

Investigation on the Incidence of Marek's Disease Virus in Backyard Chicken Flocks in Iran: Complete the Puzzle of Epidemiology of Marek Disease

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Abstract: Marek's disease (MD) is an evolving disease that affects industrial poultry and backyard chickens and causes significant economic damage. Epidemiologically studying and continuous monitoring is vital to evaluate the progression and change of the MD virus and take appropriate action timely. In this study, after MD epidemiologic research on broilers, breeders, and layers of Iran, the prevalence of Marek's disease virus (MDV) in backyard chickens of some provinces was investigated. This cross-sectional study was conducted from 2020 to 2021 in the villages located in five provinces of Mazandaran, Qazvin, Ilam, Khuzestan, and Markazi in Iran. DNA of the samples was extracted, then the prevalence of Marek's disease virus serotypes and the presence of the gB gene were detected using the real-time PCR method. gB gene analysis was performed on MDV-1 samples collected of 50 villages and each village represented one epidemiologic unit. Fifteen epidemiologic units were positive for MDV serotypes, and the overall prevalence was recorded as 30%. The provinces with more industrial poultry farms (Markazi and Mazandaran) had shown the higher prevalence of Marek's disease virus. Conclusively, MDV-1 is circulating in backyard chicken farms in Iran, and there is a need to properly plan for extensive training and vaccination.

Keywords: Marek's Disease, Backyard Chickens, gB Gene, Real-Time, Iran

1. Introduction

Marek's disease virus (MDV) belongs to the family Herpesviridae, subfamily Alphaherpesvirinae, genus Mardivirus, and the virus species: Gallid herpesvirus 2 (Serotype 1 or MDV1), Gallid herpesvirus 3 (serotype2 or MDV2), and Meleagrid herpesvirus 1 (serotype 3 or MDV3) which has affected the poultry industry [1] and it is also a cause of disease and death in backyard chicken in many parts

of the world [2-6]. MD is caused by the infection of chickens with alphaherpesvirus 2 (serotype MDV1), a highly contagious virus that affects the immune and nervous systems, causing lymphoma and eventually death [7, 8]. MDV1, a pathogenic serotype, is identified by the oncogenic *Meq* gene and other genes such as *pp38*, *vIL-8*, and *vTR*, especially in the TRL (transactivation) region. The *Meq* gene plays a role in tumorigenesis, and its removal stops the transformation of T-cell lymphocytes [9]. MDV and HVT

contain several homologs of conserved glycoproteins, including gB, gC, gD, gE, gH, gI, gK, gL, gM, and gN found in Herpes Simplex Virus-1. In *alphaherpesvirus*, glycoproteins (gB, gC, gD, gH, and gL) play a prominent role in entering the cell through fusion [10, 11].

Backyard chickens are raised as pets and a cheap source of protein in rural areas and are weak in biosafety and vaccination planning [12-14]. Marek's disease has also been reported as a cause of death in backyard chickens in several countries such as Nigeria, Italy, the United States, Brazil, Iraq, and Indonesia [6, 15-19]. Studies have been conducted in Iran on MD in industrial poultry, but no report has been published on the prevalence of Marek's disease in backyard chickens. In the present study, Marek's disease virus has been investigated using amplification of the glycoprotein B (gB) gene of serotype 1 MDV on a large scale in rural areas of Iran.

2. Materials and Methods

This cross-sectional study was conducted from 2020 to 2021 in the villages located in five provinces of Mazandaran, Qazvin, Ilam, Khuzestan, and Markazi in Iran (Figure 1). For this purpose, 50 villages and backyard chicken flocks were selected, and ten backyard chickens were selected for each village. Then, five feathers were collected and pooled from the wings and tail per backyard chicken and stored in a frozen package. Finally, the samples were transferred to the Faculty of Veterinary Medicine Laboratory, University of Tehran.

2.1. DNA Extraction

DNA from feathers was extracted. In brief, the samples were mixed with proteinase K and incubated at 55°C overnight with shaking. The next day, DNA was extracted by DNA extraction kit (Sinaclon, Iran) following the manufacturer's instructions and stored at -20°C until use. The samples were pooled so that every ten samples (a village sample as an epidemiologic unit) were mixed in a tube and considered one sample.

2.2. Detection of gB Gene by Real-Time PCR Assay

The real-time PCR assay was performed as described previously. In brief, each sample was amplified with four pairs of primers specific for the glycoprotein B (gB) gene of serotype 1 MDV; Amplifications of the gB sequence were carried out in separate reactions using the following primers and probes: gBforward, CGGTGGCTTTTCTAGGTTTCG; gBreverse, CCAGTG-GGTTCAACCGTGA. Amplifications were done using a Rotor-Gene Q (Qiagen, CA) in a 25-ml PCR reaction containing 50 ng of DNA, 0.2 mM of each primer, and SYBRH Green PCR master mix (Maxcell) that contains the appropriate buffers, nucleotides, and Taq polymerase. The reaction was cycled 50 times at 95°C denaturation for 15 sec and a 60°C combined annealing/extension for 60 sec. Fluorescence was acquired at

the end of the annealing/extension phase. The melting curves were obtained at the end of amplification by cooling the sample at 20°C/sec to 60°C and increasing the temperature to 95°C at 0.1°C/sec [20].

2.3. Statistical Analyses

Statistical analyses were carried out using Excel 2016 (Microsoft Office).

3. Results

gB gene analysis was performed on samples collected from 50 villages. Of 50 villages, 15 epidemiological units (villages) were positive for MDV1, and the overall prevalence was 30% (Table 1; Figure 1). The virus was isolated from all five studied provinces. In addition, the largest numbers of positive villages were allocated to the Markazi and Mazandaran provinces (40%). The meanest rank was related to Ilam and Khuzestan provinces, with 20%.



Figure 1. Distribution of Sampled Regions for MDV in Backyard Chickens in Iran During 2020.

Table 1. The Number of Flock Sampled and Percentage of Positive Units in Five Provinces in Iran.

province	The number of villages studied	Number of positive villages (%)
Markazi	10	4 (40%)
Ilam	10	2 (20%)
Mazandaran	10	4 (40%)
Khuzestan	10	2 (20%)
Qazvin	10	3 (30%)
Total	50	15 (30%)

4. Discussion

In 2019, about 26 billion chickens were produced globally, and Iran is ranked sixth, producing about 630 million pieces [21]. Also, more than 50 million backyard chickens were raised in the rural area of Iran. Backyard chickens provide some of the protein needs of the villagers, and the surpluses are supplied to the markets.

Owners of backyard poultry farms facilitate the transfer of GaHV2 strains due to poor management, lack of vaccination, and loss of biosecurity [22]. In addition to the economic damage to the villages in case of an MD outbreak, the transfer of backyard chickens and the short distance between the villages and the industrial poultry farms would be a potential risk for the poultry industry.

In this study, five provinces located from north to southwest with different climates and geographical conditions have been studied. Mazandaran province has a temperate climate on the north coast, unlike Khuzestan on the southwest coast, which has a hot and humid climate. While the Markazi, Qazvin, and Ilam, which are located in the middle and the west of Iran, belong to the semi-desert, cold and mountainous areas. MDV was noticeably tracked in the backyard chickens of all studied provinces. This shows that climatic conditions have not affected the prevalence of the disease, and other factors, such as the density of backyard chickens in the village or the existence of layer and broiler farms at close distances, should be considered.

Marek's disease seems to be a global evolving problem that causes disease even in industrialized countries. Mete *et al.*, In a 2007-2010 study of backyard chickens in California, reported that Marek's disease was estimated at 21.5%. They attributed the cause of the disease to the wrong vaccination strategies [6]. In a similar study, Mescolini *et al.* sampled 19 MD-suspected backyard chicken flocks from 2015 to 2017. Detection of the gB gene from feathers confirmed all those flocks were positive [18]. In 2014, Hartawan *et al.* in Indonesia examined different samples such as blood, feather, and dust from five backyard chicken farms (Kampung and Arabic chicken farms). Backyard chickens were infected with the MDV1. Blood, feather, and dust were 38.8%, 35%, and 20.8% positive, respectively [17]. A 2012 study by Wajid *et al.* in Iraq on the prevalence of Marek's disease found little difference between commercial and domestic chickens, with an overall prevalence of 49.5% [19]. Marek's disease (MD) was the most commonly diagnosed primary viral disease in a two-year prospective study of small poultry flocks (non-commercial) in Ontario, Canada (87% of primary viral diagnoses) [3].

Several studies have been performed in Iranian industrial poultry to investigate MDV1, including Mohammadi *et al.*, 2005; Farhoodi *et al.*, 2007; Hablolvarid, 2011; Ghalyanchilangeroudi *et al.*, 2022 [23-26]. In only one study published on Iranian backyard chickens, Gholami-Ahangaran sampled 50 suspected MD backyard fowls from 2015 to 2018, out of which 35 samples were positive [27]. The difference in the results of that study with the current study was in the

number of samples. Also, latent infections were not considered, and the investigation was performed only on suspicious chickens.

The results showed that the higher number of poultry farms in the provinces, the more prevalence of Marek's disease virus. This could be an alarm for industrial chicken production throughout Iran because, due to the dispersion of industrial broiler and layer farms throughout Iran, the risk of economic losses would be considerable. The current study results show that the MDV is circulating in Iranian chicken flocks, and the disease is either in the early stages or latent. On the other hand, various factors, such as markets and workers, veterinarians, trucks, etc., can facilitate the spread of the disease to industrial poultry farms.

5. Conclusion

Feathers sampling of backyard chickens in five provinces of Iran to investigate MDV1 through detection of the gB gene by real-time PCR assay showed that the mean prevalence of MDV1 was 30% and also revealed that the higher number of poultry farms in the provinces, the more prevalence of Marek's disease virus. In order to compare pathogenic strains between backyard chickens and industrial chickens in Iran, it is necessary to identify the circulating strains based on the *Meq* gene and to study them phylogenetically.

Conflict of Interests

The authors declare that they have no competing interests.

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