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Original Article

# Molecular Detection of Chicken Anemia Virus from Native Larry-breed Chickens in Chaharmahal-va-Bakhtiyari Province, Iran

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**A B S T R A C T**

**Background and Aims:** Finding prevalence of chicken anemia virus (CAV) infection in native chickens is necessary to avoid transmission of infection to commercial flocks. In this paper we attempt to describe molecular detection of chicken anemia virus in native Larry-breed chickens in Chaharmahal-va-Bakhtiyari province in Iran for the first time.

**Materials and Methods:** Blood samples were collected from 100 native Larry-breed chickens (5-8 months old) in Shahrekord, Lordegan, Brogen and Koohrang, i.e.four cities of Chaharmahal-va-Bakhtiyari province. To detect CAV, Polymerase chain reaction (PCR) was undertaken on isolated DNA from blood samples using a pair of CAV specific primers that produced a 374 base pair fragment.

**Results:** PCR analysis detected CAV in 12 of 100 (12%) tested blood samples.

**Conclusions:** The results revealed that native Larry chicks were not free from CAV infection in Chaharmahal-va-Bakhtiyari province and vaccination against CAV should be taken into account in native farms.

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## Introduction

Chicken anemia virus (CAV), a negative single-stranded DNA virus is the only member of genus *Gyrovirus* of the *Circoviridae* [1]. In 1979, Yuasa and coworkers isolated chicken infectious anemia virus and described this disease for the first time [2]. In maternal antibody-free chicks (less than 2 weeks old), CAV causes subcutaneous hemorrhage, thymic, bursal and bone marrow atrophy and severe anemia which result in lymphocyte depletion of both cortex and medulla [2, 3] and develop a profound immunosuppression with enhanced susceptibility to a wide range of viral and bacterial pathogens [4, 5]. Maternal antibody-positive chicks become resistant to the disease by one month of age [6, 7] and thus the disease appear subclinically [8]. The major economic importance caused by this virus is associated with subclinical form and might result in sever immunosuppression, poor growth, increased mortality and the cost of treatment due to secondary infections [9-11]. Three serological assays are routinely used for CAV diagnosis: ELISA-based assays, indirect immunofluorescence assays, and virus neutralization tests [12-13] but they are recommended for epidemiological study of the virus [14]. Polymerase chain reaction (PCR) assays have become the assay of choice for the detection of isolated chicken infectious anemia virus DNA in infected cell cultures, chicken tissues, archived formalin-fixed paraffin-embedded tissues, or vaccines. PCR assays have proven to be specific and much more sensitive than cell-culture isolation of the virus and facilitate sequence analysis [15-20]. The presence of CAV infection in commercial broiler chicken flocks in Iran has been reported by several investigators [21-23]. Because of high transmission rate of CAV and possibility of transmitting the infection to susceptible commercial chickens, finding its prevalence rate in native chicks and controlling CAV seem to be necessary. In this paper we describe molecular detection of CAV in native Larry-breed chickens in Chaharmahal-va-Bakhtiyari province in Iran for the first time.

## Materials and Methods

In the cross-sectional study, blood samples from 100 native Larry-breed chickens (5-8 months old) were collected from Shahrekord, Lordegan, Brogen and Koohrang, four cities of Chaharmahal-va-Bakhtiyari province in Iran. Twenty five samples were collected from Shahrekord (No. 1-25) and the remaining 75 samples were collected from Lordegan (No. 26-50), Brogen (No. 51-75) and Koohrang (No. 76-100). Serum samples were kept -70º C until used. We collected chickens randomly from the city sides where they raised native Larry fowls. The fowls had not been vaccinated against CAV, and no clinical signs indicating of CAV infection were observed in any of the flocks. Ethical clearance was taken from institutional ethical committee.

### Viral DNA extraction

For DNA extraction, all samples were processed with DNA isolation kit (Cinnagene,

Iran) according to the manufacturer's instruction.

**PCR amplification profiles**

The PCR reaction was performed in a thermocycler (Master cycler, Gradient, Germany) as follows:

94ºC for 1 min (denaturation), 57ºC for 1 min (annealing), and 72ºC for 2.5 min for 29 cycles. PCRs were finished with a final extension step of 10 min at 72ºC. We used CAV specific primers (CAV-F, CAV-R, 374 bp) as previously described by Iwata and coworkers [24]. (Table 1). PCR products were visualized by electrophoresis on an ethidium bromide-stained 2% agarose gel and images were visualized using an UV transilluminator (National Labnet Company, USA).

**Table 1.** Specific primers used for amplification and detection of DNA

| Primer | Sequence (5'-3') | Orientation | Product (bp) |
|---|---|---|---|
| **CAV-F** | TTT CAA ATG AAC GCT CTC CA | Forward | 374 bp |
| **CAV-R** | TCT TAC AGT CTT ATA CAC CT | Reverse | |

## Results

The presence of the expected amplification products obtained by PCR was confirmed by agarose gel electrophoresis and is shown in figure 1.

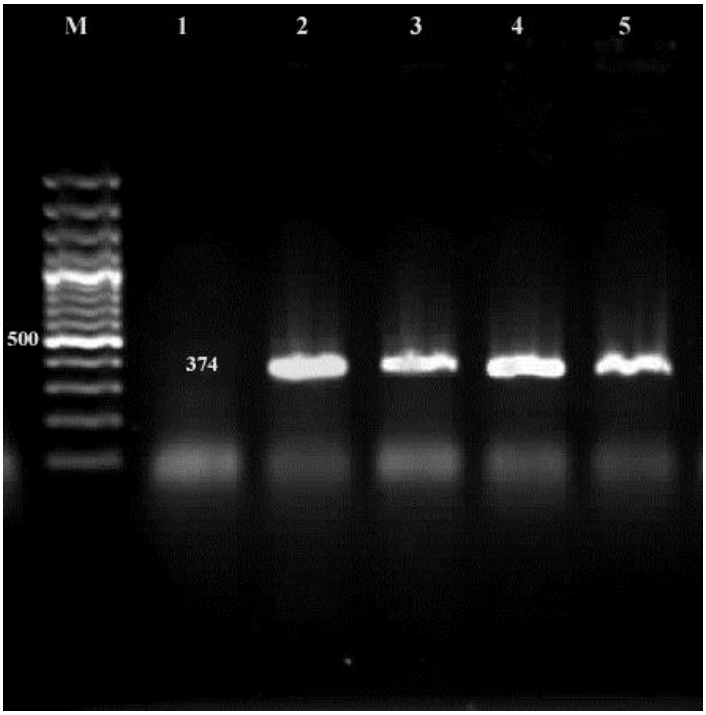


**Fig.1**. Amplicons of PCRs performed with (CAV-F, CAV-R) primers were separated by agarose gel electrophoresis. Lane M: 100 bp DNA marker, Lane 1: negative control, Lane 2: positive control (a live vaccine virus used as positive control) and Lanes 3, 4, 5 positive samples. PCR product is 374 bp.

Our results showed chicken anemia virus infection rate of 12% in non-commercial native Larry chickens subclinically in four cities of Chaharmahal-va-Bakhtiyari province. Of all 100 tested blood samples, 12 (12%) were positive for the presence of CAV (shown in table 2) and the rate of infection was shown to be 12%, 16%, 16% and 4% in Shahrekord, Lordegan, Koohrang and Brogen, respectively.

**Table 2.** Prevalence of CAV in samples from native Larry- breed chickens in four cities of Chaharmahal-va-Bakhtiyari province

| | Shahrekord | Lordegan | Brogen | Koohrang | Total |
|---|---|---|---|---|---|
| **CAV Positives** | 3 | 4 | 1 | 4 | 12 |
| **Number** | 25 | 25 | 25 | 25 | 100 |
| **Percentage** | 12 | 16 | 4 | 16 | 12 |

## Discussion

After the primary report of CAV in 1979, a large number of articles have been published about serological detection of CAV around the world. However, only a few have successfully isolated and characterized viruses. There are many reports on clinical CAV infection in virtually all countries with poultry industries [25]. In Sudan 44.3% of flocks [26]; in Japan 69% of flocks and 60% of broiler chickens [27]; in Turkey 70% of broiler farms and 20.8% of broiler chickens [28]; and in Jordan 100% of broiler chicken flocks and 82.6% of broiler chickens [29] were infected with CAV. Despite the potential importance of backyard chickens in epidemiology of CAV, few studies have been conducted on these chickens. For instance, in Nigeria, 75% of the studied native chickens were positive for serum CAV DNA [30] and 90% of native chickens evaluated in Ecuador presented CAV-specific antibodies in the serum [31] and in Brazil with 30% [32].

Although results of serological tests suggest that CAV alone has no public health significance [33] but immunosuppression in anemic chicken infectious anemia virus-infected birds has been linked to increased secondary viral, bacterial and fungal infections such as Influenza viruses, therefore, finding CAV prevalence rate among native fowls is important from this aspect. Interestingly, because of vulnerability of commercial chickens to CAV infection, control of CAV infection by encouraging farmers to perform CAV vaccination before the onset of egg production or biosecurity observance is important from veterinary aspect too.

Subclinical infections with chicken infectious anemia virus reduced development of antigen-specific cytotoxic T lymphocytes [34] and also adversely-affected macrophage function [35] thus resulted in increased susceptibility to diseases caused by other infectious agent and is one of the most significant mortality which is caused by CAV infection [28], therefore controlling of this disease could prevent many losses following opportunistic infections and lower performance [23]. Shoushtari and co-workers in 2006 have described co-infection cases of CAV with Marek and Influenza viruses in Iran [36]. Several investigations which have been carried out in different parts

of Iran reveal that CAV infection with extensive hemorrhage in skeletal muscles has been observed in commercial broiler chicken flocks [21, 22, 37], whereas in our study infection was detected in native flocks for the first time. High subclinical CAV infection prevalence in commercial broiler chicken flocks has been shown by Gholami-Ahangaran and Zia-Jahromi to exit in central areas of Iran. They suggest that high infection rate may be due to previous exposure to CAV or presence of maternal antibodies in chickens [22].

More recent pathogenecity findings of CAV virus in Iran suggest necessity of vaccine application of young chicks before their maternal immunity drops [38]. In this study, we observed occurrence of subclinical CAV infection in four cities of this province (Shahrekord, Brogen, Koohrang and Lordegan) which showed that native Larry-breed chicks in this area are not free from CAV infection. Larry-breed chicks are the main breeds in this province and there is not a special management system for breeding them in Iran. These birds live as free in the environment so this free-rang management system of local chickens expose them more to infectious agents compared to commercial fowls therefore, control and eradication of the disease must be taken into consideration in traditional farms.

## Conclusion

This study shows subclinically chicken anemia virus infection rate of 12% in non-commercial native Larry chickens in four cities of Chaharmahal-va-Bakhtiyari province, and Larry-breed fowls in the study region can be a focus for CAV and provide a source of infection for other breeding systems thus vaccination against CAV should be considered in native fowls in this area.

## Conflict of Interest

The authors declare that there is no conflict of interest.

## Acknowledgement

There is no acknowledgement to declare.

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