# Serological and molecular detection of chicken anaemia virus in Iranian poultry flocks

Majid Gholami-Ahangaran

*Poultry Diseases Department, Veterinary Medicine Faculty, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.*

** Corresponding author at: Poultry Diseases Department, Veterinary Medicine Faculty, Shahrekord Branch, Islamic Azad University, PO Box 166, Shahrekord, Iran.*
*Tel.: +98 9133231388, e-mail: mgholamia1388@yahoo.com.*

*Veterinaria Italiana* 2015, **51** (3), 211-215. doi: 10.12834/VetIt.80.253.3
Accepted: 30.06.2015 | Available on line: 30.09.2015

**Keywords**
Chicken,
Chicken anaemia virus,
Iran,
Quail,
Turkey.

**Summary**
Despite chicken being the main natural host for chicken anaemia virus (CAV), other birds may be infected by this virus too. In this study we examined chickens, turkeys, and quails for serological and molecular detection of CAV in Iran. For this study, we used 375 sera and thymus samples from broiler chickens, 100 sera and blood samples from turkeys, and 250 thymus samples from quails. The sample were collected from all over Iran between 2009 and 2010. Serum samples were examined using ELISA. DNA was extracted from thymus and blood samples and was analysed for the presence of the VP2 gene of CAV by polymerase chain reaction. Results showed that 69.07% of chickens were positive for antibody to CAV. In chickens, 58.4% were positive for CAV VP2 gene. The prevalence of CAV infection in quails was 15%, based on CAV VP2 gene detection. In turkey flocks, all turkeys (100%) were negative with respect to detection of VP2 CAV gene and CAV antibodies. It was concluded that, for the span of the time considered in this study, CAV has circulated in broiler chickens and quails throughout Iran.

## Indagine sierologica e molecolare del virus dell'anemia infettiva aviare in alcune specie di uccelli domestici in Iran

**Parole chiave**
Iran,
Pollo,
Quaglia,
Tacchino,
Virus dell'anemia infettiva aviare.

**Riassunto**
Sebbene il pollo sia l'ospite naturale del virus dell'anemia infettiva aviare (CAV), anche altri volatili possono essere infettati dal virus. In questo studio abbiamo analizzato 375 campioni di siero e timo prelevati da polli da allevamento, 100 campioni di siero e sangue di tacchino e 250 campioni di timo prelevati da quaglie per la presenza di anticorpi e del gene VP2 del CAV in Iran. I campioni sono stati raccolti in Iran tra il 2009 e il 2010. I campioni di siero sono stati esaminati con ELISA, mentre, per la rilevazione del gene VP2 del CAV, il DNA estratto da campioni di sangue e di timo è stato analizzato mediante reazione a catena della polimerasi (PCR). I risultati ottenuti mostrano che il 69,07% dei polli presenta anticorpi nei confronti del CAV, e il 58,4% a frammenti del gene VP2. La prevalenza dell'infezione da CAV nelle quaglie è risultata essere del 15%, in base alla rilevazione del gene VP2. Tutti i tacchini (100%) sono risultati negativi sia al rilevamento del gene VP2 sia al rilevamento degli anticorpi nei confronti del CAV. Pertanto, per il periodo oggetto dell'indagine, si può affermare che in Iran il CAV ha circolato infettando polli e quaglie.

## Introduction

Chicken anaemia virus (CAV) is the only member of the genus *Gyrovirus*. This virus belongs to Circoviridae family (Todd *et al.* 2007). Members of the Circoviridae family are non-enveloped, regular icosahedrons, and are the only animal viruses with a circular, single-stranded DNA genome (Twentyman *et al.* 1999). 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) (Bassami *et al.* 1998, Schat 2003).

Chicken anaemia virus was first isolated and

described in Japan by Yuasa and colleagues (Yuasa *et al.* 1979), while CAV antibodies have been detected in chicken sera world-wide (Yuasa *et al.* 1979, McNulty 1989). This virus has been found in most countries with poultry industry (Ducatez *et al.* 2006).

The virus spreads vertically from parental stock to progeny and horizontally by contact exposure with infected chickens or fomites. Infection from CAV appears both subclinically and clinically (Simionatto *et al.* 2006). Clinical disease occurs in chickens infected during the first 2 weeks of life. It can be avoided if hens transfer sufficient antibodies to their progeny. Chickens could also be infected with the virus after 2 weeks of age, although in this case they do not develop clinical signs (Canal *et al.* 2004). Both the clinical or subclinical forms of the disease in broiler chickens can cause important economic losses (McNulty *et al.* 1991, Yuasa and Imai 1986). Signs and lesions include stunting, increased mortality, anaemia, bone marrow cell depletion, subcutaneous haemorrhages, and atrophy of secondary lymphoid organs (Adair 2000). This infection is often associated with opportunistic viral and bacterial infections and vaccination failures in chicken flocks (Cloud *et al.* 1992, Simionatto *et al.* 2006).

Chicken anaemia virus was known as a much-conserved virus of 1 serotype (Yuasa *et al.* 1979) with several genetic groups (Simionatto *et al.* 2006), however an additional serotype has been recently reported (Simionatto *et al.* 2006, Spackman *et al.* 2002).

Apparently chickens are the main natural host of CAV (Schat 2003), but there are studies reporting CAV infection in other avian species including Japanese quail (Farkas *et al.* 1998) fancy chicken breeds (De Wit *et al.* 2004), jackdaws, rooks, and some rare avian breeds (Campbell 2001). In contrast, antibody to CAV has not been found in birds such as duck, pigeon, or pheasant (Schat 2003).

In order to extend our knowledge of the epidemiology of CAV infection in chicken flocks, it is necessary to evaluate the role of other avian species in circulation of CAV infection. In this study, chicken, turkey sera were tested using a commercial enzyme linked immunosorbent assay for the presence of antibodies to CAV, and whole blood and thymi were tested for detection of CAV genome in chickens, turkeys, and quails, in Iran. The present study is an extension of my research on serological and molecular investigation of CAV in chicken, ostrich and turkey (Gholami-Ahangaran *et al.* 2013). In present research the infectivity to CAV was compared in chicken, turkey and quail as three main species of poultry that growing in Iran.

## *Materials and methods*

### Flocks

Three hundred and seventy-five sera and 375 thymus samples have been collected from 25 broiler chicken flocks in Isfahan, Yazd, Tehran, and Chaharmahal-va-Bakhtiyari provinces, central areas of Iran, between 2009 and 2010. In average, 15 serum and 15 thymus samples were collected per flock. All sampled flocks had an average mortality higher than 1% per day within 10 days in growing period. All broiler chicken flocks have 3-8 week of age. One hundred sera and blood samples were also collected from 10 healthy turkey flocks of different ages all over Iran between 2009 and 2010. In the same period of time, 250 thymus samples have been collected from 50 Japanese quail flocks with ages ranging from 18 to 45 days.

The collected tissues were stored at -20°C until examination. All sampled quail 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*. At least 5 thymus samples were collected from each flock. All commercial poultry flocks (chicken, turkey, and quail), examined in this study, had not been vaccinated against CAV, and no clinical signs suggesting CAV infection were observed in any of the flocks.

### ELISA

Blood samples were collected via wing vein. The blood was allowed to clot at room temperature for 30 minutes and centrifuged (1,500 g, 15 minutes, room temperature). Serum was decanted into micro-tube and inactivated by heating at 56˚C for 30 minutes and stored at -20˚C until tested. Sera were tested by indirect ELISA using commercial CAV ELISA kit (Flockchek, CAV, IDEXX, Hoofddorp, North Holland). A serum dilution of 1:10 was used following the instructions of the manufacturer. Optical density value was read at 650 nm wavelength on ELISA reader as per manufacturer's instructions.

The presence or absence of antibody to CAV was determined for each sample by ratios between test sample and negative control. Samples with an S/N value of less than 0.6 were considered positive and those with a value equal or greater than 0.6 were considered negative. A positive sample indicates presence of CAV antibodies and previous exposure to CAV.

### Polymerase Chain Reaction

For the polymerase chain reaction (PCR), whole blood samples were collected in heparinized tube and

stored in refrigerator (2-4˚C). Thymus samples were collected after slaughtering or necropsy of poultry carcasses and stored in -20˚C until experiment.

DNA extraction from thymus and blood samples was carried out using a High Pure Viral Nucleic Acid Kit (High Pure Viral Nucleic Acid Kit, Roche, Basel, Switzerland), according to the manufacturer's instructions.

The PCR was conducted 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-3'; reverse primer: 5'-GGG GTT CGG CAG CCT CAC ACT AT-3' (Natesan *et al.* 2006). Polymerase chain reaction amplification was performed in PCR buffer containing 1.5 m*M* $MgCl_2$, 200 µ*M* each deoxynucleotide 5'-triphosphate, 10 p*M* each primer, and 1.0 unit of *Taq* polymerase (Fermentas, Glen Burnie, MD, USA) 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 minutes, following by 34 cycles of denaturation, annealing, extension at 94°C for 1 minute, 63°C for 1 minute, and 72°C for 1 minute, respectively, and a final extension at 72°C for 5 minutes. The PCR product was then analysed 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, Cuxhaven, Germany) were provided and used as a positive control while deoxyribonuclease-free water was used as a negative control.

## Results

### ELISA

All broiler chicken flocks were found to be positive at 3-8 weeks of age (in which at least 1 positive bird was detected). The prevalence rate of CAV in chicken flocks differs from 20% to 100%. The seroprevalence rate of CAV was 69.07 % in all tested chickens.

In this study, all turkey flocks (100%) were found to be negative for antibodies to CAV. The mean of CAV S/N value of examined turkey was 0.757 (Table I).

### PCR

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 (in which at least 1 positive bird was detected). The results of the PCR showed that 219 of 375 (58.4%) thymus samples from 25 broiler chicken flocks were positive to CAV. The minimum and maximum of positives in all tested flocks were 6.6% and 100%, respectively.

In the turkey flocks tested, all turkeys (100%) were negative with respect to detection of VP2 CAV gene fragment in blood samples.

The CAV genome in this study was detected in 11 of the 50 (22%) Japanese quail flocks in Iran. The CAV prevalence in all 250 thymus samples collected from 250 quails was 15% (Table II).

## Discussion

This study analysed the situation of chicken, turkey, and quail flocks with respect to CAV infection. The results show that turkeys are not infected to CAV but chickens and quails have partial high infectivity rate to CAV in Iran.

**Table I.** *ELISA results for chicken anaemia virus (CAV) in serum samples from chicken and turkey flocks collected in Iran between 2009 and 2010.*

| Species | Number of flocks | Sample size | CAV S/N Ratio (Mean±SD) | CV (%) (Mean) | Positivity |
|---|---|---|---|---|---|
| **Chicken** | 25 | 375 | 0.430 ± 0.178 | 45.03 | 69.07 |
| **Turkey** | 10 | 100 | 0.757 ± 0.154 | 29.50 | 0 |

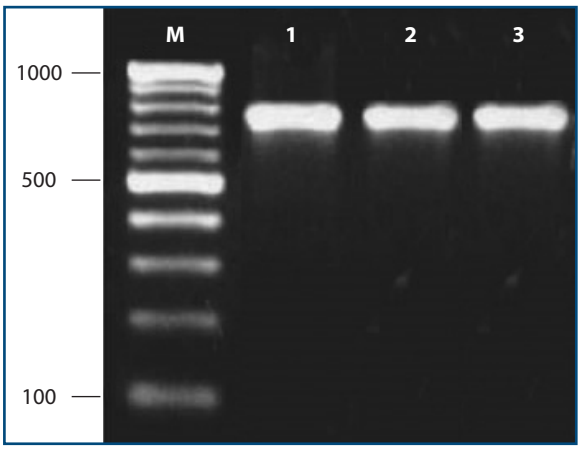


**Figure 1.** *PCR amplification of the VP2 region of CAV in thymus samples from chicken and quail collected in Iran from 2009 to 2010 (M: DNA ladder marker, lane 1: positive samples of chicken; lane 2: positive samples of quail; lane 3: positive control).*

**Table II.** *PCR results for chicken anaemia virus (CAV) in thymus and blood samples collected from chicken and turkey flocks in Iran between 2009 and 2010.*

| Species | Number of flocks | Sample size | Positivity |
|---|---|---|---|
| **Chicken** | 25 | 375 thymi | 58.4 |
| **Turkey** | 10 | 100 blood samples | 0 |
| **Quail** | 50 | 250 thymi | 15.0 |

Clinical, serological, and molecular studies confirming CAV infection in chicken flocks in Iran have already been provided (Gholami-Ahangaran and Zia-Jahromi 2012). However, during the past decade, we observed some complication in poultry production in Iran comprising failure in vaccination programs, high infectivity to some bacterial diseases (*e.g.* colibacillosis and mycoplasmosis) followed by high mortality in chickens. The hypothesis was advanced that such complication could be related to immunosuppressive agent *e.g.* CAV infection. Gholami-Ahangaran and colleagues showed that a percentage of apparently healthy chickens (24.58%) may be infected to CAV at the slaughtering time (Gholami-Ahangaran *et al.* 2011). This finding revealed that CAV has circulated through broiler chickens throughout Iran and the results provide evidence of widespread distribution of the virus and high incidence of infection among commercial broiler flocks in the country, as it has similarity been reported worldwide in all major poultry producing countries (Cardona *et al.* 2000).

In this study we found infectivity to CAV in Japanese quail flocks with PCR in Iran. 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 chicken in Phasianidae family. We conclude that although chickens have been considered a natural host for CAV, antibodies to CAV have been detected in Japanese quail in Japan (Farkas *et al.* 1998), in fancy chicken breeds in the Netherlands (De Wit *et al.* 2004), and in jackdaws, rooks, and some rare avian breeds in Ireland (Campbell 2001). The antibody to CAV was not found in turkeys and ducks in the United Kingdom (McNulty *et al.* 1998); in pigeons, ducks, and pheasants in Ireland (Campbell 2001); and in crows, pigeons, and ducks in Japan (Farkas *et al.* 1998). Recently, Gholami-Ahangaran and colleagues studied CAV infection in sparrow as one species of Passeriformes, in Iran. They clearly show that CAV is widespread in sparrows in Iran and that 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 bird-proof. These findings suggest that other birds can be a vector of CAV (Gholami-Ahangaran *et al.* 2013).

On the basis of negative results obtained in this study turkeys do not appear susceptible to CAV infection. These findings are in agreement with McNulty and colleagues, who reported that the inoculation of 1-d-old turkey poult with CAV did not lead to incidence of clinical signs of anaemia and did not develop antibodies to the virus (McNulty *et al.* 1998). However, it was concluded that CAV has circulated in broiler chickens and quails throughout Iran, while turkeys may not been infected to CAV.

## References


Adair B.M. 2000. Immunopathogenesis of chicken anaemia virus infection. *Dev Comp Immunol*, **24**, 247-255.

Bassami M.R., Berryman D., Wilcox G.E. & Raidal S.R. 1998. Psittacine beak and feather disease virus nucleotide sequence analysis and its relationship to porcine circovirus, plant circoviruses, and chicken anaemia virus. *Virology*, **249**, 453-459.

Campbell G. 2001. Investigation into evidence of exposure to infectious bursal disease virus (IBDV) and chick infectious anaemia virus (CIAV) in wild birds in Ireland. *In* Infectious bursal disease and chicken infectious anaemia (T. Van Den Berg, eds). Proc. Second International Symposium, Rauischholzhausen, 15 July 2001. Institut für Geflügelkrankheiten Press, Justus Liebig University, Giessen, 230-233.

Canal C.W., Ferreira D.J., Macagnan M., Fallavena L.C.B., Moraes H.L.S. & Wald V.B. 2004. Prevalence of antibodies against CAV in broiler breeder in southern Brazil. *Pesq Vet Brasil*, **24**, 89-92.

Cardona C., Lucio B., Oconnell P., Jagne J. & Schat K. 2000. Hurmonal immune responses to chicken infectious anaemia virus in three strains of chickens in a closed flock. *Avian Dis*, **44**, 661-667.

Cloud S.S., Rosenberger J.K. & Lillehoj H.S. 1992. Immune dysfunction following infection with chicken anaemia agent and infectious bursal disease virus. II. Alterations of *in vitro* lymphoproliferation and *in vivo* immune responses. *Vet Immunol Immunopathol*, **34**, 353-366.

De Wit J.J., van Eck J.H., Crooijmans R.P. & Pijpers A. 2004. A serological survey for pathogens in old fancy chicken breeds in central and eastern part of The Netherlands. *Tijdschr Diergeneesk*, **129**, 324-327.

Ducatez M.F., Owoade A.A., Abiola J.O. & Muller C.P. 2006. Molecular epidemiology of chicken anemia virus in Nigeria. *Arch Virol*, **151**, 97-111.

Farkas T., Maeda M., Sugiura H., Kai K., Hirai K., Ostuki K. & Hayashi T.A. 1998. A serological survey of chickens, Japanese quail, pigeons, ducks and crows for antibodies to chicken anaemia virus (CAV) in Japan. *Avian Pathol*, **27**, 316-320.

Gholami-Ahangaran M., Fathi-Hafshejani E. & Seyed-Hosseini R. 2013. Seromolecular study of chicken infectious anemia in chickens, ostriches, and turkeys in Iran. *J Appl Poult Res*, **22**, 404-409.

Gholami-Ahangaran M., Momtaz H., Zia-Jahromi N. & Momeni M. 2011. Genomic detection of the chicken anaemia virus from apparently healthy commercial broiler chickens in Iran. *Revue Méd Vét*, **162**, 604-606.

Gholami-Ahangaran M. & Zia-Jahromi N. 2012. Chicken anemia virus infection in broiler chickens vaccinated and not vaccinated for avian influenza in Iran. *J Appl Poult Res*, **21**, 413-417.

Gholami-Ahangaran M., Zia-Jahromi N. & Rahimi E. 2013. Molecular detection of chicken anaemia virus (CAV) in house sparrow (*Passer domesticus*) in Iran. *Revue Méd. Vét*, **164**, 487-490.

McNulty M.S., Connor T.J., McNeilly F., Kilrkapatrick K.S. & McFerran J.B. 1988. A serological survey of domestic poultry in the United Kingdom for antibody to chicken anaemia agent. *Avian Pathol*, **17**, 315-324.

McNulty M.S., Cormor T.J. & McNeilly F. 1989. A survey of specific pathogen-free chicken flocks for antibodies to chicken anemia agent, avian nephritis virus and group A rotavirus. *Avian Pathol*, **18**, 215-220.

McNulty M.S., McIlroy S.G., Bruce D.W., Todd D. 1991. Economic effects of subclinical chicken anaemia agent infection in broiler chickens. *Avian Dis*, **35**, 263-268.

Natesan S., Kataria J.M., Dhama K., Rahul S. & Baradhwaj N. 2006. Biological and molecular characterization of chicken anaemia virus isolates of Indian origin. *Virus Res*, **118**, 78-86.

Schat K.A. 2003. Chicken infectious anaemia. *In* Diseases of poultry, 11th Ed. (Y.M. Saif, H.J. Barnes, J.R. Glisson, A.M. Fadly, L.R. McDougald and D.E. Swayne, eds). Iowa State University Press, Iowa, 182-202.

Simionatto S., Lima-Rosa C.A.V., Binneck E., Ravazzol, A.P. & Canal C.W. 2006. Characterization and phylogenetic analysis of Brazilian chicken anaemia virus. *Virus Genes*, **33**, 5-10.

Spackman E., Cloud S.S., Pope C.R. & Rosenberger J.K. 2002. Comparison of a putative second serotype of chicken infectious anemia virus with a prototypical isolate I. Pathogenesis. *Avian Dis*, **46**, 945-955.

Todd D., Scott A.N.J., Fringuelli E., Shivraprasad H.L., Gavier-Widen D. & Smyth J.A. 2007. Molecular characterization of novel circoviruses from finch and gull. *Avian Pathol*, **36**, 75-81.

Twentyman C.M., Alley M.R., Meers J., Cooke M.M. & Duignan P.J. 1999. Circovirus-like infection in a southern black-backed gull (*Larus dominicanus*). *Avian Pathol*, **28**, 513-516.

Yuasa N., Taniguchi T. & Yoshida I. 1979. Isolation and some characteristics of an agent-inducing anaemia in chicks. *Avian Dis*, **23**, 366-385.

Yuasa N. & Imai K. 1986. Pathogenicity and antigenicity of eleven isolates of chicken anaemia agent (CAA). *Avian Pathol*, **15**, 639-645.