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Molecular analysis of the chicken anemia viruses isolated from broiler farms of East Azerbaijan province using PCR-RFLP

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Abstract

Anemia was first detected in Japan. Since then, the CAV virus has been reported in broiler and oviparous chicken breeds in many countries, both in viral isolation and serologically. One of the major immunosuppressive viruses is infection anemia virus. Infections with the CAV virus appear to be clinically prominent, including symptoms of bone marrow cells, subcutaneous hemorrhage, atrophy of lymph nodes, and increased mortality. The virus has a circular DNA genome of 2,300 bp, including three protein sequences (VP1, VP2, and VP3). The 50 kDa (VP1) viral protein is the only isolated protein from viral objects. The study examined the prevalence, traceability, isolation and identification of an infective anemia virus in broiler flocks within East Azarbaijan province. For this purpose, 100 samples of slaughtered broiler flocks were collected at slaughterhouses in the East Azerbaijan province. To confirm the presence of CAV virus in the samples, PCR was performed and the results were studied with RFLP technique. This study showed that poultry infectious anemia virus in broiler farms in East Azarbaijan province is widespread. There are very few differences in virus between isolates and isolates from various parts of the world.

Introduction

Immunosuppressive viral diseases are associated with high mortality and economic losses related to poultry industry, which are often accompanied by raised sensitivity to secondary infections and reduced response to vaccination. Such viral immunosuppressive diseases are infectious anemia of poultry chicken anemia virus (CAV). CAV is a member of the circovirus family, icosahedron, without membrane and with a thickness of 25 to 26/5 nm (1). Their genomes are circular and have single-stranded DNA with the size of 2.3 kbp containing three overlapping open reading frame sections. VP2 is a scaffold protein with a specific phosphatase specific protein, whereas VP1 is the main structural protein of the CAV virus with high variability (2). VP3 is an unconstructive protein called apoptin (13.6 kDa), which has apoptotic properties (3). Two of the primary proteins to neutralize antibodies are VP2 and VP1 (4). In Japan, in 1976, an outbreak of chicken infected anemia was isolated (5). Infectious anemia is one of the most important immunosuppressive diseases in day-old chicks. Severe anemia, pancytopenia, bleeding in the legs and chest muscles, necrosis cen-

-ters in the liver, and the neck, and general atrophy of the lymphoid tissue bone marrow, thymus, and bursa of Fabricius are characteristic features of this disease (6). Chicken infectious anemia virus has a high resistance to most disinfectants (7).

To diagnose the disease, anti-CAV antibodies can be detected by serological methods such as indirect Immunofluorescence, ELISA and serum neutralizing (SN) (8).Therefore, to detect and characterize CAV different molecular techniques have been developed such as polymerase chain reaction (PCR), hybridization assay, restriction enzyme analysis, and sequencing (4).The most appropriate component of the virus for molecular characterization is VP1, the main protein and the only structural protein of the CAV with the most variability and also responsible for the nucleotide events between genotype and intracellular (9).

Materials and Methods

Sample collection and virus detection

One hundred liver samples were collected from slaughterhouses in East Azarbaijan province from different cities, the liver was transferred to the laboratory under sterile condition on ice and stored in the freezer (-80°C) until DNA was extracted.

DNA extraction

Liver tissue has been homogenized in a sterilized honeycomb with PBS buffer and centrifuged for 5 minutes at 3500 rpm. In sterile microtubes, the supernatant has been taken for DNA extraction. According to the instructions given by the kit manufacturer, DNA extraction has been performed using a high-purity viral genome extraction kit.

Amplification of the VP1 gene

For amplification of the VP1 gene of CAV, two primers targeting 1390 bp comprising the whole VP1 gene of CAV were used. The forward primer (5'-AGC CGA CCC CGA ACC GCA AGAA-3') was described by Farhoodi et al., (10) and the reverse primer (5'-TCA GGG CTG CGT CCC CCA GTA CA-3') was previously described by Mohamed (11). The PCR was carried out in 25 ml PCR mixtures in a mixture of 25 ml carried out using Mastermix (Yekta Tajhiz Azma). Cycle conditions for amplification were used, first incubation at 94°C for 2 min followed by 35 cycles of incubation at 94°C for 45 seconds, 62°C for 45 seconds and 72°C for 110 seconds with a final extension up to 72°C for 5 minutes. In the 1% agarose gel and in the photo taken using an ultraviolet transilluminated spectrophotometer, the resulting PCR products have been broken down.

Restriction fragment length polymorphism (RFLP)

sprouting The XbaI restriction endonuclease, selected on the basis of a simulated limit map for amplified PCR products using BioEdit software, has been used to digest purified PCR products. Digestion reaction was carried out in 25µl mixtures containing 10µl purified PCR product, 5 units of XbaI endonuclease (Takapuozist) and 2.5µl XbaI buffer and 12µl. The resulting mixtures were placed in a 37°C temperature bath for 20 minutes, followed by an additional 20 minutes at 65°C to deactivate the enzyme. After enzyme digestion, the resulting DNA fragments were separated by 1.5% agarose gel and photographed using ultraviolet light.

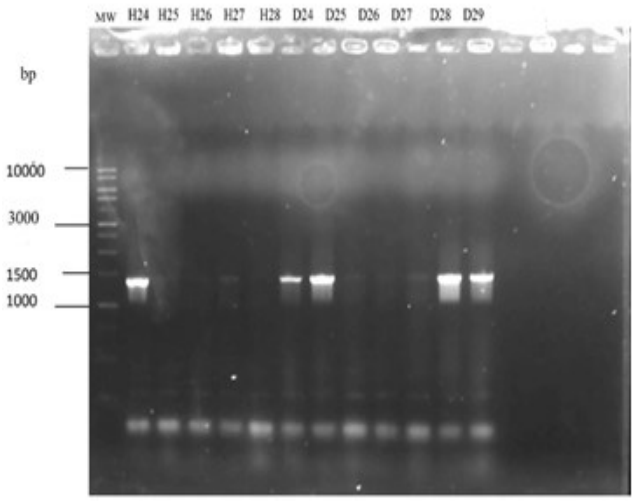

Figure 1. Proliferation of 1,390 bp fragment containing Vp1 gene of 12 samples

Results

38 samples out of 100 samples suspicious for CAV (from an aviculture of East Azarbaijan province) were tested and the 1,390 bp segment containing the Vp1 gene were proliferated with PCR, successfully (Figure 1).

Results of enzyme digestion

The RFLP pattern of ten strains, generated by enzyme digestion of the Vp1 gene with Alu1 restriction enzyme, created ten different patterns which can be seen in Figure 2. The RFLP pattern of ten strains, generated by enzyme digestion of the Vp1 gene with XbaI restriction enzyme, created seven different patterns

analyzed by gel electrophoresis.

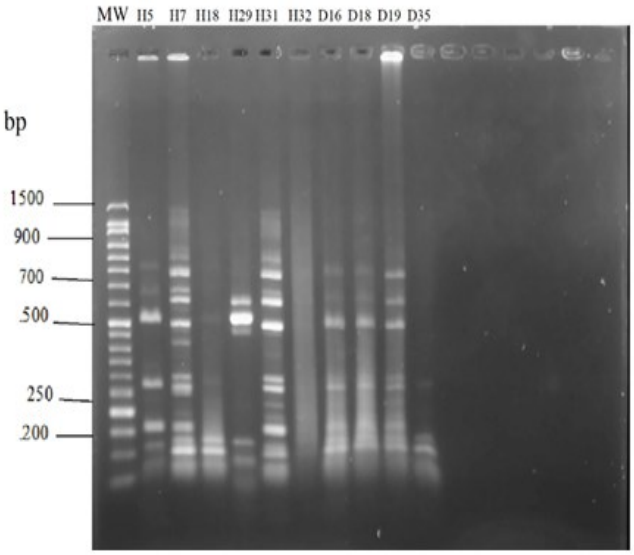

Figure 2. Gel electrophoresis of proliferated Vp1 gene, digested with AluI. H1-D35 are different RFLPpatterns.

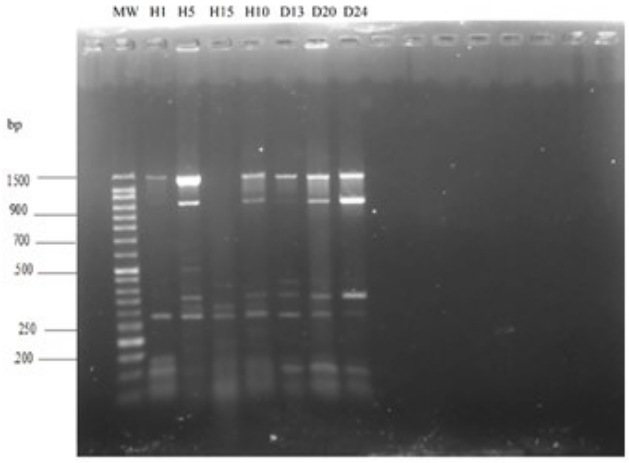

Figure 3. Gel electrophoresis of proliferated Vp1 gene, digested with XbaI. H1-D24 are different RFLP patterns.

Discussion

The main reason for limiting the growth of chickens in Iran and in other countries is considered to be a large number of viral diseases affecting chickens. CAV is a major disease in poultry around the world. It is mainly a disease of the immature chick's immune system, but it can have an impact on chicks at any age. CAV is a member of the Anelloviridae family, which includes Gyrovirus. It's a single-stranded DNA with a 2.3 kbp genome, one of the smallest bird viruses. The disease is associated with severe anemia, bone marrow aplasia and lymphoid atrophy and is accompanied by immunodeficiency, which results in secondary infections in young chicks that are usually less than 4 weeks of age. The host's sensitivity to all bacteria, viruses and fungi is increased when the immune system is weakened by this virus leading to an outbreak of serious disease. In India, the disease has been reported from Namakkal veterinarian based on antigen detection with non-immunoxidase testing, which was later confirmed by PCR diagnosis in clinical specimens. Following this report, the virus was detected using PCR technique and was also isolated in other countries. Bahmaninejad et al., (12) reported the first CAV infection in Iran using the PCR technique for amplification of the Vp1 gene, and 440 samples were confirmed positive for CAV.To highlight the importance of this important economic and pathogenic bird disease virus in a country, it was, therefore, necessary to identify its molecular characteristics in that area to help establish an appropriate control strategy. Bird diseases from the pathology department, IVRI, are actively involved in the detection and identification of this important economic pathogen. The potential of pathogenicity of CAV in chicks has been studied; different isolates at molecular levels have been sequenced using PCR, RE and sequencing. Therefore, the present work involves the detection of PCR-RFLP from CAV DNA in field samples to show the occurrence of CAV with comparisons with other known strains in the world. Molecular detection is currently used to diagnose and confirm the CAV infection. These tests are more reliable, highly sensitive, and specific in terms of results that can be obtained. The sensitivity of the PCR to classical serological methods is said to be very high. To some extent, the detection of virus DNA has been carried out only through PCR or in combination with enzyme degradation.

Therefore, in this study, PCR was used to diagnose CAV from suspected tissues. To identify the PCR, the VP1 F and VP1 R primer set was designed. Duplicated PCR products were tested by agarose gel electrophoresis with standard DNA markers. This finding also agrees with other researchers who have identified PCR from different tissues of DNA CAV (13-16), CAV from MSB-I cells were identified. Imai et al., used PCR technique to diagnose the CAV DNA of infected liver tissue with MDCC-MSB1 which were fixed with formalin and paraffin. With high sensitivity and rapid detection of PCR, it is an important tool for disease confirmation. Moreover, the amplification of the PCR of virus viral genome directly from the target tissue, eliminate the need for virus isolation.

from axons to make new connections with other neurons or target cells. This is important for the nervous system to recover, grow, and change, especially after damage or disease. The things that control axonal sprouting are complicated and have many parts, such as signals, proteins, and interactions between cells. These are chemicals that can pull or push axons and he

-lp them find their right targets. Some examples of these chemicals are netrins, semaphorins, ephrins, and slits. These chemicals can attach to certain receptors on the axon surface, such as DCC, neuropilins, ephs, and robo, and start signals inside the cells that control how the cell structure, growth cone movement, and branching change (18,19).

Extracellular matrix components are chemicals that give structure and signals to the axons and the growth cones. Some examples of these chemicals are laminin, fibronectin, collagen, and hyaluronic acid. These chemicals can work with integrins and other receptors on the axon surface, and change how the axons stick, move, and branch out (20). Cell-to-cell interactions are when the axons touch or communicate with other cells, such as glial cells, target cells, or nearby axons. These interactions can affect how the axons live, grow, and branch out through different ways, such as giving them support, sending messages, connecting electrically, and releasing small packets (21).

Axon sprouting is when new branches grow from axons to make new connections with other neurons or target cells. This is important for the nervous system to recover, grow, and change, especially after damage or disease. We can use the ways that axon sprouting is controlled for therapy by doing different things, such as Genetic engineering means changing the genes of stem cells or neurons to make them sprout and live better in the nervous system that is hurt or sick. For example, a study by Burke Neurological Institute and Weill Cornell Medicine showed that turning on MAP2K signaling by genetic engineering helped corticospinal tract axon sprouting and function recovery after spine injury in mice (16). Cell therapy is a method of transplanting cells that can either become neurons or help new axons grow in the nervous system. For example, a review by Springer explored how stem cells that have been modified by genes could be used for spinal cord injury. These cells could reduce the damage, lower inflammation, protect neurons, and promote axon regeneration (22). Physical stimulation is a technique of applying methods that can either be non-invasive or invasive to the nervous system and cause new axons to grow and plasticity to increase. a study by Frontiers demonstrated that acupuncture can trigger the formation of new neurons, stimulate the regeneration and sprouting of axons, and enhance the structure and function of synapses after a stroke (23). Pharmacological intervention is a method of giving drugs or molecules that can change the signaling pathways, extracellular matrix components, and cell-to-cell interactions that control axon sprouting. a study by Nature revealed that Cellatoz Therapeutics is creating new cell therapies by using its own cells, called A-to-Z cells, for different therapeutic areas, such as musculoskeletal disorders, peripheral nerve injury, and Charcot-Marie-Tooth disease (24). These are some of the methods that can use the axon sprouting regulatory.

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