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Research article

The survey on the circulation of Marek's disease virus in local chickens in Tra Vinh province, Vietnam

Huynh Ngoc Trang[1]*, Nguyen Le Hung Phong[2], Nguyen Tran Phuoc Chien[1], Ho Thi Viet Thu[1]

[1]Department of Veterinary Medicine, College of Agriculture, Can Tho University, Can Tho 900000, Vietnam
[2] Master student, Department of Veterinary Medicine, College of Agriculture, Can Tho University, Can Tho 900000, Vietnam

Abstract

The study was conducted to determine the circulation of Marek's disease virus in local chickens in Tra Vinh province. A total of 343 feather follicle samples were collected from 49 unvaccinated chicken flocks for Marek disease in Tra Vinh City, Cang Long, Tra Cu, and Tieu Can districts. The samples were tested by polymerase chain reaction to detect specific *meq* gene of Marek's disease virus serotype 1 (MDV-1). Information on hygiene and vaccination programs was also collected. The results showed that 45 out of 343 samples were positive for MDV-1, accounting for 13.12%. The prevalent MDV-1 in chickens in Cang Long was 17.85%, followed by Tra Vinh City (15.38%). The same prevalence of MDV-1 (9.52%) was recorded in chickens in the Tieu Can and Tra Cu districts. The differences in the prevalence of MDV-1 in chickens among surveyed sites were not statistically significant (P>0.05). There was no significant difference in the prevalence of MDV-1 by chicken breeds. The prevalence of MDV-1 in chickens from 3 to 6 months old was highest at 34.04%, followed by chickens from 1 to less than 3 months old (11.06%) and older 6 months of age (5.71%). The risk of MDV -1 infection in vaccinated chickens against Avian influenza, Newcastle disease and Gumboro disease was lower than that of unvaccinated chickens (OR=0.277, P=0.044). The combination of sanitation and disinfection decreased the risk of MDV-1 infection in chickens (OR=0.502; P=0.031).

Keywords: Marek's disease, *Meq* gene, Local chickens, Tra Vinh province

***Corresponding author:** Huynh Ngoc Trang, Department of Veterinary Medicine, College of Agriculture, Can Tho University, Can Tho 900000, Vietnam. Email: hntrang@ctu.edu.vn

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INTRODUCTION

Marek's disease (MD) is a contagious oncogenic disease of chickens caused by *Gallid herpesvirus* 2 (GaHV-2) or Marek's disease virus serotype 1 (MDV-1). The virus belongs to the genus *Mardivirus*, a member of the subfamily of *Alphaherpesvirinae* under the family of *Herpesviridae* (Osterrieder and Vautherot, 2004). MD has caused economic losses due to lower feed conversion, weight loss, and decreased egg production (Rozins and Greenhalgh, 2019). It has also induced immunosuppression, which has led to animals being more susceptible to secondary infections (Gimeno and Schat, 2018; Rozins and Greenhalgh, 2019). Chickens have been infected by inhalation of contaminated dust from the poultry houses, and the virus will be shed from the feather follicles of infected chickens (Baigent and Davison, 2004). MDV-1 has been considered a major cause of mortality in backyard chickens, and the virus has persisted in the environment (Pohjola et al., 2015; Mete et al., 2016). The backyard and local chickens with small scale were not generally vaccinated, and the applied biosecurity was not constantly concerned. This has allowed the virus to be present in the environment, and it has caused a threat to industrial chicken farms nearby (Cecchinato et al., 2011). The *meq* gene is specific to GaHV-2, which is considered one of the genes related to an increase in virulent GaHV-2 (Trimpert et al., 2017). Thu et al. (2022) detected MD in chicken flocks in Tra Vinh province. However, investigations of circulation MDV-1 in local chickens were not reported. Therefore, the study was carried out to survey the status of MDV-1 infection in unvaccinated, apparently healthy local chickens for MD.

MATERIALS AND METHODS

Survey method

The study was carried out by a cross-sectional survey to investigate the prevalence of MDV-1 in chickens. Information on the chicken breed, age, raising method, present other animals, and drinking water was collected on sampled chicken flocks. In addition, hygiene status such as sanitation, disinfection, and vaccinations was recorded by questionnaire.

Sample collection

The chickens were treated followed the Vietnam's Animal Husbandry Law (32/2018/QH14). The feather follicle samples were collected from unvaccinated healthy chicken flocks for Marek's disease in Tra Vinh City, Cang Long, Cau Ke, and Tieu Can districts in Tra Vinh province (Figure 1). Each flock randomly chose 7 chickens, and 343 samples were collected from 49 flocks. Every chicken obtained 5 feather follicles from its wings (López-Osorio et al., 2019). All the feather follicles from each chicken were pooled.

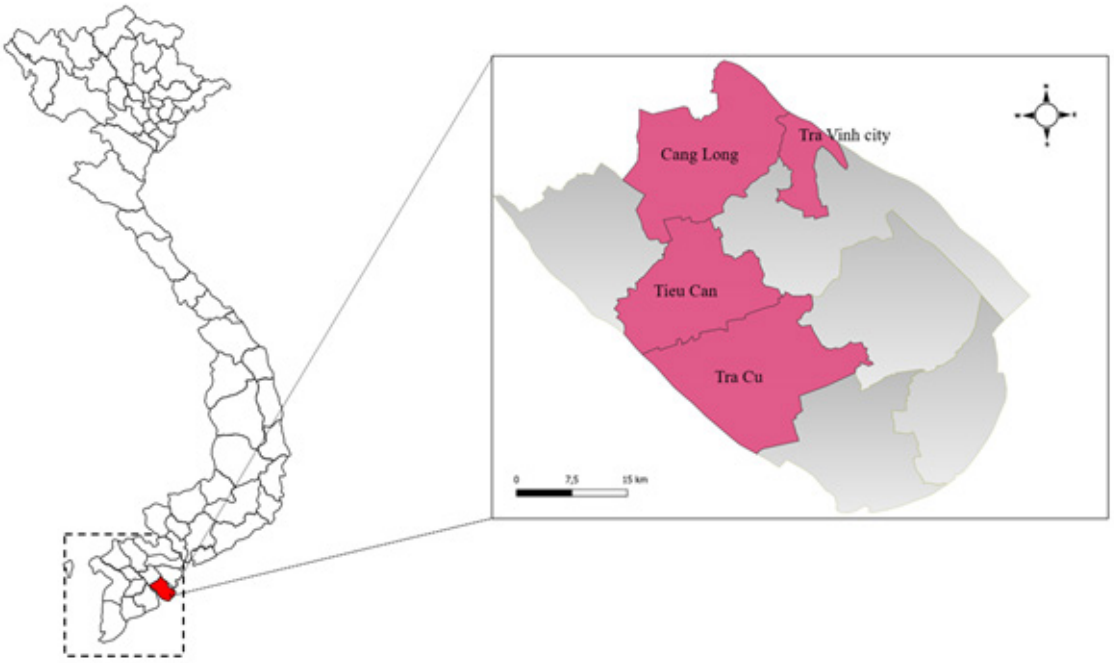

Figure 1 Location of Tra Vinh province, where the sample collection was carried out. Tra Vinh province is indicated in red on the map of Vietnam. Sampling sites are indicated in pink on the map of Tra Vinh

DNA extraction

The total DNA was extracted from the pulp of feather follicles using a commercial kit, TopPURE® Tissue viral extraction (ABT Biomedical, Solution Company, Vietnam). The DNA extraction process was performed according to the instructions of the manufacturer.

PCR amplification of *meq* gene

The samples were examined to detect the specific *meq* gene of serotype 1 by PCR, and the primer was shown in Table 1. The *meq* gene (1,148 bp) of GaHV-2 was amplified, according to López-Osorio et al. (2017). The PCR reaction was carried out in a final volume of 25 µl, containing 12 µl of Master Mix 2X Tracking dye (2X), 3 µl of template DNA, 8 µl of H_2O for molecular biology, 1 µl of forwarding primer, 1 µl of the reverse primer. Conditions of the PCR test contained initial denaturation at 94 °C for 5 min. This was followed by 35 cycles of denaturation at 94 °C for 1.5 min, annealing at 57 °C for 1 min, and extension at 72 °C for 2 min. A final elongation step at 72 °C for 5 min completed the reaction. Products of PCR were separated on a 1.5% agarose gel, which was prepared with TAE 1X (40 mM Tris, acetic acid, 2 mM EDTA) buffer and stained with safe dye (Phusa biochem, Vietnam) (1 mg/ml). The gel was visualized under ultraviolet light after electrophoresis at 150 V and 400 mA for 40 min. Positive samples for MDV-1 by amplification of products were 1,148 bp.

Table 1 Primer set used for the PCR for detection of the serotypes 1 of MDV

Primer	Target gene	Sequences	Product size (bp)
GaHV-2 (MDV-1)	*meq*	F: 5'-CCGCACACTGATTCCTAGGC-3' R: 5'-AGAAACATGGGGCATAGACG-3'	1,148

Data analysis

The comparison of the prevalence of MDV-1 in chicken flocks among location, breed, raising type, and age was statistically analyzed by Chi-square test using Minitab (version 16.0) software and Yates correction in Microsoft Excel 2010. The odds ratio was calculated according to Thrusfield (2007).

RESULTS

Prevalence of MDV-1 in chickens by location, breed, age, and raising type

The prevalence of MDV-1 in the chicken flocks among survey sites ranged from 9.52% to 17.85%. The prevalent MDV-1 in chicken flocks in the Cang Long district was 17.85%, followed by Tra Vinh City with 15.38%. The same prevalence of MDV-1, with 9.52%, was recorded in chickens in Tieu Can and Tra Cu districts (Table 2).

Table 2 Prevalence of MDV-1 infection in chickens by location

Location	No. of samples	No. of positive samples (%)	*P*
Cang Long	84	15 (17.85)	0.265
Tra Vinh City	91	14 (15.38)	
Tieu Can	84	8 (9.52)	
Tra Cu	84	8 (9.52)	
Total	343	45 (13.12)	

Besides, the results in Table 3 showed that the prevalence of MDV-1 in crossbreed Noi and crossbreed Tau was 14.29% and 9.52%, respectively, and no significant difference in the prevalence of MDV-1 by chicken breeds ($P>0.05$). In addition, the highest prevalent MDV-1 was recorded in chicken from 3 to 6 months old at 34.04%, followed by 1 to less than 3 months (11.06%) and older than 6 months (5.71%). The differences in the prevalence of MDV-1 in chickens by age were statistically significant ($P\leq0.05$) (Table 3). The results in Table 3 also presented that the prevalent MDV-1 in semi-captive chickens was highest at 21.25%. This was followed by a lower prevalence of captive chickens (15.94%), and the lowest prevalence was recorded in free-range chickens with 4.76%. There were significant differences in the prevalent MDV-1 among raising types.

Table 3 Prevalence of MDV-1 infection in chickens by variables

Variables	No. of examined samples	No. of positive samples	Infection rate (%)	*P*
Breed				
Crossed Noi	259	37	14.29	0.261
Crossed Tau	84	8	9.52	
Age (month)				
1 - <3	226	25	11.06[a]	0.01
3 - 6	47	16	34.04[b]	
>6	70	4	5.71[a]	
Raising type				
Semi-captive	127	27	21.25[a]	
Captive	69	11	15.94[a]	0.01
Free-range	147	7	4.76[b]	

[a,b]: Infection rates in the same column for each parameter with different superscripts are different at P≤0.05

Relation of risk factors with MDV-1 infection

The relationship between MDV-1 infection and certain factors was investigated. The results are presented in Table 4.

Table 4 Relation of risk factors with MDV-1 infection

Risk factors	No. of positive samples	No. of negative samples	Odds ratio (OR)	*P*
Vaccinated other vaccines[1]				
Vaccinated	3	61	0.277	0.044
Non-vaccinated	42	237		
Hygiene status				
Combination of sanitation and disinfection	20	183	0.502	0.031
Only sanitation	25	115		
Present animals[2]				
Present	29	146	0.530	0.053
Absent	16	152		
Drinking water				
Groundwater	17	135	0.733	0.344
Tap water	28	163		

[1]: vaccines against avian influenza, Newcastle disease, infectious bursal disease (Gumboro disease)[2]: animals like pigs, cattle

Some chickens were vaccinated against common infectious diseases such as infectious bursal disease, Newcastle, and avian influenza. The risk of MDV-1 infection in the vaccinated chickens for these diseases was lower than in the unvaccinated chickens (OR=0.277, *P*=0.044). Besides, chicken flocks that applied a combination of sanitation and disinfection had a lower risk of MDV-1 infection than chicken flocks that only used sanitation (Table 4). The risk of present animals (OR=0.530, *P*=0.053) and the source of drinking water (OR=0.733, *P*=0.344) related to MDV-1 infection showed no significant difference (Table 4).

DISCUSSION

Prevalence of MDV-1 in chickens by location, breed, age, and raising type

All 4 investigation locations recorded infection chickens with MDV-1 by PCR test. The chickens were not vaccinated for MD, so they were infected virus from the environment. Cang Long district had 8 out of 12 chicken flocks infected by MDV-1. Cang Long district had a high density of chicken raising; therefore, MDV-1 was transmitted easily. The infected chickens shed the virus into the environment from the feather follicles that have been considered a major source for transmission to other chickens in nature (Couteaudier and Denesvre, 2014). MDV-1 transmits by the respiratory route, and chickens have been infected by inhalation of dust contamination (Baigent and Davison, 2004). Chickens also have been infected by contact with dander or father follicles of infected chickens (Beasley et al., 1970). Calnek et al. (1970) have reported that cells of feather follicles can keep virus and virus transmission for a long time. Besides, the prevalence of MDV-1 in chickens was not significantly different between crossbreed Tau and crossbreed Noi. However, according to Boodhoo et al. (2016), the susceptible chickens to MDV-1 are related to breeds because of genetic differences. Both crossbreed Tau and crossbreed Noi have been local chicken breeds, and they have been crossed from other local breeds. Therefore, they can have similar susceptibility and genetic characteristics.

On the other hand, the prevalent MDV-1 in chicken from 3 to 6 months old was highest (Table 3). Marek's disease can occur at various age stages from 1 month or older, most commonly between 12-30 weeks (WOAH, 2018). According to Schat and Nair (2008), MD occurred in chickens from 1.5 to 10 months of age, with the majority being 3-6 months old. Other studies reported that MD could occur at 6 or 10-12 weeks old (Okwor and Eze, 2011). In addition, prevalent MDV-1 in chickens had different significance among raising types. MDV-1 can be transmitted by direct contact between infected chickens and healthy chickens or indirectly by the air (Schat and Nair, 2013). Captive and semi-captive chickens were easily infected with MDV-1 by direct contact. Biosecurity performance in small-scale flocks was improper, so it was risky to near industrial chicken flocks (Cecchinato et al., 2011; Mescolini et al., 2019).

Relation of risk factors to MDV-1 infection

The chickens were vaccinated against infectious bursal disease (Gumboro), Newcastle, and avian influenza to reduce the risk of infection of MDV-1. Chickens were affected by immunosuppression pathogens like Gumboro virus that increased the susceptibility of chickens to MDV-1 (Baigent and Davison, 2004). When chickens were early infected Gumboro virus, they had a higher risk of MD outbreak (Hoerr, 2010). Besides, applying a combination of sanitation and disinfection decreased the risk of MDV-1 infection in chickens. Survival of MDV-1 in dust for 44 days (Jurajda and Klimes, 1970) and in litter for 28-42 days (Schat and Nair, 2008). Performance of sanitation and disinfection reduced transmission of MDV-1 by respiratory route (Read et al., 2015). A variety of common chemical disinfectants could inactivate the infectivity of the virus within 10 minutes (Calnek et al., 1973;

Hlozanek et al., 1977). The risk of present mammals, pigs, and cattle in the chicken flocks, as well as a source of drinking water to MDV-1 infection was not statistically significant. Marek's disease has occurred in chickens, and other species like quail and ducks have been susceptible to infection (Schat and Nair, 2013). Besides, Marek's disease virus is transmitted by the respiratory tract, and chickens can be infected by inhalation of contaminated dust from dander. The infected chickens have shed the virus from the feather follicles (Baigent and Davison, 2004).

CONCLUSIONS

The investigation illustrated the circulation of MDV-1 in local unvaccinated chickens for Marek's disease. There were significant differences in the prevalence of MDV-1 in the chickens by age and raising type. The vaccinated chickens against other contagious diseases and a combination of sanitation and disinfection reduced the risk of MDV-1 infection.

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AUTHOR CONTRIBUTIONS

This work was carried out with the contribution of all authors. **Huynh Ngoc Trang, Nguyen Le Hung Phong**, and **Nguyen Tran Phuoc Chien** collected and analyzed the samples. **Huynh Ngoc Trang** and **Ho Thi Viet Thu** interpreted the data and prepared the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

We have no conflict of interest.

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