Molecular detection of chicken anaemia virus (CAV) in house sparrow (Passer domesticus) in Iran

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SUMMARY

Until now, chickens were considered as the only natural and main host for chicken anaemia virus (CAV), but this virus was also recently detected in other bird species such as Japanese quail, jackdaw, rook and rare avian breeds. In this study, the occurrence of CAV was investigated in sparrow thymuses (n = 100) from 2 central provinces in Iran (Isfahan and Chaharmahal-va-Bakhtiyari) using specific PCR for the VP2 gene amplification. Result showed that 38% of samples were positive for CAV. It was concluded that CAV was widespread in sparrows in Iran and that sparrows may be considered as an important reservoir for the viral infection.

Keywords: Chicken anaemia virus, sparrow, VP2 gene, PCR, Iran

Introduction

Circoviridae family contains two genuses. Chicken anaemia virus (CAV) is the only member of the genus Gyrovirus, while the genus Circovirus currently comprises porcine circoviruses type 1 and 2 (PCV1, PCV2), psittacine beak and feather disease virus (BFDV), pigeon circovirus (PiCV), canary circovirus (CaCV), goose circovirus (GoCV) and duck circovirus (DuCV) [24]. Members of circoviridae family are non-enveloped, regular icosahedrons and are the only animal viruses with a circular, single-stranded DNA genome [27]. These viruses share many epizootiological and pathological similarities (i.e. young age of affected animals, particular tropism for lymphoid tissue and organs, related acquired immunosuppression and secondary infections) [2, 21].

Chicken anaemia virus was first isolated and described in Japan by YUASA et al. [30] but CAV antibodies were detected in chicken sera world-wide [16, 31]. This virus has been found in most countries with a developed poultry industry [7]. The virus spread vertically from parent to progeny and horizontally by contact exposure to infected chickens or fomites. CAV infection induces either clinical or subclinical signs [22]. This virus can cause economically important losses in either clinical or subclinical forms of disease in broiler chickens [17, 29]. Signs and lesions include stunting, increased mortality, anaemia, bone marrow cell depletion, subcutaneous haemorrhages, and atrophy of secondary lymphoid organs [1]. This infection is often associated with opportunistic viral and bacterial infections and vaccination failures in chicken flocks [5, 22].

Although until now, the chicken was considered to be the only natural and main host of CAV [21] but there are some distributed reports of CAV infection in other avian species including infection of Japanese quail [9], fancy chicken breeds [6], jackdaws, rooks, and some rare avian breeds [3]. In contrast, antibody to CAV was not found in some birds e.g. duck, pigeon, and pheasant [21]. Until now, there is no report of CAV infection in Passeriformes. In this examination CAV genome by PCR was detected in house sparrow (Passer domesticus), in Iran. The role of sparrow as a reservoir of CAV that could pose economic threats to commercial poultry operations was evaluated.

Materials and methods

A total of 100 sparrows dead for miscellaneous causes were collected from different areas of Isfahan and Chaharmahal-va-Bakhtiyari provinces, central areas of Iran, during 2010-2011. DNA extraction from thymus samples was carried out using a commercial DNA extraction kit (High Pure Viral Nucleic Acid Kit, Roche, Germany), according to the instructions of manufacturer.
A 713 bp fragment of CAV VP2 gene was amplified by PCR using specific primers published by NATESAN et al. [19] [forward primer: 5'- GCG CAC ATA CCG GTC GGC AGT; reverse primer: 5'-GGG GTT CGG CAG CCT CAC ACT AT]. The PCR amplification was performed in PCR buffer (1.5 mM MgCl₂, 200 μM of each dNTPs, 10 pM of each primers) and 1.0 U of Taq polymerase (Fermentase) in 25 μl of total reaction volume. The amplification was carried out under following conditions in a thermal cycler (Mastercycler, Gradient, Germany) with an initial denaturation of 94°C for 4 minutes following by 34 cycles of denaturation, annealing, extension at 94°C for 1 minute, 63°C for 1 minute, 72°C for 1 minute, respectively, and final extension was carried out at 72°C for 5 minutes. 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, Cux-1 strains of CAV (THYMOVAC Vaccine, Lohmann Animal Health, Germany) were provided and used as positive control and DNase free water was used as negative control.

Result

A 713 bp fragment of CAV VP2 gene was amplified as in positive control (figure 1) in 38 of 100 (38.0%) thymus samples from sparrows. On the other hand, 38% of sparrows were infected by CAV.

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

Discussion

Until now, there is no information of CAV infection in Passeriformes and this study is the first report of CAV infection in sparrow. In this study, the infectivity rate of sparrows to CAV was 38%. This study clearly shows that sparrows can be a major reservoir of CAV infection and may play main role in transmission of CAV to growing chickens in commercial poultry houses.

Previously, 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 [3, 6, 9]. These findings suggest that other birds can be reservoir of this virus. For example, antibodies to CAV have been detected in Japanese quail in Japan [9], fancy chicken breeds in the Netherlands [6], jackdaws, rooks and some rare avian breeds in Ireland [3]. By contrast, antibody to CAV were not found in turkey and duck in UK [15], in pigeon, duck and pheasant in Ireland [3] in crows, pigeon and duck in Japan [9]. Also, one day turkey poults inoculated with CAV did not show clinical sign of anaemia and did not develop antibodies to the virus [14]. Recently, GHOLAMI-AHANGARAN et al. [11] 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.
Until now, no information to CAV infection in sparrows was available and this study is the first report of molecular detection of CAV in sparrow, as one species of Passeriformes. This work clearly shows that CAV is widespread in sparrows in Iran and that this bird specie 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.

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References

24. TODD D., SCOTT A.N.J., FRINGUELLI E., SHIVRAPRASAD H.L., GAVIER-WIDEN D., SMYTH


