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Research Article

Occurrence of Marek's disease in household chicken flocks in the Mekong delta

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Abstract

Marek's disease (MD) is a common lymphomatous and neuropathic disease of chicken caused by alphaherpesvirus, it has been caused significant economic losses to the chicken production as a result of the mortality and morbidity. The aim of the study was to determine the occurrence of Marek's disease virus infections in chicken household farms in the Mekong delta of Vietnam, and to analyze clinical cases occurring in the years 2018–2019. This study was carried out from August 2018 to September 2019, during this time, 16 MD suspicious chicken flocks were observed, and 40 chickens were subjected to anatomopathological examination, and PCR testing for confirmation of MD and MDV (Marek's disease virus) serotyping. All of examination flocks (16/16) were confirmed being involved with MD, nearly all cases were in acute form, with typical lesions of visceral lymphomas in internal organs, especially in the liver. Besides the presence of MDV-1(Marek's disease virus serotype1) from 100.0% of tested chicken flocks (16/16), MDV-3 (Marek's disease virus serotype3) were also found from 7/16 (43.75%) of MD flocks. Morbidity and mortality at sampling time varied from 1.0% to 42.11%, and 0.6% to 10.0%, respectively. Chicken flocks with MD vaccination have lower morbidity and mortality. These first finding data confirm endemic MD in the Mekong delta and prove it continues to be a threat to chicken production, in general. Thus, it is essential to develop sustainable vaccine strategies, and strict biosecurity practice in order to prevent losses from this disease.

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- Serotype

INTRODUCTION

Marek's disease (MD) *is a common lymphomatous and neuropathic disease of chicken caused by alphaherpesvirus, named Marek's disease virus* (MDV). This disease was first reported by József Marek (1907). Then, outbreaks were reported as early as 1914 in United State, subsequently the disease was recognized in many poultry producing countries in the world and have caused great losses in chicken production. MD has been causing significant economic losses to the poultry industry as a result of the morbidity and mortality, particularly in chicken. Prior to the usage of vaccines, loss of the infected flocks was estimated range from 25-30% and occasionally as high as 60% (Schat & Nair, 2008). After the first vaccine was introduced, a vaccination is an optional service in prevention and controls this disease (Biggs & Nair, 2012). Then, the problems of MD morbidity and mortality receded, but vaccine now seems to be less effective due to continuing evolution of virulence and emergence of more virulent MDV pathotype (Biggs & Nair, 2012). In Vietnam, MD was first reported in 1970 (Vu-Dinh-Chinh,1970) and rapidly spread in1980s, such as outbreaks in Chau Thanh breeding chicken farming enterprise 1982 (Nam Dinh province), in Cau Dien 1984 (Ha Noi city); and all chickens flocks had to be destroyed (Phan Van Luc *et al.,* 2006). Since 1993, the condition trend to decline by many strategies of prevention and vaccination. However, in recent years, although majority of farm operations using vaccine for MD prevention, MD have still occurred in some places. Reports from national animal health department announced that Marek's disease appeared in birds in Long An and Tien Giang, southern provinces of Vietnam. Especially, in alone Cho Gao district of Tien Giang province there were 120,000 birds in 49 farming households suspected of suffering from MD, around 40,000 died. Failure of MD prevention in chickens due to many factors, especially lack of vaccination, difference types of field viruses with vaccine viruses, latent carriers in infected flocks, and poor biosecurity. In the Mekong delta, most of chickens are raised in household farming type with indigenious breeds, it has a significant economic contribution among the agricultural sectors and indigenous chicken meat is one of the most consumed foods among the urban and rural communities in this region. Although, MD is known to be found in most poultry farms worldwide, the status of this disease in the Mekong delta is poorly understood. Therefore, this study aimed to investigate the occurrence of Marek's disease (MD) in chickens of household farms in the Mekong delta and to determinate the types of MDVs circulating in disease chicken flocks by postmortem examination, histopathology, and polymerase chain reaction (PCR).

MATERIALS AND METHODS

Study time and site

The study was conducted in a year, during the period from 8/2018-7/2019, by examination 16 chicken flocks which were suspicious of being ill with MD by clinical signs and macrolesions from 5 provinces (Long An, Vinh Long, Tra Vinh, Can Tho, Hau Giang) in the Mekong delta. The diagnosis of MD is based on a combination of necropsy and histopathology findings, along with viral DNA confirmation by conventional PCR.

Postmortem examination and sample collection

Postmortem (PM) examination was carried out on 40 chickens from 16 flocks (2-5 chickens per flocks) which had signs suggestive of MD. The chicken's carcasses were thoroughly inspected, and gross pathological conditions were identified and recorded. The samples taken were comprised of the heart, liver, spleen, kidney, proventriculus, and intestine which had pathololigical changes. Of the samples collected, one part was placed on ice and the other part was placed in 10% phosphate buffered formalin fixative and transported to the laboratory of Veterinary Medicine department of College of Agriculture, Can Tho University for histopathological examination and PCR testing. A detailed survey was undertaken using the questionnaires to acquire the epidemiological data from 16 MD flocks such as flock populations, chicken ages, number of birds affected and died, vaccination status (with or without MD vaccination, types of MD vaccine), disinfection (with or without), bird breeds, type of raising operation.

Histopathological examination

Samples of different organs were kept in 10% formalin until fixation, dehydrated in ethanol (70%–100%), cleared in xylene and embedded in paraffin. Five-micron thickness of paraffin sections were prepared and labeled appropriately and thereafter, deparaffinized, routinely stained with hematoxylin and eosin (H and E) dyes. Finally, histopathological sections were examined under a light microscope at 100X, 200X and 400X high-powered fields.

Dectecting of MDVs by polymerase chain reaction

The internal organs suggestive of MD were pooled in to one sample for each chicken. DNA was extracted from homogenates of the tissue samples mixed using DNA TopPURE® Tissue viral extraction (ABT Biomedical, Solutions Company, Vietnam). Totally, 36 processed samples were subjected to PCR, using serotype 1, 2, 3 specific primers. Commercial vaccines (CVI988/ Rispens, SB-1, and HVT) were used as positive controls for the MDV serotypes and synthetic DNA fragments (Invitrogen™ GeneArt™ Strings™), sequences of primer pairs for detecting of three serotypes (1, 2, 3) of MDVs based on research of López-Osorio *et al.* (2017).

*Conventional PCR was used to detect specific genes of MDV. S*ets of specific oligonucleotide primers (GaHV-2 *Meq*, GaHV-3 *gD*, MeHV-1 *sORF 1* gene (**Table 1**) were used to detect each of the MDV serotypes (MDV-1, MDV-2, MDV-3). The final volume of each PCR reaction was 25µl comprising 12µl of My Taq mix buffered (2X), 1µl of forward primer (10pM) 1µl of reverse primer (10pM), 2µl of DNA template and 9 ml of deionized water. The samples were analyzed with 1 % agarose gel electrophoresis using ethidium bromide. Amplifications of GahV-2 and GaHV-3 DNA was carried out by initial denaturation at 94°C for 5 mins, followed by 35 cycles of denaturation at 94°C for 1.5 mins, annealing at 57°C for 1 min and extension at 72°C for 1.5 mins with final extension at 72°C for 5 mins. For MeHV-1, the amplification was performed using 30 cycles of 94 ◦C for 1.5 min, 60 ◦ C for 1 min, and 72 ◦ C for 1.5 mins. PCR products were separated on a 1.5% agarose gel and stained with ethidium bromide (1 mg/ml). The gel electrophoresis was carried out and viewed using a Gel DocTM XR (BioRad, USA). Samples were considered positive for MDV-1, MDV-2, and MDV-3 by amplification of products of 1148, 1040 and 350bps, respectively.

Table 1: Primers used for the PCR for detection of the 3 serotypes of MDV.

Primers	Gene target	Sequence (5'-3')	Product size (base pairs,bp)
GaHV-2 (MDV- 1)	Meq	F: CCG CAC ACT GAT TCC TAG GC R: AGA AAC ATG GGG CAT AGA CG	1148
GaHV-3 (MDV-2)	gD	F: TTCTTCGGACACCTTTCGCCT R: TTCCTGGACGGGCGTTGAGG	1040
MeHV-1 (MDV-3)	sORF 1	F: AAGCGCTTGTATGTGTAGG R: TATGGACGTCATGCAGTTGG	350

RESULTS

Postmortem (PM) examination

For detecting of Marek's disease from chicken flocks, postmortem examination is a crucial work due to chickens with MD currently exhibit few or nonspecific signs such as weight loss, paleness, diarrhea or laboured breathing, and some have normal signs or become depressed or comatose prior to death. So that for suspected diagnosis of MD, typical lesions are very important. In this present study, PM examination of 40 suspicious chickens revealed viceral lymphomas in one or more of variety of chicken internal organs, the most frequently was reported from liver with multi-sized tumourous nodules or enlargement grayish-whitish appearance with many time bigger than normal (Figure 1A, 1B), the second popular organ involved was lung (Figure 1C), then spleen (Figure 1D), kidneys (Figure 1E), proventriculus (Figure 1G), heart (Figure 1F), and instestine, hemorrhage occasionally be seen at the tumors of proventriculus (Figure 1G) and intestines (Figure 1H). There was only chicken which was collected from a flocks at ending outbreak period had grayish mild enlargement of sciatic nerve.

The histopathological examination

The histopathologic changes observed from tissue sections of internal organs (liver, lung, kidney, heart, gizard and instestine) obtained from infected chickens included infiltration and aggregation of lymphocytes at different levels from mild to severe. They gathered in a mass (circle) or disperse (arrow) among parenchymal or tissue of internal organ cells. Undermicrospe, sections of internal organs (Figure 2) at 200X magnification presented extensive infiltration round neoplastic cells; at higher magnication (400X), there was proliferation of pleomorphic

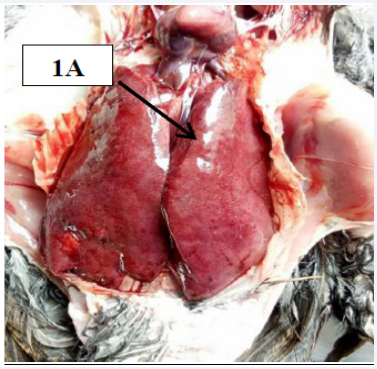

Figure 1a Enlargement whitish appearance with many times bigger than normal occupied whole abdominal cavity.

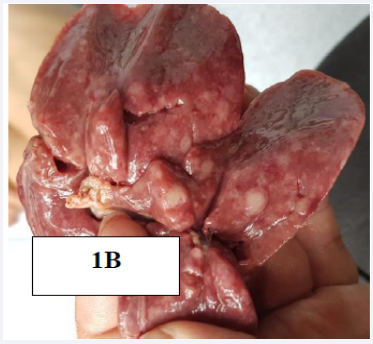

Figure 1b Lung.

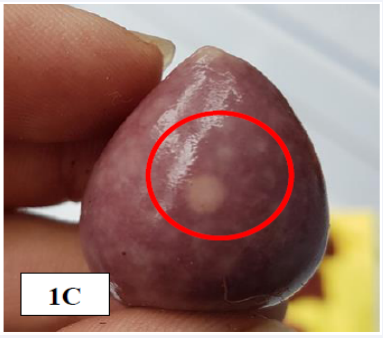

Figure 1c Spleen.

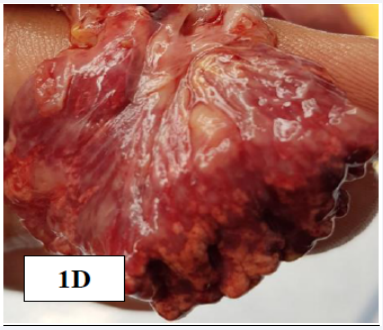

Figure 1d With multi-sized (circle) tumorous nodules; kidney.

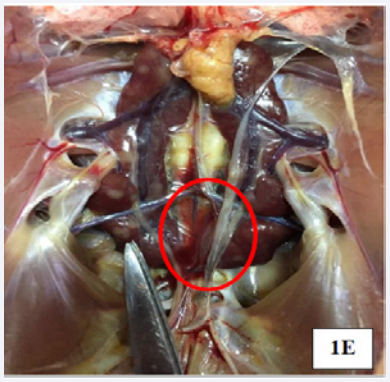

Figure 1e and heart.

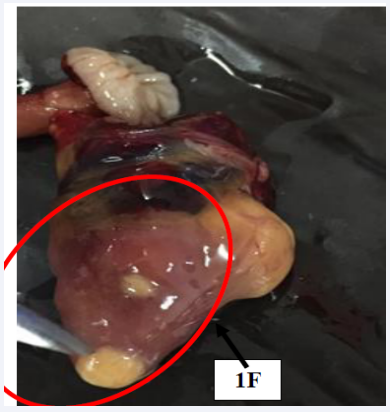

Figure 1f with lymphocyte tumors (circle) which made the architecture deformed; Multiple lymphomas in proventriculus.

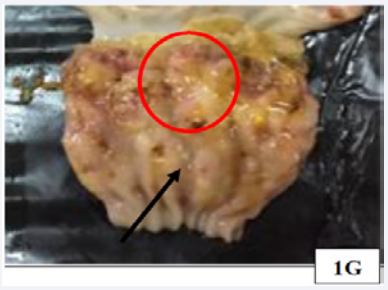

Figure 1g intestine.

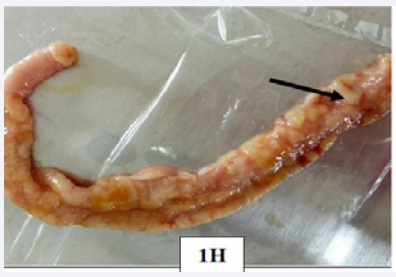

Figure 1h and hemorrhage at the tumors of proventriculus and intestines (arrow).

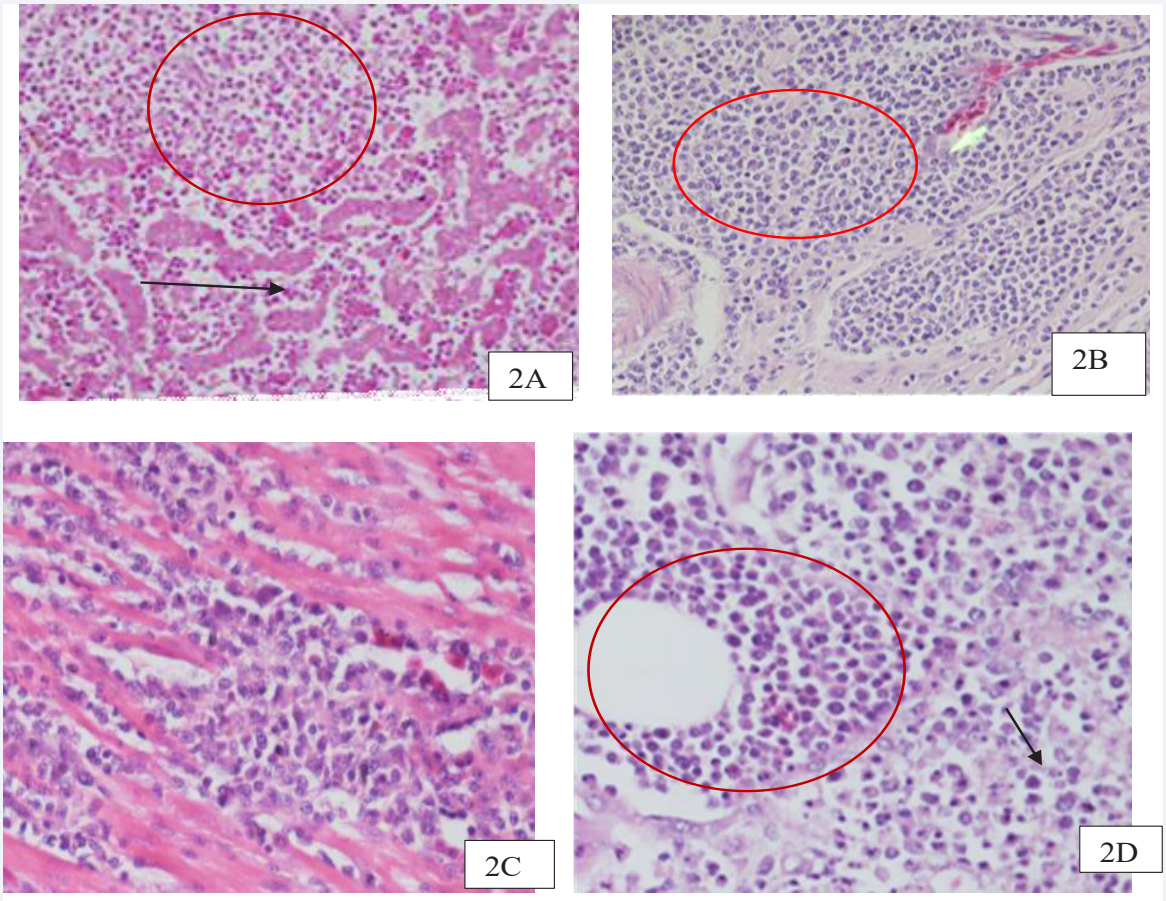

Figure 2 Microscopic lesions of interanl organs; at 200X magnification, there were intensive infiltrations of lympocytic cells which focused (circle) or dispersed (arrow) among hepatic parenchymas (Fig. 2A), and submucosa of the gizard (Fig 2B); at higher magnification (400X), heart section (3C) showing infiltration of pleomorphic lymphocytes in the myocardium, lung section (2D) showing the proliferation of pleomorphic lymphocytes in the alveoli (arrow) and around bronchiole (circle).

tumor cells which replaced and compressed the parenchyma or tissue cells lead to distortion of tissue architecture.

Dectecting of MDVs by polymerase chain reaction

In the current study, 40 pooled internal organ samples of 40 MD suspicious chickens from 16 chicken flocks were subjected to screen MDV-1, MDV-2, and MDV-3 by conventional PCR. The results revealed that the MDV-1 oncogenic strain (Figure: 3A) and the MDV-3 non oncogenic strain (Figure: 3B) were amplified from theses pooled samples.

The results of PCR assay were presented in the Table 2 confirmed that all of 16 suspective flocks (100%) were involved with MD by detecting MDV-1 from samples of internal organs having tumors. There were 32/40 (80.0%) chickens positive with MDV-1. In addition, MDV-3 was also detected from 7 of 16 MDV-1 flocks (43.75%), but MDV-2 was not detected.

Vaccination status and other epidemiological data were collected from 16 MD flocks under this study are shown in **Table 3**.

The data from **Table 3** showed that the age of affected birds ranged from 29 days to 210 days, and morbidity and mortality rates varied from 1 to 42.11% and 0.6 to 10.0%, respectively. However cumulative morbidity and mortality would be higher due to these data were collected only one -time-only at sampling, such studies on surveillance need to be conducted on regular and continuous basis in order to assess more precise measure of the economic losses from actual MD.

DISCUSSION

Postmortem (PM) and histopathological examination

In the past, Marek's disease was firstly described by József Marek (1907) in four adult roosters with leg paralysis in Hungary and he called it polyneuritis, this is the classical form (Marek's form) which is chronically with signs of asymmetric progressive paralysis related to peripheral nerve dysfunction, particular characteristic is bird with one leg straight forward and the other back, or signs of distorted pupil caused by iridocyclitis. Then, Pappenheimer *et al*. (1926) reported an association of lymphoid tumors in the peripheral nerves as well as visceral organs and used the name "*neurolymphomatosis gallinarum*". After that, several articles reported that disease gradually increased in severity (Benton and Cover, 1957; Biggs *et al*., 1965; Purchase and Biggs, 1967). According to these authors, the disease was characterized

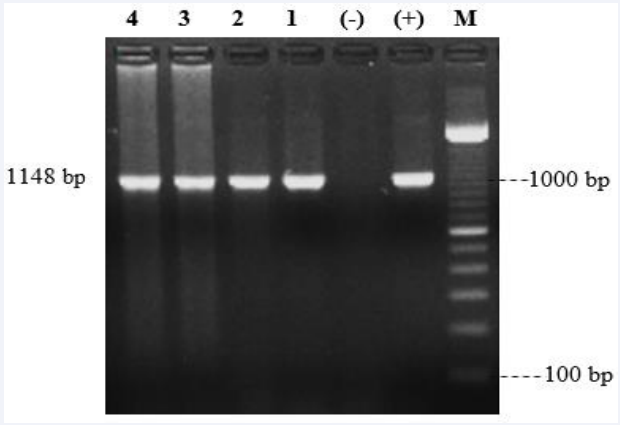

Figure 3a Agar gel electrophoresis of PCR products of MDV-1; M: Marker 100bp, well 1, 2,3,4: positive samples, (-): negative control, (+): positive control.

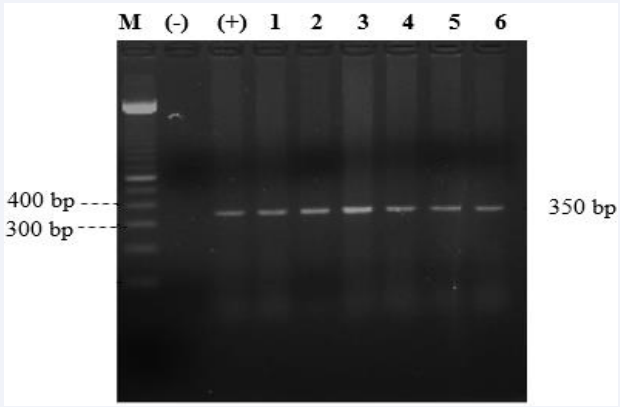

Figure 3b Agar gel electrophoresis of PCR products of MDV-3; M: Marker 100bp, well 1, 2,3,4,5,6: positive samples, (-): negative control, (+): positive control.

by an unusually high rate of incidence and mortality in a flock and a high incidence of lymphoid tumors in the visceral organs. So, the name "acute Marek's disease" was proposed for this "visceral lymphomatosis" form and "neurolymphomatosis" as "classical Marek's disease." Classical and acute Marek's disease, however, are considered fundamentally to be the same disease, except for differences in the degree of severity.

The results of post-mortem (**Figure 1)** from diseased chickens showed that chickens had lymphomatous tumors in visceral organs from every flocks, nearly no lesion of neuritis. In addition, the best method for confirming the clinical cases of MD is histopathological observation **(OIE, 2017)** which was also realized in this study, and the specific microlesions of MD also were found from tumors of visceral organs **(Figure 2)** of diseased chickens by infiltration and aggregation of lymphocytes. These results suggested that all of these examined flocks were affected by "visceral lymphomatosis" or acute MD form.

Detecting of MDVs by polymerase chain reaction

MDV belongs to the genus *Mardivirus* that includes three species (serotypes) designated as *Gallid herpesvirus 2* (*serotype 1*) or MDV-1, *Gallid herpesvirus 3* (serotype 2) or MDV-2 and *Meleagrid herpesvirus 1* or *herpesvirus* of turkeys (HVT) (serotype 3) or MDV-3. MDV-1 includes all the virulent strains and some attenuated vaccine strains. MDV-2 includes the naturally avirulent strains, some of which are used as vaccines. MDV-3 is the antigenically related *Meleagrid Herpesvirus*-1 (herpesvirus of turkey- HVT), it is also used as vaccine against MD. So, the flock was diagnosed involving with MD if it had suspicious chicken positive with MDV-1. Since, MD can be recognized by clinical signs such as paralysis of legs and wings related to peripheral nerve disfunction, iridocyclitis that renders the bird unable to accommodate the iris in response to light and causes a distorted pupil; especially lesions of lymphomatous tumors in multiple organs of the liver, gonads, spleen, kidneys, lungs, proventriculus and heart, and enlarged peripheral nerves are often sufficient to make a positive diagnosis. But in some circumstances, MD can be misjudged with reticuloendotheliosis and lymphoid leukosis or other cancer diseases. The agents of these diseases widespread in commercial poultry, often resulting in simultaneous infections (David and Boreinstein, 1999). While PCR is a quick performed and accurate assay which can demonstrate the presence of virus in the tumor that is sufficient to make positive diagnosis. In addition, PCR tests enable differentiation of oncogenic (serotype 1 MDVs) and nononcogenic strains (MDVs strains of serotypes 2 and 3) (Becker *et al.,* 1992; Bumstead *et al.,* 1997; Handberg *et al.,* 2001; Zhu *et al.,* 1992).

Serotype-1 only is capable of inducing tumors. So that the presence of MDV-1 from chicken bearing tumor can be concluded that it was MD chicken. As the data in the **Table 2**, MDV-1 positive chicken was found in all 16 flocks (100%). All of chickens from the flocks in Can Tho, Hau Giang and Vinh Long are positive, but some ones in Longan and Tra Vinh was negative, this might suggest that there were other cancer diseases such as conditions caused by retroviruses, reticuloendotheliosis virus (REV), avian leukosis virus (ALV) which concurrent affected in these flocks (Davidson and Borensteins, 1999; Chacón *et al.,* 2019), other studies should be conducted to clarify this situation. The presence of MDV-3 (HVT) may be from natural infection, it was also from latent infection from vaccinal virus, report of Rémy and his co-researchers showed that most of the birds showed a persistent vaccinal HVT infection of feathers over 41 weeks with moderate viral loads (Rémy *et al,* 2020); similar findings also were found from several articles (Islam and Walkden-Brown, 2007; Fakhrul Islam *et al.,* 2008; Baigent *et al.,* 2005, Denesvre *et al.,* 2015, Nguyen *et al.,* 2019) which reported persistent contamination of vaccinal MDVs in feathers, spleens and other tissues of chickens.

In the Mekong delta, chicken production mainly based on middle and small scales with indigenous chicken breeds for broilers and exotic breeds for layers. MD happened in chicken might due to lack of vaccination, because it was not commonly realized by small chicken flock holders, but MD outbreaks were not rare in vaccinated flocks. The result data in the **Table 3** showed that 6/16 of MD positive flocks were vaccinated earlier. Vaccinations of birds were mostly done by administering the vaccine into day-old birds immediately after hatching with MDV-3 type (cell free HVT) or MDV3 combined with MDV-1 (CVI988/ Rispens) vaccines and no booster was done. However, the reason why the chickens were not protected against MD although they

Table 2: Results of confirmed Marek's disease diagnosis by PCR and distribution of MD outbreaks by places.

Place	No. MDV-1 infected flocks (%)	MDV-1 infected Birds (%)	No. MDV-2 infected flocks (%)	MDV-2 infected Birds (%)	No. MDV-3 infected flocks (%)	No. MDV-3 infected Birds (%)	No. MDV-1 and MDV-3 infected Birds (%)
Cantho	4/4 (100%)	8/8 (100%)	0/4 (0%)	0/8 (0%)	1/4 (25%)	2/8 (25%)	2/8 (25%)
Haugiang	3/3 (100%)	4/4 (100%)	0/3 (0%)	0/4 (0%)	2/3 (75%)	2/4 (50%)	2/4 (50%)
Longan	2/2 (100%)	7/13 (53.85)	0/2 (0%)	0/13 (0%)	2/2 (100%)	7/13 (53.85%)	7/13 (53.85%)
Travinh	4/4 (100%)	10/11 (90.90)	0/4 (0%)	0/11 (0%)	2/4 (50%)	4/11 (36.36%)	4/11 (36.36%)
Vinhlong	2/2 (100%)	4/4 (100%)	0/2 (0%)	0/4 (0%)	0/2 (0%)	0/4 (0%)	0/4 (0%)
Total	16/16 (100%)	32/40 (80.0%)	0/16 (0%)	0/40 (0%)	7/16 (43.75%)	15/40 (37.50%)	15/40 (37.50%)

Table 3: Epidemiological measures of disease occurrence in MD affected flocks.

No. Flock	Place	Type of chicken	Total strength	No. affected (%)	No. died (%)	deaths/diseased (%)	Vaccination status	Age of chickens	Type of MDV infection
1	Tra Vinh	Noi -Binhdinh	1,000	100 (10%)	20 (20%)	20%	cell free HVT	65	1&3
2	Tra Vinh	Noi Binhdinh	2,000	70 (3.5%)	40 (2%)	57.14%	cell free HVT	72	1
3	Tra Vinh	Noi Binhdinh	2,000	200 (10%)	150 (7.5%)	75%	None	75	1
4	Tra Vinh	Noi	700	200 (28.57%)	50 (7.14%)	25%	None	170	1&3
5	Hau Giang	Noi	300	49 (16.33%)	10 (3.33%)	20.41	None	45	1
6	Hau Giang	Noi	46	4 (8.7%)	2 (4.35%)	50%	None	35	1&3
7	Hau Giang	Noi	500	100 (20%)	50 (10%)	50%	None	105	1&3
8	Vinh Long	Noi	1,000	60 (6%)	10 (1%)	16.67%	None	200	1
9	Vinh Long	Noi	10	1 (10%)	1 (10%)	100%	None	120	1
10	Long An	Noi	1000	100 (10%)	100 (10%)	100%	None	65	1&3
11	Long An	IsaBrown	50,0000	1,000 (2%)	500 (1%)	50%	HVT + CVI988/Rispens	180	1&3
12	Long An	IsaBrown	49,600	500 (1.01)	300 (0.6%)	60%	HVT +CVI988/ Rispens	210	1&3
13	Can Tho	Noi Binhdinh	2,000	20 (1%)	20 (1%)	100%	cell free HVT	90	1&3
14	Can Tho	Noi Binhdinh	3,000	50 (1.67%)	30 (1%)	60%	HVT +CVI988/ Rispens	29	1
15	Can Tho	Noi	38	16 (42.11%)	3 (7.89%)	18.75%	None	40	1
16	Can Thơ	Noi	30	4 (13.33%)	3 (10%)	75%	None	40	1
Total			113,224	2,474 (2.19%)	(1.14%)	52.1%			

were vaccinated was not investigated in this study. Similar findings were reported by many researchers such as Handberg *et al*. (2001) reported that vaccination with either MDV-1 or MDV-3 vaccine did not protect the layer flocks of chicken against MD, Jayalakshmi and Selvaraju (2016) found that MD happened in 12 vaccinated commercial layer flocks in which 3 was booster within one week of primary vaccination, Bell *et al.* (2019) demonstrated the presence of MDV-1 in clinical MD flocks despite they were vaccinated, Kamaldeep *et al.* (2007), Othman and Aklilu (2019) detected MDVs-1 in broiler and layer flocks which was all vaccinated. Failure of vaccination may come from mistakes in the preparation, storage handling and administration of vaccine. Besides, mistakes in poultry management such as the heavy and very early exposure to infection, chicken houses not being well disinfected or birds being reared for many cycles in the same place without cleaning or disinfecting, etc.,...It was also reported that vaccinated chickens may shed virulent virus into the environment because of its immunosuppressive abilities, and MDV-1 has evolved to become more competent in immune system depression or evasion (Davison & Nair, 2005; Gimeno, 2008; Burnside & Morgan, 2011; Torres *et al.,* 2019) and evolution of MDV virulence remains the major challenge for the control of the disease (Gimeno, 2008; Nair, 2018).

The higher morbidity and mortality were found in unvaccinated flocks (**No. 3 - No. 10, No. 15, N. 16**). Shortly after the isolation of MDV in the late 1960s vaccines were developed in many countries, and HVT vaccine was widely used in industrial poultry production (Schat, 2016). The vaccination reduced the incidence of MD by 99% and was the first successful vaccine against naturally occurring virus-induced cancer (Boodhoo *et al.,* 2016), but in the late 1970s, HVT showed limited effect on viral infection and transmission. Hence, vaccinated birds continue to get infected and transmit the virus to the environment encouraging the evolution of MDV towards increased virulence (Gimeno, 2008). As a consequence, the emergence of the viruses classified into mild (mMDV), virulent (vMDV), very virulent (vvMDV) and very virulent plus (vv+MDV) were reported from several poultry production countries (Dunn *et al.,* 2014). Currently, only attenuated MDV strain, CVI988-Rispens is effective in providing protection against the very virulent MDV.

In this study, morbidity and mortality of chickens in flocks vaccinated with bivalent vaccine (flock **No. 11, No. 12, No. 14**) were lowe compared to the others. This suggested there were very virulent (vvMDV) and very virulent plus (vv+MDV) strains in the poultry farm environment. Our data also provided evidence that MD have caused big loss for chicken production in the Mekong delta and vaccination only can't totally protected chicken from MDV. Since, most of chickens were raised in backyard or middle scale, and the producers had limited knowledge in disease prevention and control, especially biosecurity. Hence, there were lot of hazards of raising backyard chickens such as no reliable chicken sources, chicken coops not or seldom being disinfected, no quarantine unit, multi-age birds in the farms, bird being raised continuously without interval break, lacking of veterinary care such as vaccination and deworming. These matters make heavy environmental population and bird immune suppression which favor animals to exposure and infect pathogens. We also witnessed the MD happening from flocks with subclinical infectious bursal disease (IBD) in the past, and all birds in flocks gradually died due to MDV and other pathogens (unpublished data).

In this study, we also found that loss from MD of cross-breed (from two indigenous chicken lines), Noi- Binh Dinh lesser than that of pure Noi chicken flocks. The considerable variability in the susceptibility to MD of different genetic strains of chickens affecting losses was also reported from many researchers (Sharma and Stone, 1972; Bacon, 2001; Emara *et al.,* 2001). Nowadays, the increasing virulence of MDV may pose as a threat to the standard MD prevention strategy, progressively reducing the success of vaccine protection, the genetic resistance to MD should be again considered, especially in small and medium scale chicken production in the Mekong delta. Besides, revaccination should be realized, some studies reported that revaccination (prime-boost), particularly with the cell-associated MDV vaccine (CVI988/Rispens) improve protection against the disease and increases the magnitude of anti-MDV T cell responses as demonstrated by enhancement of anti-MDV neutralizing antibody and proliferation of CD4+ and CD8+ and CD3+Tcells (Wu *et al.,* 2009; Gimeno, 2008).

CONCLUSION

These first finding data confirm MD in the Mekong delta and prove it continues to be a threat to chicken production, in general. Thus, it is essential to develop sustainable vaccine strategies, and strict biosecurity practice. This has been achieved by adopting all-in all-out methods of production, high standards of husbandry and good sanitation, and biosecurity, especially prevention of early MDV exposure.

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