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PCR Amplification of Chicken Anaemia Virus VP2 Gene in Chicken and Pigeon, in Iran

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The situation of chicken anaemia virus (CAV) infection in different species of birds, exception of chickens is unclear. For this, in this study 375 thymus samples have been collected from 25 broiler chicken with high mortality in Iran. Furthermore, 200 blood samples were collected from 20 apparently healthy pigeon flocks with different ages. After DNA extraction, PCR was carried out to amplify a fragment of 713 bp from the viral protein 2 (*VP2*) gene of CAV. Results showed that, 58.4% thymus samples from 25 broiler chicken flocks were positive to CAV. In the pigeon flocks, all pigeons (100%) were negative with respect to detection of VP_2 CAV genome in blood samples. This concluded that in reverse of chicken, the healthy pigeon cannot be infected to subclinical CAV, in Iran.

Key words: Chicken, Pigeon, VP2, PCR, Iran.

Chicken anaemia virus (CAV) is the only member of *Gyrovirus*[1]. The CAV has been detected worldwide by isolation, serology or DNA amplification in chickens[2] in many countries with a poultry industry[3] and described properly. The virus spreads vertically from parental stock to progeny and horizontally by contact exposure with infected chickens or fomites[2]. CAV infections induce either clinical or subclinical signs and both result in important economical losses[2]. The clinical disease is mainly seen in young chicks at 10 to 14 d of age, which usually acquire the infection vertically[4]. The infection is accompanied by immunosuppressive effects, such as poor vaccine responses and increased susceptibility to secondary infections[5, 6, 7]. Until now, the chicken was considered to be the only natural host and the main host of CAV[8], but CAV infection has been reported in other avian species, including Japanese quail[9], fancy chicken breeds[10], jackdaws, rooks, and some rare avian breeds[11]. In reverse, the antibody to CAV was not found in some birds, such as the duck, pigeon, or pheasant[9]. Therefore, in this study, we evaluated the CAV genome detection in chickens and pigeons in Iran.

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MATERIALS AND METHODS

Sampling

375 thymus samples have been collected from 25 broiler chicken flocks in all over Iran, from 2009-2011. Average 15 thymus samples were collected from each chicken flock. Averagely, all sampled flocks had mortality higher than 1% per day for at least 5 days during the growing period. Also, 200 blood samples were collected from 20 apparently healthy pigeon flocks with different ages.

All sampled flocks (chicken and pigeon), examined in this study, had not been vaccinated

against CAV, and no clinical signs suggestive of CAV infection was observed in any of the flocks. The collected tissues were stored at -20°C until assayed.

Polymerase chain reaction (PCR)

DNA extraction from tissue and blood samples was carried out using commercial DNA extraction kit (High Pure Viral Nucleic Acid Kit, Roche, Germany), according to the manufacturer's instructions.

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 CAC ACT AT[12]. PCR amplification was performed in PCR buffer containing 1.5 m*M* MgCl2, 200 µ*M* each dNTPs, 10 p*M* each primer, and 1.0 unit of *Taq* polymerase (Fermentas, Germany) in a 25µL 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.

RESULTS

A 713 bp fragment of CAV VP2 gene was amplified as in positive control (figure 1). In this study all broiler chicken flocks, (100%) were positive to CAV. PCR results showed that 219 of 375 (58.4%) thymus samples from 25 broiler chicken flocks were positive to CAV (Table 1). The minimum and maximum of positives in all tested flocks were 6.6% and 100%, respectively.

In the pigeon flock tested, all birds (100%) were negative with respect to detection of VP_2 CAV genome in blood samples.

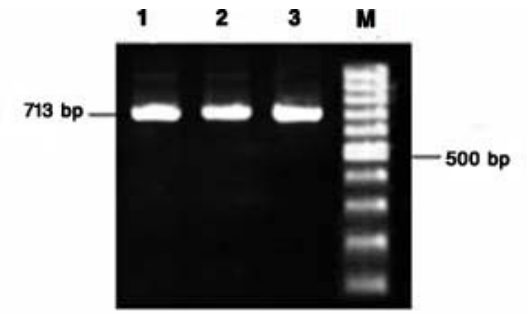

Fig. 1. PCR amplification of the VP2 region of CAV (chicken anaemia virus) in thymus samples from chickens (lanes 1 and 2: positive samples; lane 3: positive control, M: DNA ladder marker)

DISCUSSION

In this study, further examination of commercial chicken flocks to CAV infection, the situation of pigeon flocks was evaluated for infection to CAV. Results represent although pigeons are not infected to CAV but chickens have high infectivity rate to CAV in Iran. The infection rate of chickens to CAV reported previously in many countries with industrial poultry production[13]. In Iran the history of CAV infection reflected to 2003 that Toroghi et al reported heamorrhagic subcutaneous and muscles in chickens in some slaughterhouses[14]. The clinical finding was accompanying with serological evidence[15] and after this, some molecular studies confirmed correlation of this clinical feature with CAV infection[16]. In present decade, we observed some complications in poultry production in Iran e.g. failure in vaccination programs, high infectivity to some bacterial diseases (e.g. colibacillosis and mycoplasmosis) and followed with high mortality in chickens. In reason of this complications, the hypothesis was proposed that it may be related to immunosuppressive agent e.g. CAV infection. After this, Gholami-Ahangaran et al. showed that a percent of apparently healthy chickens (24.58%) may be infected to CAV until slaughtering time[4]. This finding revealed that CAV is widespread in broiler chicken throughout the Iran and the results provide evidence of widespread distribution of the virus and high incidence of infection among poultry commercial broiler flocks in Iran, as it has similarity been documented to occurs worldwide in all major poultry producing countries[13].

Previously, reported that chickens are the main and natural host of CAV[8] but present studies demonstrated the infection to CAV in some species other than chickens and refused in some another avian species. In current study we evaluated the CAV infection in pigeon in Iran using PCR and did not found infectivity of pigeon to CAV. In this along, antibodies to CAV have been detected in Japanese quail in Japan[9], in fancy chicken breeds in the Netherlands[10], and in jackdaws, rooks, and some rare avian breeds in Ireland[11]. In contrast, the antibody to CAV was not found in turkeys and ducks in the United Kingdom[17]; in pigeons, ducks, and pheasants in Ireland[11]; and in crows, pigeons, and ducks in Japan[9]. Recently, Gholami-Ahangaran *et al.* [17] 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 *et al.,*[18] 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 further more, Zia-Jahromi and Gholami-Ahangaran[19] revealed quail can be as a host of CAV and it is necessary to take control strategy for preventing of CAV infection between chicken and quail flocks. In current study, the sampling from chickens was done in birds with highly mortality while in pigeon flocks; the sampled birds were apparently healthy. Therefore, the negative result in CAV detection in pigeon may be related to host specificity in CAV susceptibility. Hence, previously Gholami-Ahangaran et al. represent the subclinical infection of CAV in apparently healthy chickens in Iran[4] but this phenomenon may be not occur in some other bird species e.g. pigeon. Furthermore, the type of samples in pigeon sampling may be effect the outcome of this molecular assay. In this study, blood was collected from pigeon while it is approved that the target organ of CAV is thymus[8] and the load of this virus may be very low in blood samples. However, in base of this study we concluded that in reverse of chicken, the healthy pigeon cannot be infected to CAV, in Iran.

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