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Short Communication

Evidence of possible vertical transmission of duck circovirus

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ABSTRACT

To test the hypothesis that duck circovirus (DuCV) may be vertically transmitted from infected breeder ducks to their ducklings, we investigated 120 newly hatched ducklings, 30 dead duck embryos and 80 non-embryonated duck eggs with the duplex polymerase chain reaction (PCR). DuCV DNA was present in 15 newly hatched ducklings, 4 duck embryos and 3 non-embryonated eggs. Four ducklings from two flocks were co-infected by DuCV-1 and DuCV-2, three ducklings from three flocks were DuCV-1 single infection, and eight ducklings from six flocks were DuCV-2 single infection. One duck embryo and one non-embryonated egg were positive for both DuCV-1 and DuCV-2 DNAs, one embryo for DuCV-1 DNA, and two embryos and two non-embryonated eggs for DuCV-2 DNA. The findings provide evidence of possible vertical transmission of DuCV and simultaneous transmission of DuCV-1 and DuCV-2 from breeder ducks to ducklings.

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1. Introduction

Duck circovirus (DuCV), one member of the genus *Circovirus* of the *Circoviridae* family, is a small, non-enveloped, 15–16 nm in diameter, icosahedral virus (Hattermann et al., 2003). The genome of DuCV is a single-stranded circular DNA of about 1.99 kb, which contains three major open reading frames (ORFs), ORF1, ORF2 and ORF3 (Hattermann et al., 2003; Xiang et al., 2012). Based on the analysis of capsid gene and genomic sequences, DuCV was classified into two genotypes, DuCV-1 and DuCV-2 (Wang et al., 2011; Zhang et al., 2012; Zhang et al., 2013; Wen et al., 2014).

DuCV infection was characterized by feathering disorders, poor body condition and low body weight (Hattermann et al., 2003), and caused lymphocyte depletion, necrosis, and histiocytosis in the bursa of Fabricius (BF) of ducks (Soike et al., 2004). There were multiple local lesions in the spleen, thymus and BF of ducks infected with DuCV (Liu et al., 2010b). Surveys would suggest a high prevalence of the virus in flocks experiencing morbidity and mortality as well as those that appear clinically normal (Fringuelli et al., 2005; Chen et al., 2006; Banda et al., 2007; Jiang et al., 2008; Zhang et al., 2009; Liu et al., 2010a).

Based on findings from other avian circoviruses, it is possible that DuCV can be transmitted both horizontally and vertically. It has been reported that there was a high prevalence of DuCV in symptom-free duck populations in China, and the DuCV-seropositive rate of long-term farmed breeder flocks was significantly higher than that of meat duck flocks, indicating that DuCV was transmitted horizontally (Liu et al., 2010a). However, the vertical transmission of the virus thus far remains unclear. The aim

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of the work was to investigate the possible transmission of DuCV from breeder ducks to newly hatched ducklings using the polymerase chain reaction (PCR).

2. Materials and methods

On the basis of the whole genome sequence alignments of DuCV sequences retrieved from GenBank (http://www.ncbi.nlm.nih.gov), four primers for DuCV were selected. DuCV-F (CGG GAA ATG ACG TAG TCG TCA TG, position 640–662) and DuCV-R (GGA(C) C(T)TG(A) AAC ATG AGA TGG GC, position 1653–1672) were designed to amplify a 1032 base pair (bp) fragment for DuCV-1 and DuCV-2. DuCV-1F (GTT CAC TCC G(T)GT TGT GTT GTC C(T)GG, position 1226–1249) was designed with DuCV-R to amplify a 446 bp fragment for DuCV-1 typing, and DuCV-2R (GAT AAT GCG ACC(T) GGC GAC G, position 1219–1239) was designed with DuCV-F to amplify a 599 bp specific fragment for DuCV-2 strains.

Using the four primers, a duplex PCR assay was performed in a total volume of 25 μl, included 1 μl of DNA, 1× PCR buffer (50 mM KCl, 10 mM Tris–HCl [pH 8.3]), 1.5 mM $MgCl_2$, 0.4 mM of each dNTP, 1 μM of the each primers and 1.0 U Taq DNA Polymerase (TaKaRa, Dalian, China). PCR parameters were as follows: a denaturation step at 95 °C for 5 min and then 35 cycles of a denaturation step at 95 °C for 45 s, an annealing and extension step of 68 °C for 90 s, with a final extension at 72 °C for 10 min. The PCR products were analyzed by electrophoresis in 0.8% agarose gel.

DuCV-1 strain FJ0601 (EF370476) (Jiang et al., 2008), DuCV-2 strain WF0701 (EU022375) (Zhang et al., 2012), duck plague virus (DPV), egg drop syndrome virus (EDSV), duck hepatitis B virus (DHBV), Muscovy duck parvovrius (MDPV), *Riemerella anatipestifer* (RA), *Salmonella* and *Escherichia coli* (*E. coli*) were used to determine the specificity of the duplex PCR. Two plasmids, pDuCV-1 including the complete genomic DNA of DuCV-1 strain FJ0601 and the plasmid pDuCV-2 including the complete genomic DNA of DuCV-2 strain WF0701, were purified with a QIAprep Spin Miniprep kit (Qiagen, Chatsworth, CA, USA). Quantitation of the plasmid DNAs was performed spectrophotometrically at 260 nm, and a mixture of 1 μl of 10^8 to 10^0 copies of the plasmid DNAs was used to detect the sensitivity of the duplex PCR.

Total of 120 1-day-old ducklings from 8 breeder Cherry Valley duck flocks (15 ducklings per flock) in eastern China were collected to investigate the presence of DuCV infection. The antibodies to DuCV in breeder duck sera were detected with the established iELISA method (Liu et al., 2010a). The positive rates of DuCV-specific antibody ranged from 40% to 64.7% among the 8 breeder duck flocks. The mortality of pre-hatching ducklings was 2–3%. Newly hatched ducklings were obtained within 10 min of hatch. The liver, spleen, kidney, thymus and BF samples from ducklings were collected. According to the manufacturer's instructions, DuCV DNA was extracted by Dneasy tissue kit (Qiagen, Hilden, Germany).

In addition, 30 duck embryos and 80 non-embryonated duck eggs from one of the three DuCV-1 and DuCV-2 co-infection flocks were also detected by the PCR. The embryos died in the final stage of incubation, and the non-embryonated eggs were obtained within 1 h of laying. DNA derived from embryonic tissues was isolated using the Dneasy tissue kit (Qiagen, Hilden, Germany). DNA isolation from non-embryonated eggs was carried out following the established procedures (Rahaus et al., 2008). Briefly, the non-embryonated eggs were opened using a sterile scalpel, glair and yolk were separated and introduced into a phenol/chloroform extraction, and ethanol was used for precipitation of DNA.

All the samples were detected by the pan DuCV PCR and the two type specific PCRs respectively. The expected DuCV-1-specific and DuCV-2-specific products amplified from clinical samples were sequenced on a commercial service (Shanghai Sangon Biological Engineering Technology & Service Co., Ltd, Shanghai, China).

3. Results

The duplex PCR amplified two DNA fragments of 1032 bp and 446 bp from DuCV-1 DNA, two DNA fragments of 1032 bp and 599 bp from DuCV-2 DNA, and three DNA fragments of 1032 bp, 446 bp and 599 bp from DuCV-1 and DuCV-2 mixed DNAs (Fig. 1). Under the same conditions, no amplification occurred using nucleic acids from the 7 other duck pathogens (DPV, EDSV, DHBV, MDPV, RA, *Salmonella* and *E. coli*) and negative control (healthy duck) (Fig. 1). Using 10^8 to 10^0 copies of the plasmid DNAs of DuCV-1 and DuCV-2 as templates, the duplex PCR assay was able to detect 10 copies of DuCV-1 and DuCV-2 DNAs (Fig. 2).

From 120 newly hatched ducklings from 8 anti-DuCV antibody positive breeder duck flocks, 3 (37.5%) flocks were identified as mixed infection of DuCV-1 and DuCV-2, and other 3 (37.5%) flocks were DuCV-2 single infection (Table 1). Among them, 3.33% (4/120) of duckling were detected as mixed infection of DuCV-1 and DuCV-2, 2.5% (3/120) of duckling were DuCV-1 single infection, and 6.67% (8/120) of duckling were DuCV-2 single infection. DuCV-1 was detected in seven BF samples from three duck flocks. DuCV-2 was found in nine liver samples from five duck flocks and four thymus samples from three duck flocks. All spleen and kidney samples were negative for DuCV-1 and DuCV-2.

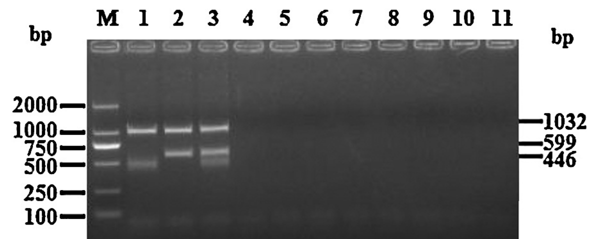

Fig. 1. The specificity of the duplex PCR for simultaneous detection of the two genotypes of DuCVs. Lane M, DNA marker DL2000; lane 1, DuCV-1 strain FJ0601; lane 2, DuCV-2 strain WF0701; lane 3, DuCV strains FJ0601 and WF0701; lane 4, DPV; lane 5, EDSV; lane 6, DHBV; lane 7, MDPV; lane 8, RA; lane 9, *Salmonella*; lane 10, *E. coli*; lane 11, negative control (healthy duckling sample).

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