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Abstract

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Detection of infectious bursal disease virus (IBDV) genome in free-living pigeon and guinea fowl in Africa suggests involvement of wild birds in the epidemiology of IBDV

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Abstract Infectious bursal disease (IBD) virus (IBDV) serotype 1 is the causative agent of IBD, a highly contagious immunosuppressive disease of young chickens. In this study, we examined IBDV infection in apparently healthy 21 guinea fowls and 20 pigeons obtained in Tanzania and Zambia by virus neutralization test (VNT) and reverse transcription polymerase chain reaction (RT-PCR) for the VP2 hypervariable region (VP2-HVR) of IBDV. Two guinea fowls (9.5%) in Tanzania were RT-PCR and VNT positive for IBDV, and 1 pigeon (5%) in Tanzania was RT-PCR positive and VNT negative. Phylogenetic analysis based on the nucleotide sequences of the PCR products indicated that segment A of IBDV detected from one guinea fowl and a pigeon belonged to the very virulent genotype of European/Asian type, while the other IBDV detected from a guinea fowl belonged to the classical genotype. To our knowledge, this is the first report of detection of the IBDV genome in free-living pigeons and guinea fowls. The detection of IBDV from apparently healthy guinea fowls and pigeons elucidates the role of wild birds in the epidemiology of IBDV.

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Introduction

Infectious bursal disease virus (IBDV) is the causative agent of infectious bursal disease (IBD) of young chickens [6]. IBDV is classified into the genus *Avibirnavirus* of the family *Birnaviridae* [4]. The virus is extremely lymphocidal and infects IgM-bearing B-lymphocytes in the bursa of Fabricius [19] leading to immunosuppression. Two distinct serotypes, 1 and 2, exist in IBDV. Serotype 1 viruses are pathogenic to chickens and are further classified into classical virulent, antigenic variant, and very virulent (VV) IBDVs based on their virulence or antigenicity. Serotype 2 viruses are mainly isolated from turkey and are non-pathogenic to chickens [8].

IBDV genome consists of two segments of double-stranded RNA (dsRNA), segments A (3.4 kb) and B (2.8 kb). The large segment A encodes two viral proteins, a 17–21-kDa non-structural viral protein 5 (VP5) and a 110-kDa precursor polyprotein (NH_2–VP2–VP4–VP3–COOH), which is processed into mature VP2 (37 to 42 kDa), VP3 (32 to 35 kDa), and VP4 (24 to 28 kDa). The smaller segment B encodes VP1 (90 kDa), an RNA-dependent RNA polymerase. The hypervariable region (HVR), which spans amino acids from position 206 to 350 within VP2 (VP2-HVR), is known to be critical for determination of the conformational epitopes responsible for recognition of virus neutralizing antibodies in VP2 [2, 25]. The VP2-HVR has the highest amino acid sequence variation among serotype 1 strains [10, 12], and the nucleotide and deduced

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amino acid sequences within this region are widely used for molecular diagnosis and genotyping of IBDVs [9].

Since 1987, pathotypic IBDV variants with enhanced virulence, called very virulent IBDVs (VV-IBDVs), emerged in Europe, and have spread to many places of the world. These strains were called as “European VV-IBDV.” Recently, Kasanga et al. [12] described the existence of the VV-IBDV variants in Tanzania, which were genetically different from the European VV-IBDVs. These Tanzanian VV-IBDVs were closely related to some strains isolated in the western part of Africa and were thus called “African VV-IBDV” [11, 12]. However, the origins of the European and African VV-IBDVs are not clearly known.

There is some speculation that unknown IBDVs distributed in wild birds contributed to the evolvement and/or emergence of new IBDV strains [7, 28]. In addition, Nigerian researchers reported that VV-IBDVs originated from Africa [18]. However, there is no information about the distribution of IBDV in wild birds in Africa. The only information presently available is based on a serological survey [16]. Furthermore, chickens in many parts of Africa are likely to be in contact with captive guinea fowls and free-living pigeons.

In this study, we conducted a serological and molecular epidemiological survey of IBDV in captive and free-living guinea fowls and pigeons in selected parts of Tanzania and Zambia to investigate the involvement of wild birds in the evolution and epidemiology of IBDV. Our findings indicate that IBDVs detected in guinea fowls and pigeons are genetically closely related to some chicken isolates in the VP2-HVR, suggesting that wild birds could be involved in evolution and epidemiology of IBDV in Africa.

Materials and methods

Birds, sera, and viruses

We examined 21 apparently healthy guinea fowls (*Numida meleagris*) and 20 apparently healthy pigeons (*Columba livia*) for IBDV infection in 2005. Ten of the guinea fowls were obtained in Zambia and all the other birds were obtained in Tanzania. All birds were obtained from different geographical regions in Tanzania and Zambia. The birds obtained were free-living and captive in areas where no IBD outbreaks were reported to occur (Table 1).

Serum was prepared from blood collected in birds prior to the postmortem examination. Each serum sample was absorbed to a Nobuto blood filter strip (Advantec, Tokyo, Japan), air-dried according to the manufacturer’s instructions, and transported to Japan for serological studies.

Bursa tissues from clinically healthy birds were smeared on tissue sampling filter papers, inactivated with 99.5% ethanol, air-dried, and transported to Japan. The inactivation of the virus was done as described previously [15].

Complementary DNA (cDNA) synthesis

Total RNA was isolated from bursa-smeared filter paper using TRIZOL (Invitrogen, Carlsbad, CA, USA) as reported previously [15]. The first strand cDNA was synthesized using Rever Tra Ace reverse transcriptase (RT) (Toyobo, Osaka, Japan) and random primer Pd(N)$_6$ (Toyobo). Briefly, 3 µl of resuspended dsRNA in RNase-free water was mixed with 1.5 µl of dimethyl sulfoxide (DMSO), incubated for 5 min at 97°C, then immediately chilled on ice. To this was added 2.5 µl of 5 × RT reaction buffer, 2.5 µl of 2.5 mM dNTPs, 0.5 µl of 25 mM Pd(N)$_6$ random primer, 0.5 µl of RNase inhibitor, 0.25 µl of Rever Tra Ace reverse transcriptase enzyme (100 U/µl), and 0.25 µl of RNase-free water to a final volume of 11 µl. Reactions were incubated for 45 min at 42°C, then for 5 min at 94°C, and withheld at 4°C. The synthesized cDNAs were used as templates for reverse transcription polymerase chain reaction (RT-PCR).

Reverse transcription polymerase chain reaction (RT-PCR) for VP2-HVR

For detection of the VP2-HVR by RT-PCR, forward primer V1 (position 737–754: 5′-CCA GAG TCT ACA CCA TAA-3′) and reverse primer V2 (position 1189–1208: 5′-CCT GTT GCC ACT CTT TCG TA-3′) were used (numbering based on segment A nucleotide sequence of 52–70 classical strain (GenBank accession: D00869)). The V1V2 primer set was tested and found to be highly sensitive and specific for IBDV VP2-HVR since it could detect the Acc I-Spe I region of VP2-HVR in bursa tissue, embryonated chicken eggs, dried filter papers, and transformed *E. coli* without amplification of any non-specific gene(s) or DNAs. The cDNA templates were amplified using *Thermus aquaticus* Ex-Taq DNA polymerase (Takara, Shiga, Japan) and V1V2 primers in a TAKARA PCR Thermo Cycler GP (Takara) as previously described [12]. Amplified products were run on 1.2% agarose gel and visualized with ethidium bromide staining.

RT-PCR for part of VP1

The forward and reverse primers used to amplify segment B encoding 332 amino acids of the VP1 N-terminus were BF1 (position 1–22: 5′-CCT CTT CTT GAT GAT TCT ACC A-3′) and BR1 (position 1021–1040: 5′-GAC CAT ATG TTA CGG GTC TT-3′), where the position numbers are based on the Lukert classical strain (GenBank accession: AY918947). The BF1 and BR1 primers were

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