

Original Article

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

Neda Eskandarzade^{1*} Ph.D., Alireza Heydarnezhad² M.Sc., Yousef Valizadeh² M.Sc., Mona Shokouhi² M.Sc., Alireza Shafizadeh Kholenjani² M.Sc.

¹Department of Biochemistry, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

²Research Committee Student, Ilam University of Medical Sciences, Ilam, Iran.

ABSTRACT

Article history

Received 19 Apr 2015

Accepted 25 Jun 2015

Available online 8 Aug 2015

Key words

Chicken Anemia Virus

Larry-breed

PCR

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.

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 severe 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 -70°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:

94°C for 1 min (denaturation), 57°C for 1 min (annealing), and 72°C for 2.5 min for 29 cycles. PCRs were finished with a final

extension step of 10 min at 72°C. 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.

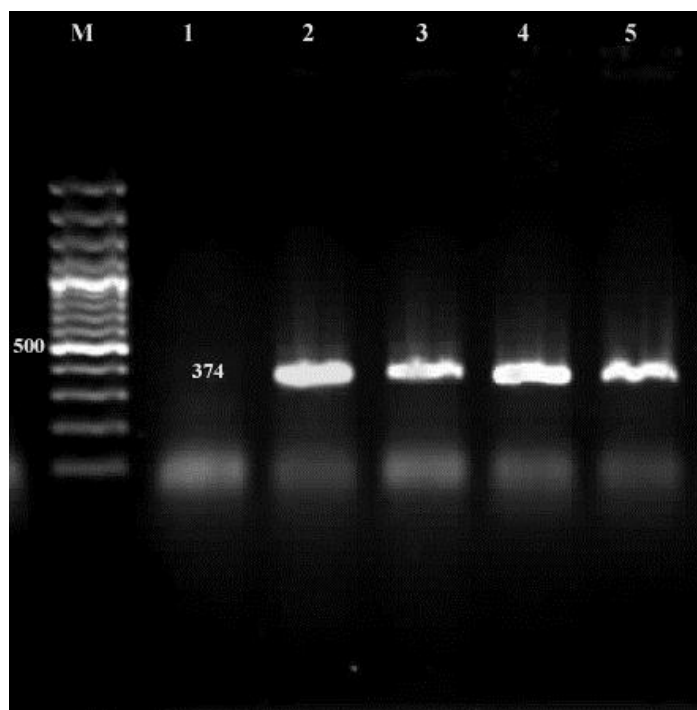


Fig.1. Amplicons of PCRs performed with (CAV-F, CAV-R) primers were separated by agarose gel electrophoresis. Lane M: 100 bp DNA marker, Lane 1: negative control, Lane 2: positive control (a live vaccine virus used as positive control) and Lanes 3, 4, 5 positive samples. PCR product is 374 bp.

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 exist 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 pathogenicity 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, Brojen, 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.

References

- [1]. Von Bulow V, Schat KA. Chicken Infectious Anemia. In: Diseases of Poultry. Chapter 2. Iowa State University Press: Ames IA; 2013: 249.
- [2]. Yuasa N, Taniguchi T, Yoshida I. Isolation and some characteristics of an agent inducing anemia in chicks. Avian Dis. 1979; 23:366-85.
- [3]. Miller MM, Schat KA. Chicken infectious anaemia virus: an example of the ultimate host-parasite relationship. Avian Pathol. 2004; 48:734-45.
- [4]. Rosenberger JK, Cloud SS. The effects of age, route of exposure and coinfections with infectious bursal disease virus on the pathogenicity and transmissibility of chicken anemia agent (CAA). Avian Dis. 1989; 33:753-59.
- [5]. Cloud SS, Rosenberger JK, Lillehoj HS. Immune dysfunction following infection with chicken anemia agent and infectious bursal disease virus. II. Alterations of in vitro lymphoproliferation and in vivo. Vet Immunol Immunopathol. 1992; 34:353-66.
- [6]. Rosenberger JK, Cloud SS. Chicken anaemia virus. Poult Sci. 1998; 77:1190-192.
- [7]. Yuasa N, Imai K. Pathogenicity and antigenicity of eleven isolates of chicken anemia agent (CAA). Avian Pathol. 1986; 15:639-45.

- [8]. Yuasa N, Noguchi T, Furuta K, Yoshida I. Maternal antibody and its effect on the susceptibility of chicks to chicken anemia agent. *Avian Dis.* 1980; 24:197-202.
- [9]. Chettle NJ, Eddy RK, Wyeth PJ, Lister SA. An outbreak of disease due to chicken anemia agent in England. *Vet Rec.* 1989; 124:211-15.
- [10]. McIlroy SG, McNulty S, Bruce DW, Smyth JA, Goodall EA, Alcorn MJ. Economic effects of clinical chicken anemia agent infection on profitable broiler production. *Avian Dis.* 1992; 36:566-74.
- [11]. McNulty MS, McIlroy SG, Bruce DW, Todd D. Economic effects of subclinical chicken anemia agent infection in broiler chickens. *Avian Dis.* 1991; 35: 263-68.
- [12]. Von Bulow V, Fuchs B, Bertram M. In vitro studies of the causative agent infectious anemia (CAA) in chickens: multiplication, titration, serum neutralization test and the indirect immunofluorescent test. *Zentralbl Veterinar med.* 1985; 32:679-93.
- [13]. Schat KA, Van Santen V. Chicken infectious anemia. In: *Diseases of Poultry*, 12th ed. Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK, Swayne DE, eds. Wiley-Blackwell: Ames IA; 2008: 211-235.
- [14]. Todd D, Creelan JL, Mackie DP, Rixon F, McNulty MS. Purification and biochemical characterization of chicken anemia agent. *J Gen Virol.* 1990; 71:19-23.
- [15]. Dren CN, Koch G, Kant A, Verschuere CAJ, Van der Eb AJ, Noteborn MHN. A hot start PCR for the laboratory diagnosis of CAV. In: *International symposium on infectious bursal disease and chicken infectious anemia.* Germany: Rauischholzhausen. 1994:413-420.
- [16]. Noteborn MHM, Verschuere CAJ, Van Roozelaar DJ, Veldkamp S, Van der Eb AJ, De Boer GF. Detection of chicken anaemia virus by DNA hybridisation and polymerase chain reaction. *Avian Pathol.* 1992; 21:107-18.
- [17]. Soine C, Watson SK, Rybicki E, Lucio B, Nordgren RM, Parrish CR, Schat KA. Determination of the detection limit of the polymerase chain reaction for chicken infectious anemia virus. *Avian Dis.* 1993; 37:467-76.
- [18]. Tham KM. Polymerase chain reaction analysis of chicken anemia virus DNA in fresh and paraffin-embedded tissues. In: *PCR: protocols for diagnosis of human and animal virus diseases.* Becker Y and Darai G, eds. Berlin: Springer-Verlag; 1995: 555-63.
- [19]. Todd D, Mawhinney KA, McNulty MS. Detection and differentiation of chicken anemia virus isolates by using the polymerase chain reaction. *J Clin Microbiol.* 1992; 30:1661-66.
- [20]. Tham KM, Stanislawek WL. Detection of chicken anaemia agent DNA sequences by the polymerase chain reaction. *Arch Virol.* 1992; 127:245-55.
- [21]. Toroghi R, Shoushtari AH, Charkhkar S, Neyazi MH. The first report of Chicken Infectious Anaemia occurrence among Iranian broiler flocks, *Proceedings of 13th Iranian Veterinary Congress, Iran Vet Association* 2003: 240.
- [22]. Mahzounieh M, Karimi I, Zahraei Salehi T. Serologic evidence of chicken infectious anemia in commercial chicken flocks in Shahrekord, Iran. *Int Poult Sci.* 2005; 4:500-503.
- [23]. Gholami-Ahangaran M, Zia-Jahromi N. Serological and molecular identification of subclinical chicken anaemia virus infection in broiler chickens in Iran. *African Journal of Microbiol Res.* 2012; 6:4471-474.
- [24]. Iwata N, Fujino M, Tuchiya AK, Iwata A, Otaki Y, Ueda S. Development of an enzyme-linked immunosorbent assay using recombinant chicken anaemia virus proteins expressed in a baculovirus vector system. *J Vet Med Sci.* 1998; 60:175-80.
- [25]. Cardona C, Lucio B, Oconnell P, Jagne J, Schat K. Humeral immune responses to chicken infectious anemia virus in three strains of chickens in a closed flock. *Avian Dis.* 2000; 44:661-67.
- [26]. Ballal A, Elhussein AM, Abdelrahim ISA. Serological survey of chicken infectious anemia in commercial chicken flocks in Khartoum state, Sudan. *J Anim Vet Adv.* 2005; 4:666-67.
- [27]. Farkas T, Maeda K, Sugiura H, Kal K, Hirai K, Otsuki K, Hayashi TA. Serological survey of chickens, Japanese quail, pigeons, ducks and crows for antibodies to chicken anemia virus in Japan. *Avian Pathol.* 1998; 27:316-20.
- [28]. Hadimli HH, Erganis O, Guler L, Uan US. Investigation of chicken infectious anemia virus infection by PCR and ELISA in chicken flocks. *Turk J Vet Anim Sci.* 2008; 32:79-84.
- [29]. Roussan DA. Serological survey on the prevalence of CIVA in commercial broiler chicken flock in Northern Jordan. *Int Poult Sci.* 2006; 5:544-46.
- [30]. Oluwayelu DO, Todd D. Rapid identification of chicken anemia virus in Nigerian backyard chickens by polymerase chain reaction combined with restriction

- endonuclease analysis. *African J Biotech.* 2008; 7:271-75.
- [31]. Hernandez-Divers SM, Villegas P, Prieto F, Unda JC, Stedman N, Ritchie B, et al. A survey of selected avian pathogens of backyard poultry in northwestern Ecuador. *J Avian Med Surg.* 2006; 20:147-58.
- [32]. Barrios PR, Marin SY, Resende M, Rios RL, Resende JS, Horta RS, et al. Occurrence of chicken anemia virus in backyard chickens of the Metropolitan region of Belo Horizonte, Minas Gerais. *Brazilian J of Poult Sci.* 2009; 11:135-38.
- [33]. Von Bulow V, Schat KA. Chicken infectious anemia: In: *Diseases of Poultry*, 10th ed. Calnek BW, Barnes JH, Beard CW, McDougald LR, Saif YM, eds. Iowa: Iowa State University Press Ames; 1997: 739-56.
- [34]. Markowski-Grimsrud CJ, Schat KA. Infection with chicken anemia virus impairs the generation of antigenspecific cytotoxic T lymphocytes. *Immunol.* 2003; 109:283-94.
- [35]. McConnell CD, Adair BM, McNulty MS. Effects of chicken anemia virus on cell-mediated immune function in chickens exposed to the virus by a natural route. *Avian Dis.* 1993; 37:366-74.
- [36]. Shoushtari AH, Ezzi A, Bozorgmehri MH, Gudarzi H. Coinfection of broilers with Influeza, chicken infectious anemia and Marek viruses. 3rd Iranian Congress of Virology 2006.
- [37]. Farhoodi M, Toroghi R, Bassami MR, Kianizadeh M. Chicken infectious anemia virus mong broiler flocks in Iran. *Archive of Razi Institute* 2007; 62: 1-6.
- [38]. Ezzi A, Shoushtari A, Marjanmehr H, Toroghi R, Tavasoly A, Bahmani-nejad MA. Experimental studies of pathogenecity of chicken infectious anaemia virus (3 isolates) in Iran. *Archives of Razi Institute* 2012; 67: 13-19.