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Molecular Detection of Infectious Anaemia Virus in Quail, in Iran

Noosha Zia-Jahromi[1] and Majid Gholami-Ahangaran[2]*

[1]Basic Sciences Faculty, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.
[2]Clinical Sciences Group, Veterinary Medicine Faculty, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.

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Chicken infectious anaemia is one immunosuppressive agent that chicken was considered to be the natural host of chicken anaemia virus (CAV). For detection of this virus in commercial Japanese quail flocks, 250 thymus samples were taken from 50 quail flocks in Isfahan and Yazd provinces, Iran. Thymus samples were taken by history recording and direct observation of quail carcasses in quail flocks with mortality. Viral DNA was extracted by DNA extraction kit and amplified a fragment of VP2 gene. The results showed the prevalence of CAV infection in 50 quail flocks was 22%. This study revealed quail can be as a host of CAV and it is necessary to take controlling strategy for preventing of CAV infection between chicken and quail flocks.

Key word: Infectious Anaemia, Quail, PCR, Iran.

The chicken anaemia virus, which is the only member of the genus *Gyrovirus*, is belonging to the *Circoviridae* family[1]. The virus was first isolated and described by Yuassa *et al.* in Japan[2]. Chicken anaemia virus (CAV) is a small, non-enveloped, with single-stranded circular DNA genome[3,4]. The CAV could be transmitted both vertically and horizontally in chickens[5]. CAV infection was described in chicken properly. The disease appear clinical and subclinically[6]. The clinical disease is mainly seen in young chicks at 10 to 14 d of age, which usually acquire the infection vertically[7]. The infection is accompanied by immunosuppressive effects, such as poor vaccine responses and increased susceptibility to secondary infections[8-10]. Clinical disease occurs when chicks are infected during the first 2 wk of life, but it can be avoided if hens transfer sufficient antibodies to their progeny. After 2 wk of age, chicks can be infected with the virus but do not develop clinical signs[10]. The major economic importance of this virus is associated with the subclinical form and may result in severe immunosuppression, poor growth, increased mortality attributable to secondary infections, and the cost of treatment to control secondary infections[11-13]. CAV has been found in many countries with a poultry industry[14]. It has been detected worldwide by isolation, serology or DNA amplification in chickens[6]. Until now, the chicken was considered to be the only natural host and the main host of CAV[5], but CAV infection has been reported in other avian species, including Japanese quail[15], fancy chicken breeds[4], jackdaws, rooks, and some rare avian breeds[16]. In contrast, the antibody to CAV was not found in some birds, such as the duck, pigeon, or pheasant[5]. Therefore, in this examination evaluated the CAV genome detection in Japanese quail (*Coturnix japonica*), in Iran for economic threat to commercial poultry industry.

* To whom all correspondence should be addressed.
E-mail: mgholamia@yahoo.com

MATERIAL AND METHODS

Sampling

A total of 250 thymus samples have been collected from 50 Japoness quail flocks in Isfahan and Yazd, central provinces in Iran, with ages ranging from 18 to 45 days of age, between 2010 and 2011. The collected tissues were stored at -20°C until assayed. The commercial flocks have no clinical signs suggestive of the CAV infection. All sampled flocks had mortality higher than 1% per day for at least 5 days during the growing period. All commercial flocks were reared in cages, and feed and water were supplied ad libitum. From each flock collected at least 5 samples. For Polymerase chain reaction (PCR), all samples from each flock mixed and prepared one stock for each flock.

DNA Extraction

The DNA extraction from each flock sample was carried out using commercial DNA extraction kit (High Pure Viral Nucleic Acid Kit, Roche, Germany), according to the manufacturer's Instructions.

PCR method

PCR was carried out to amplify a fragment of 713 bp from the viral protein 2 (*VP2*) gene of CAV. The sequence of the primers was as follows: forward primer: 52 -GCG CAC ATA CCG GTC GGC AGT; reverse primer: 52 -GGG GTT CGG CAG CCT CAC ACT AT [17]. PCR amplification was performed in PCR buffer containing 1.5 m*M* $MgCl_2$, 200 µ*M* each dNTPs, 10 p*M* each primer, and 1.0 unit of *Taq* polymerase (Fermentas, Germany) in a 25-µL total reaction volume. The amplification was carried out in a thermal cycler (Mastercycler Gradient, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) under the following conditions: initial denaturation of 94°C for 4 min, followed by 34 cycles of denaturation, annealing, and extension at 94°C for 1 min, 63°C for 1 min, and 72°C for 1 min, respectively, and a final extension at 72°C for 5 min. The PCR product was then analyzed by electrophoresis in 1% agarose gel and visualized under UV light after staining with ethidium bromide. In this study, Cuxhaven-1 strains of CAV (THYMOVAC Vaccine, Lohmann Animal Health, Germany) were provided and used as a positive and DNase free water was used as a negative control.

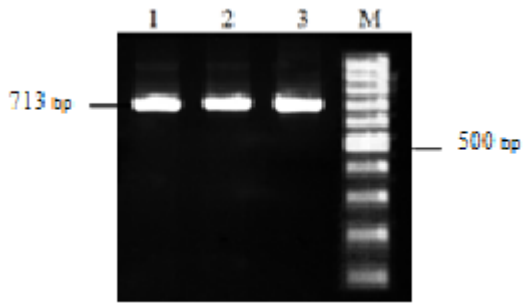

Fig. 1. PCR amplification of the VP2 region of CAV (chicken anaemia virus) in thymus samples from quails (lanes 1 and 2: positive samples; M: DNA ladder marker, lane 3: positive control)

RESULTS

A 713 bp fragment of CAV VP2 gene was amplified as in positive control (figure 1). The CAV genome in this study was detected in 11 of the 50 (22%) thymus stocks collected from 50 Japanese quail flocks in Iran.

DISCUSSION

Until now, the chicken was known as the only natural host for CAV but serological and molecular researches showed susceptibility of some other birds to this virus[4, 15, 16]. In present study we found infectivity to CAV in Japanese quail flocks with PCR, in Iran. Therefore, according to our findings, the quail can be considered a reservoir of CAV. To our knowledge, this is the first report of the molecular detection of CAV in species other than the chicken in Phasianidae family. We conclude, based on our review of the related literature, that although the chicken has been considered as the natural host for CAV, antibodies to CAV have been detected in Japanese quail in Japan[15], in fancy chicken breeds in the Netherlands[4], and in jackdaws, rooks, and some rare avian breeds in Ireland[16]. In contrast, the antibody to CAV was not found in turkeys and ducks in the United Kingdom[18]; in pigeons, ducks, and pheasants in Ireland[16]; and in crows, pigeons, and ducks in Japan[15]. Furthermore, 1-d-old turkey poults inoculated with CAV did not show clinical signs of anemia and did not develop antibodies to

the virus[18]. Recently, Gholami-Ahangaran and Zia-Jahromi[19] studied CAV infection in ostrich and turkey in Iran and reported susceptibility of ostrich to CAV infection but no evidence for CAV infection in turkey. Also, Gholami-Ahangaran and Zia-Jahromi[20] studied CAV infection in sparrow as one species of Passeriformes, in Iran. They clearly show that CAV is widespread in sparrows in Iran and this bird species can be a major reservoir of CAV and it may play a main role in transmission of the virus to growing chickens in commercial poultry houses that are not birdproof. These findings suggest that other birds can be reservoir of chicken anaemia virus.

Whether CAV causes clinical disease in quails and whether the virus in these birds is CAV or another circovirus with cross-reacting properties need further investigation. As far as is known, there are no reports showing that the circovirus of quails does cross-neutralize with CAV. however, it is uncertain whether the quails was actually infected with the virus or whether the virus was obtained from ingested plant matter[21]. Furthermore, in the study, the CAV was detected from quails that dead with miscellaneous causes. Whether CAV causes death in the quails or not, is however not clearly evidenced.

Until now, no molecular information to CAV infection in quails was available and this study is the first report of molecular detection of CAV in quail, as one species in Phasianidae family. This work clearly shows that CAV is widespread in quail in Iran and that this bird species can be a reservoir of CAV and it may play a main role in transmission of the virus to growing chickens in commercial poultry houses.

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