Molecular Detection of Infectious Anaemia Virus in Quail, in Iran

Noosha Zia-Jahromi1 and Majid Gholami-Ahangaran2*

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

(Received: 04 April 2013; accepted: 15 May 2013)

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 family1. The virus was first isolated and described by Yuassa et al. in Japan2. Chicken anaemia virus (CAV) is a small, non-enveloped, with single-stranded circular DNA genome3,4. The CAV could be transmitted both vertically and horizontally in chickens5. CAV infection was described in chicken properly. The disease appear clinical and subclinically6. The clinical disease is mainly seen in young chicks at 10 to 14 d of age, which usually acquire the infection vertically7. The infection is accompanied by immunosuppressive effects, such as poor vaccine responses and increased susceptibility to secondary infections8-10. 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 signs10. 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 infections11-13. CAV has been found in many countries with a poultry industry14. It has been detected worldwide by isolation, serology or DNA amplification in chickens6. Until now, the chicken was considered to be the only natural host and the main host of CAV5, but CAV infection has been reported in other avian species, including Japanese quail15, fancy chicken breeds1, jackdaws, rooks, and some rare avian breeds16. In contrast, the antibody to CAV was not found in some birds, such as the duck, pigeon, or pheasant1. Therefore, in this examination evaluated the CAV genome detection in Japanese quail (Coturnix japonica), in Iran for economic threat to commercial poultry industry.
MATERIALS 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: 5'-GCG CAC ATA CCG GTC GGC AGT; reverse primer: 5'-GGG GTT CGG CAG CCT AAC ACT AT 17. PCR amplification was performed in PCR buffer containing 1.5 mM MgCl₂, 200 µM each dNTPs, 10 pM 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 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 virus4, 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 Japan15, in fancy chicken breeds in the Netherlands4, and in jackdaws, rooks, and some rare avian breeds in Ireland16. In contrast, the antibody to CAV was not found in turkeys and ducks in the United Kingdom15; in pigeons, ducks, and pheasants in Ireland16; and in crows, pigeons, and ducks in Japan15. Furthermore, 1-d-old turkey poults inoculated with CAV did not show clinical signs of anemia and did not develop antibodies to

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)
the virus\(^1\). Recently, Gholami-Ahangaran and Zia-Jahromi\(^9\) 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\(^9\) 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\(^2\). Furthermore, in the study, the CAV was detected from quails that died 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.

ACKNOWLEDGMENTS

This study has been supported financially by Islamic Azad University, Shahrekord Branch, Iran.

REFERENCES


J PURE APPL MICROBIO, 8(1), FEBRUARY 2014.


