Genomic detection of the chicken anaemia virus from apparently healthy commercial broiler chickens in Iran

M. GHOLAMI-AHANGARAN1*, H. MOMTAZ2, N. ZIA-JAHROMI3, M. MOMENI4

1Poultry Diseases Department, Veterinary Medicine Faculty, Islamic Azad University, Shahrekord Branch, P.O. Box: 166 Shahrekord, IRAN.
2Veterinary Microbiology Department, Veterinary Medicine Faculty, Islamic Azad University, Shahrekord Branch, P.O. Box: 166 Shahrekord, IRAN.
3Basic Sciences Faculty, Shahrekord Branch, Islamic Azad University, P.O. Box: 166, Shahrekord, IRAN.
4Biotechnology Research Center, Veterinary Medicine Faculty, Islamic Azad University, Shahrekord Branch, P.O. Box: 166 Shahrekord, IRAN.

*Corresponding author: gholami@iaushk.ac.ir

SUMMARY

In this study, the presence of the Chicken anaemia virus (CAV) in apparently healthy broilers from the Isfahan province (central Iran) was directly investigated using PCR method in order to determine the prevalence of the subclinical viral infection. For that, a total of 240 thymuses were collected from birds belonging to 15 different flocks regrouping 223 000 apparently healthy broilers at slaughtering and the PCR amplification of the gene coding for the viral VP2 protein was performed on thymus samples. The individual viral prevalence was 24.58% and the rate of positive flocks (in which at least one animal was detected as positive) reached 73.33%. These results clearly show the high prevalence of the subclinical CAV infection in central Iran and emphasize the requirement for developing control strategies to prevent economical losses in poultry.

Keywords: Chicken Anaemia Virus, broiler chickens, Iran, subclinical infection, PCR, flock prevalence, individual prevalence.

RÉSUMÉ

Détection génomique du virus de l’anémie du poulet chez des poulets de chair apparentemment sains en Iran

La présence du virus de l’anémie du poulet (VAP) a été directement recherchée par PCR chez des poulets apparentemment sains de la province Isfahan (région centrale de l’Iran) afin de déterminer la prévalence de l’infection virale subclinique. Pour cela, 240 thymus au total ont été prélevés lors de l’abattage sur des oiseaux provenant de 15 troupeaux différents regroupant 223 000 poulets de chair apparentemment en bonne santé et l’amplification par PCR du gène codant pour la protéine virale VP2 a été réalisée à partir des échantillons de thymus. La prévalence individuelle du virus a été de 24.58 % et le taux de troupeaux positifs (comprenant au moins un animal infecté) a atteint 73.33 %. Ces résultats démontrent une forte prévalence de l’infection virale subclinique chez les poulets du centre de l’Iran et soulignent la nécessité de développer des stratégies de contrôle permettant de prévenir les pertes économiques dans l’industrie aviaire.

Mots clés : Virus de l’anémie du poulet, poulet de chair, Iran, infection subclinique, PCR, prévalence par troupeau, prévalence individuelle.

Introduction

Chicken anaemia virus (CAV) is a small, non-enveloped, 23 to 25 nm in size, with single-stranded circular DNA genome and negative sense [11, 16]. The CAV genome structure is about 2.3 kb with three partially overlapping open reading frames (ORFs) coding VP1, VP2 and VP3 proteins. VP1 and VP2 are the main targets of neutralizing antibodies and VP3 induce apoptosis [3].

The chicken anaemia virus, which is the only member of the genus Gyrovirus, is belonging to the Circoviridae family [13]. The virus was first isolated and described by YUASSA et al. [20] in Japan. The CAV could be transmitted both vertically and horizontally in chickens [1]. CAV infections induce either clinical or subclinical signs and both result in important economical losses [17]. The clinical disease is mainly noticed in young chicks of 10-14 days of age, which usually acquire the infection vertically [10].

CAV has been found in many countries with a poultry industry [3]. It has been detected worldwide by isolation, serology or DNA amplification in chickens [17]. The PCR has been applied as a rapid diagnostic tool for the detection of virus in DNA extracted from fresh tissues [14]. In Iran, there are some reports for detection of CAV from affected chicken flocks with clinical signs and lesions. Until now, there is no report about subclinical CAV infection in Iran. Consequently, the aim of the present study was to investigate the CAV prevalence by the PCR method in apparently healthy broiler chicken flocks from the central Iranian province, Isfahan.

Material and Methods

FLOCKS

A total of 240 thymus samples have been collected from 15 broiler chicken flocks in different slaughterhouses of Isfahan, a central province in Iran, between 2009 and 2010. The collected tissues were stored at -20°C until assayed. The commercial broiler chicken flocks have not been vaccinated against CAV, and no clinical signs suggestive of the CAV infection were observed in any flocks.
PCR METHOD

The DNA extraction from each thymus sample was carried out using commercial DNA extraction kit (High Pure Viral Nucleic Acid Kit, Roche, Germany), according to the manufacturer’s instructions.

For the PCR method, the primers used allow the amplification of a 713 bp fragment of the CAV VP2 gene and their corresponding sequences were the following: for the forward primer: 5’- GCG CAC ATA CCG GTC GGC AGT and for the reverse primer: 5’-GGG GTT CGG CAG CCT CAC ACT AT according to NATESAN et al. [10]. The PCR amplification was performed in PCR buffer containing 1.5 mM MgCl2, 200 μM of each dNTPs, 10 pM of each primers and 1.0 unit of Taq polymerase (Fermentas) in 25 μL of total reaction volume. The amplification was carried out under following conditions in a thermal cycler (Mastercycler, Gradient, Germany): initial denaturation at 94°C for 4 minutes, 34 cycles of denaturation (94°C for 1 minute), annealing (63°C for 1 minute), extension (72°C for 1 minute) and final extension 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 controls and DNase free water was used as a negative control.

Results

As shown in Table I, among the 240 thymuses collected from 15 healthy broiler chicken flocks representing a total of 223,000 birds, the CAV genome was detected by the PCR method in 59 tissue samples. The individual CAV prevalence was 24.58%.

Eleven flocks contained at least one bird in which the gene coding for the VP2 protein was evidenced in thymus and these flocks were considered as positively CAV infected flocks. The flock CAV prevalence was 73.33% in the apparently healthy flocks from the Isfahan province (Table I). Within the CAV infected flocks, the CAV positivity rate ranged from 7.14% to 80.00% (Table II).

Discussion

The Chicken anaemia virus is responsible for an infectious anaemia in chickens characterized by subcutaneous haemorrhages coupled to a general immuno-suppression due to the atrophy of the lymphoid tissues [8]. The disease primarily occurs in young chickens [10]. The major economical losses caused by the viral infection are linked to a markedly delayed growth and a severe immuno-suppression leading to an increased mortality due to secondary infections [2, 7]. Therefore, the CAV

<table>
<thead>
<tr>
<th>Total population</th>
<th>Sample size</th>
<th>Positivity</th>
<th>CAV prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>223 000 birds</td>
<td>240 thymuses</td>
<td>59</td>
<td>24.58%</td>
</tr>
<tr>
<td></td>
<td>15 flocks</td>
<td>11</td>
<td>73.33%</td>
</tr>
</tbody>
</table>

**Table I**: Individual and flock CAV prevalences determined by PCR amplification of the VP2 gene on thymus samples in 15 healthy broiler chicken flocks from the Isfahan province, Iran.

<table>
<thead>
<tr>
<th>Flock</th>
<th>Flock size</th>
<th>Sample size</th>
<th>Positive samples</th>
<th>CAV prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>n°1</td>
<td>20 000</td>
<td>18</td>
<td>4</td>
<td>22.22%</td>
</tr>
<tr>
<td>n°2</td>
<td>9 000</td>
<td>13</td>
<td>8</td>
<td>61.54%</td>
</tr>
<tr>
<td>n°3</td>
<td>21 000</td>
<td>20</td>
<td>5</td>
<td>25.00%</td>
</tr>
<tr>
<td>n°4</td>
<td>11 500</td>
<td>14</td>
<td>2</td>
<td>14.29%</td>
</tr>
<tr>
<td>n°5</td>
<td>9 500</td>
<td>17</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>n°6</td>
<td>8 500</td>
<td>15</td>
<td>5</td>
<td>33.33%</td>
</tr>
<tr>
<td>n°7</td>
<td>10 000</td>
<td>14</td>
<td>1</td>
<td>7.14%</td>
</tr>
<tr>
<td>n°8</td>
<td>10 000</td>
<td>14</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>n°9</td>
<td>18 500</td>
<td>17</td>
<td>3</td>
<td>17.65%</td>
</tr>
<tr>
<td>n°10</td>
<td>30 000</td>
<td>20</td>
<td>6</td>
<td>30.00%</td>
</tr>
<tr>
<td>n°11</td>
<td>12 500</td>
<td>15</td>
<td>8</td>
<td>53.33%</td>
</tr>
<tr>
<td>n°12</td>
<td>11 000</td>
<td>15</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>n°13</td>
<td>13 500</td>
<td>15</td>
<td>12</td>
<td>80.00%</td>
</tr>
<tr>
<td>n°14</td>
<td>10 000</td>
<td>17</td>
<td>0</td>
<td>0.00%</td>
</tr>
<tr>
<td>n°15</td>
<td>28 000</td>
<td>16</td>
<td>5</td>
<td>31.25%</td>
</tr>
</tbody>
</table>

**Table II**: CAV prevalence determined per apparently healthy chicken flock from the Isfahan province, Iran (n = 15), using PCR amplification of the VP2 gene on thymus samples.
can be considered as an economical important poultry pathogen in worldwide with highly immunosuppressive nature [10].

The clinical CAV prevalence rate in chickens has been reported to be high in many countries. For example, the CAV seroprevalence in commercial chicken flocks was 86% in Nigeria [12], 82.61% in Northern Jordan [15] and 62% in Khartoum state, Sudan [1]. In Iran, the disease was firstly reported in 2003 by TOROGHI et al. [19]. They found extended haemorrhages in subcutaneous and intramuscular tissues after broiler slaughtering and have confirmed the viral infection by detecting the CAV genome in these clinical cases [19]. Furthermore, MAHZOUNIEH et al. [5] have investigated in 2005 the CAV seroprevalence using ELISA in broiler chickens flocks from the Shahrekord province, western area of Iran, in which at least one bird have exhibited clinical signs and lesions and they have reported that all farms were infected and that 87.7% of chickens were positive for CAV antibodies. Later, FARHOODI et al. [4] confirmed in 2007 the large prevalence of the virus within various provinces in Iran and they reported that 71.7% of broiler chickens with muscular haemorrhages were positive to CAV infection. However, these high CAV prevalences throughout Iran were determined from flocks in which clinical signs and haemorrhagic lesions were evidenced in some birds and consequently in which the CAV infection was highly probable. In the present study, the CAV prevalence was determined using a PCR method from flocks of apparently healthy chickens; the frequency of the individual subclinical CAV infection was 24.58% and the mean flock virus prevalence was 73.33%, reaching 80.00% in a flock (n°13). These high scores obtained using a molecular method which directly detects the virus presence (and not the secondary markers of the infection as antibodies) indicate a natural exposure of the apparently healthy broilers to the virus and a continuous field challenge. They also provide evidence of the widespread distribution of the virus and the considerably high incidence of the subclinical infection in central area of Iran.

Nevertheless, the high detection rate of CAV genome in the thymus of apparently healthy chickens was not unexpected since it was previously reported the high frequency of anti-CAV antibodies in specific pathogen free flocks [8]. It seems that the CAV infection in broiler flocks is ubiquitously present, all over the world. The clinical disease due to CVA is however uncommon whereas the subclinical infection of commercial broiler appears more frequent [9]. In chickens older that 2 weeks, the infection is considered as subclinical but leads to immunosuppressive effects [6, 18] that increase the bird susceptibility to other poultry pathogens, resulting in reduced performance, delayed growth and economic losses [9].

As a conclusion, the prevalence of the CAV subclinical infection is dramatically high even in apparently healthy broiler flocks. Therefore, further molecular and pathogenic CAV characterizations are required to find out suitable control strategy to prevent losses in the poultry industry.

Acknowledgment

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References