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Prevalence of Duck Circovirus Infection of Subclinical Pekin Ducks in South Korea

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ABSTRACT. An investigation was carried out to determine the prevalence and infection pattern of duck circovirus (DuCV) in subclinical Pekin ducks on South Korean duck farms. A total of 147 samples collected from 92 duck farms in five provinces were examined from 2011 to 2012. The overall prevalence of DuCV PCR-positive pooled bursa of Fabricius and liver samples was 21.8% (32/147). The prevalence of DuCV PCR-positive samples increased significantly in 3-week-old ducks compared with that in 1-week-old ducks ($P<0.05$). DuCV in association with *Riemerella* and *Salmonella* infections (10.9%; 16/147) occurred at the same level as infection with DuCV alone (10.9%; 16/147). In comparison of the relationship between bacterial diseases (salmonellosis, *Riemerella* infection) and morbidity in farms with and without DuCV, morbidity was higher in circovirus-positive farms (50%; 16/32) than in circovirus-negative farms (26.1%; 30/115). Our findings provide baseline information on the degree of DuCV infection and distribution and pattern of DuCV in ducks, and it is evident that DuCV can be associated with subclinical diseases and that subclinical infection could be economically important.
KEY WORDS: duck circovirus, Pekin duck, prevalence, subclinical.

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Duck circovirus (DuCV), clustered in the genus *Circovirus* of the family *Circoviridae,* was first described in mulard ducks from a farm in Germany in 2003 [5]. The mulard ducks in Germany had been purchased from a French breeder and exhibited poor growth and marked feather dystrophy, which was particularly noticeable over the dorsum, and hemorrhaging was noted on feather shafts [10]. Ducks infected with DuCV exhibit feathering disorders, growth retardation and low body weight. A histopathological examination of the bursa of Fabricius demonstrated lymphocyte depletion, necrosis and histiocytosis [10].

A similar DuCV was detected in Muscovy, Pekin and mule ducks with stunting and feather abnormalities in Taiwan [2]. Since then, DuCV infections have been reported in Hungary, the US and China [1, 4, 12]. Although high morbidity has been shown in China [11, 12], Korea and Japan, neighboring country, have not reported high morbidity. Moreover, the South Korean duck industry has been growing dramatically in the past several years according to the FAO [3], as duck meat is presumed to be a healthy food (http://www.duckhealth.com/foodvalu.html). However, management and sanitation on duck farms are poor compared with chicken farms. Recently, cases in which ducks show no noticeable symptoms and do not grow weak have increased, and the reason for these cases is unknown. As a rule, avian circovirus infections are either clinically silent or are characterized by a combination of feather abnormalities and secondary infections subsequent to a compromised virus-induced immune response [1, 4]. Most studies of DuCV have focused on clinical cases, and few studies have been conducted to find subclinical infection of DuCV. There is no way to know how damage derived from subclinical infection can result in reduced production efficiency and economic losses. Although it is possible that DuCV may predispose ducks to other diseases and co-infection of DuCV with *Riemerella anatipestifer*(RA), *Pasteurella multocida*, *Staphylococcus aureus* and duck hepatitis virus (DHV) has been observed [1, 2, 4, 12], little is known about the natural prevalence of DuCV infection in subclinical ducks. The purpose of the present study was to investigate the prevalence of DuCV in a population of subclinical Pekin ducks in South Korea by detection of viral DNA.

Pekin ducks were collected from 92 farms in South Korea including 41 in Gyeonggi, 14 in Chungnam, 44 in Chungbuk, 29 in Chonbuk and 19 in Chonnam in 2011 and 2012. The sizes of the different flocks varied from 5,000 to 100,000 ducks. Approximately 12–15 Pekin ducks (1, 3, and ≥3 weeks old) were chosen randomly per farm. The affected ducklings did not display typical clinical symptoms of a duck circovirus infection. Among these 92 farms, 55 farms were selected at random and were sampled two times, i.e., when the ducks were approximately 1 and 3 weeks old, respectively. The collected live Pekin ducks were placed in safety cages, and dead ducks were placed on ice and transported to the laboratory within 5 hr of collection. All Pekin ducks were euthanized by cervical dislocation, and samples were collected at necropsy.

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Portions of the bursa of Fabricius and liver were prepared as 10% suspensions by placing approximately 1 g of tissue in a screw-topped container containing sea sand and 10 m*l* PBS containing 1% antibiotic-antimycotic (Invitrogen, Carlsbad, CA, U.S.A.) followed by vigorous shaking in a mechanical shaker. The same organs from the same farm were pooled and cultured for virus and bacteria isolation. The samples were centrifuged at low speed after freezing and thawing, and the supernatant was kept at −80°C until use.

Salmonella was isolated according to standard methods [6]. Cloacal swab samples were aseptically inoculated into 10 m*l* each of Rappaport–Vassiliadis broth (Oxoid, Basingstoke, Hampshire, U.K.) for enrichment and incubated at 42°C for 24 hr. A loopful of each broth culture was then inoculated simultaneously onto xylose lysine deoxycholate agar (Difco Laboratories, Detroit, MI, U.S.A.), Brilliant Green Agar or Brilliance *Salmonella* Agar Base (Oxoid). Presumptive *Salmonella* colonies were further tested using a *Salmonella* Latex Test Kit (Oxoid) and identified by API 20E strips (bioMerieux, Marcy l'Etoile, France), according to the manufacturer's instructions. All isolates were serogrouped by the slide agglutination test using O antiserum for serogroups A, B, C1, C2–C3, D, E and G and were serotyped by the tube agglutination test using H antiserum according to the Kauffman and White schema (Difco). RA was isolated from pharyngeal and cloacal swab samples that were collected from ducks and aseptically smeared on blood agar plates (Hanil Komed, Seongnam, South Korea) containing 5% sheep blood. After incubation at 37°C for 36 hr in an atmosphere containing 5% CO_2, RA was identified by polymerase chain reaction (PCR), and sequencing was subsequently performed as described previously [9]. The forward primer 190f (5′-GTA TTGAAAGCTCTGGCGG-3′) and reverse primer 843r (5′- TCGCTTAGTCTCTGAACCC-3′) were used to amplify a 654-base pair product.

Total viral DNA/RNA was extracted from tissue homogenates using a Viral Gene-Spin™ Viral DNA/RNA Extraction Kit (iNtRON Biotechnology, Daejeon, South Korea). DNA/RNA of the tissue homogenates was used in PCR or RT-PCR assays to detect potential pathogens, such as duck circovirus and DHV. The common gene was detected using common DuCV detection primers (408 bp; DuCVaF 5′-MGAGCTGCCGCCCTTGAG-3′ and DuCVaR 5′-TCCCGAGTAACCGTCCCACCAC-3′) [1]. A set of primers specific for the common gene was used for routine DHV detection (467 bp; DHV comF 5′-AAGAAGGAGA-AAATYAAGGAAGG-3′ and DHV comR 5′-TTGATGT-CATAG CCCAASACAGC-3′) [7]. Nucleotide sequences of each PCR product were determined using an ABI PRISM 310 Genetic Analyzer autosequencer (Applied Biosystems, Foster City, CA, U.S.A.).

All statistical analyses were performed using SPSS version 12.0 (SPSS, Chicago, IL, U.S.A.). The chi-square test was used to assess the association between the prevalence of the isolates and region, age and antimicrobial resistance. *P*-values<0.05 were considered statistically significant.

Of the 147 samples collected from the 92 farms in South Korea, 32 samples (21.8%) were PCR positive for DuCV. As shown in Table 1, the 32 PCR-positive DuCV samples were from five provinces, and 6.8%, 32.8% and 60% of the DuCV PCR-positive samples were found in 1-, 3- and ≥3-week-old Pekin ducks. Statistical analyses showed a highly significant difference in the number of PCR-positive ducks between 1 and 3 weeks of age ($P<0.05$).

The prevalence of DuCV PCR-positive samples during the rearing period (1 and 3 weeks) on 55 randomly selected farms was determined. A time-course evolution was observed for the PCR-positive farms; five farms were PCR positive for DuCV based on samples from 1-week-old ducks, and one farm was persistently positive based on samples from 3-week-old ducks; however, the number of positive farms increased to 12, with 11 new positive farms being added when the ducks were 3 weeks old. Prevalence increased significantly in 3-week-old ducks ($P<0.05$).

Among the 32 DuCV PCR-positive samples, co-infection with RA and *Salmonella* Enteritidis was detected, but DHV was not detected. Samples positive for DuCV only were found in 10.9% of the cases, and co-infection occurred in 10.9% of the cases, among which double and triple infections occurred in 9.5% and 1.4% of the cases, respectively (Table 2).

In this study, we detected the DuCV gene in subclinical Pekin ducks and analyzed the prevalence and infection pattern according to region, age and co-infection. The results of PCR revealed that 32 of the 147 samples (21.8%) from five provinces were positive for DuCV; these five provinces represented, by farm duck population, the duck industry in South Korea, comprising>91% of the total duck industry [8]. When we compared the relationship between morbidity and bacterial diseases (salmonellosis, *Riemerella* infection) for farms with and without DuCV, morbidity was higher in circovirus-positive farms (50%; 16/32) than in circovirus-negative farms (26%; 30/115). Our results suggest that DuCV has spread successfully in subclinical Pekin ducks in South Korea probably due to poor breeding management and poor environmental sanitation conditions on some farms. Various levels of infections in clinical ducks have been reported in other countries. DuCV infections are highly prevalent in Hungary (84%) and are likely to be widespread in farmed ducks [4]. Comparatively lower level of DuCV has been reported in Germany (46%), Taiwan (38.2%), the U.S. (6.1%) and China (33.3%) [1, 2, 10, 12]. However, we suggest that similar levels of infection may be shown in subclinical ducks, and attention is being increasingly focused on the effects of subclinical DuCV infections.

We demonstrated that ducklings ≥3 weeks of age had a significantly higher prevalence of DuCV than in 1-week-old Pekin ducks. This suggests that subclinical infections are common in older ducks and that subclinical DuCV infections in Pekin ducks could be the result of immunosuppression [1]. Growth retardation of DuCV-positive ducks infected with RA, *E.coli* or DHV-1 was more obvious than in DuCV-negative birds [10, 12]. In the present study, the DuCV PCR-positive rate for the 147 samples was 21.8% including single DuCV infections and co-infections of DuCV with RA and *Salmonella* Enteritidis. The co-infection rates of RA and

Table 1. Duck circovirus (DuCV) positive rates and differences by region and age of domestic Pekin ducks

Region	Age of ducks (weeks)			Total
	1	3	≥ 3	
Gyeonggi	5.0% (1/20) [a)]	50% (9/18)	66.7% (2/3)	29.3% (12/41)
Chungnam	16.5% (1/6)	0% (0/6)	100% (2/2)	21.4% (3/14)
Chungbuk	4.8% (1/21)	26.3% (5/19)	25.0% (1/4)	15.9% (7/44)
Chonbuk	13.3% (2/15)	21.4% (3/14)	nt [b)]	17.2% (5/29)
Chonnam	0% (0/11)	57.1% (4/7)	100% (1/1)	26.3% (5/19)
Total	6.8% (5/73)	32.8% (21/64)	60.0 (6/10)	21.8% (32/147)

a) PCR-positive rate (%): total number of positive samples/tested samples. b) Not tested.

Table 2. Analysis of the duck circovirus (DuCV) infection pattern and co-infection with *Riemerella anatipestifer* and *Salmonella* Enteritidis

Pattern of infection	Etiologic agents [a)]	Positive samples [b)]
Single	DuCV only	10.9% (16/147)
Double	DuCV + *Riemerella anatipestifer*	4.1% (6/147)
	DuCV + *Salmonella* Enteritidis	5.4% (8/147)
Triple	DuCV + *Riemerella anatipestifer* + *Salmonella* Enteritidis	1.4% (2/147)
Total		21.8% (32/147)

a) *Riemerella anatipestifer* and *Salmonella* Enteritidis infection was detected by culture method and confirmed by polymerase chain reaction. b) Positive rate (%): total number of positive samples/tested samples.

Salmonella Enteritidis were the same as the rate of single infection with DuCV. To determine whether DuCV is a factor that increases susceptibility to *Salmonella* and RA infection, a more comprehensive rigid methodology is needed in the development of challenge studies. However, at this moment, these challenge studies are not possible, because DuCV has not yet been isolated with current technique.

From the findings of this study, it is evident that DuCV can be associated with subclinical diseases and that subclinical infection could be economically important. These data might be alarming for farmers and veterinarians and suggest that a duck farm monitoring program is necessary to control these diseases. A further study is needed to intensively analyze the pathogenesis and transmission of DuCV.

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