic areas from eight governorates of Egypt. We classified all wild bird species that were included in the analysis into three different families, which reflected both their taxonomy and their ecology. These families were *Corvidae* (*C. splendens*, n = 102), *Ardeidae* (*B. ibis*, n = 99) and *Anatidae* (*A. crecca*, n = 96) (Table 1). These samples were individually screened using the RT-PCR targeting the S1 and F genes of the ACoV and APMV1, respectively. This screen yielded a 5.4% (16 out of 297) and 1.7% (5 out of 297) positive samples for ACoV and APMV1, respectively among all tested tracheal and faecal/cloacal samples. Among positive samples for APMV1, a single isolate shown high genetic similarity to a vaccine strain whereas two out of 16 ACoV shown vaccine-alike characteristics. Pathotyping of the APMV1 isolate (NDV/Teal/VRLCU-EG/2015) showed MDT (96 h) and ICPI (0.4375) characteristics for lentogenic strains of NDV.

3.2. Sequencing and phylogeny

All positive samples, detected based upon the RT-PCR for both ACoV and APMV-1, were sequenced and analyzed. A set of sequences, representing previously reported APMV1 and ACoV genotypes in Egyptian poultry sectors were aligned with sequences presented in this study using the ClustalW algorithm in BioEdit [17]. Comparison of nucleotide identity percentages revealed that one APMV1 isolate was genetically identical to the genotype II

Table 1
Proportion and positivity of sampled wild birds across different regions.

Family	Species (Genus)	Sharqia	Dakahlia	Kafr El Sheikh	Gharbia	Qalubia	Menofia	Giza	Benisuef	Total	Positive%	
											APMV-1 (1.7%)	ACoV (5.4%)
Corvidae	*C. splendens (Corvus)*	10	14	19	10	21	8	9	11	102	0	2
Passeridae	*P. domesticus (Passer)*	11	9	21	14	13	10	12	9	99	1	5
Anatidae	*A. crecca (Anas)*	13	12	18	13	9	10	11	10	96	4	7
Total = 3	**Total = 3**	**34**	**35**	**58**	**37**	**43**	**28**	**32**	**30**	**297**	**5**	**16**

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