Molecular and pathological investigations of Marek's disease outbreaks in vaccinated poultry farms in Plateau State, North Central-Nigeria

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Summary
Marek's disease (MD) is a devastating neoplastic disease of poultry caused by MD virus (MDV). MD is one of the several diseases limiting the thriving Nigerian poultry industry. MD is mostly diagnosed in Nigeria based on history and gross lesions without laboratory investigations leading to underreporting of the disease. This study investigated MD outbreaks in poultry farms using polymerase chain reaction (PCR) and histopathology. Tumourous visceral organs were collected from dead chickens presented to veterinary clinics from 110 farms in Plateau State, North Central Nigeria from April 2013 to August 2014. Clinical signs observed in affected chickens were paralysis, stunting and uneven growth. Whilst the gross lesions observed were hepatomegaly, splenomegaly with lymphoma, prominent peripheral nerves and cachexia. The meq gene of MDV-1 was detected by PCR in 55.0% (n = 11/20) of broilers and 71.1% (n = 64/90) of vaccinated layer chicken samples collected. Microscopy revealed severe diffuse lymphocytic infiltrations in the heart, spleen and liver of chickens with tumourous gross lesions. Based on history, gross lesions, detection of meq gene of MDV-1 by PCR and histopathology results, MD was confirmed in the affected farms. Despite vaccination, outbreaks of MD still occurs in poultry farms in Nigeria. This study represents the first confirmatory diagnosis of MD in vaccinated poultry in Nigeria

Introduction

Marek's disease (MD) is a highly contagious and lymphoproliferative neoplastic disease of poultry associated with severe economic impact caused by the ubiquitous MD virus (MDV) (Biggs and Nair 2012, Bertzbach *et al.* 2020). MDV belongs to the order *Herpesvirales*, family *Herpesviridae*, subfamily *Alphaherpesvirinae* and genus *Mardivirus* (Davison 2010, Gimeno and Schat 2018). There are three members of the genus *Mardivirus*; Gallid herpesvirus 2 or MDV serotype 1 (MDV-1), Gallid herpesvirus 3 or MDV serotype 2 (MDV-2) and herpesvirus of turkeys (HVT) or MDV serotype 3 (Nair 2005, Gimeno and Schat 2018). All virulent or oncogenic strains of MDV belong to serotype 1 (Nair *et al.* 2020). Furthermore, pathogenic MDV serotype 1 or MDV-1 are classified into four pathotypes i.e. mild MDV (mMDV), virulent MDV (vMDV), very virulent MDV (vvMDV) and very virulent plus MDV (+vv MDV) (Payne 2004, Gimeno and Pandiri 2013). Chickens are known to be the most important natural and susceptible host to MD, although the disease has been reported in quails, turkeys, pheasants, game fowls, ducks, sparrows, partridges, pigeons, and red crown cranes (Murata *et al.* 2012, Schat and Nair 2013, Schock *et al.* 2016, Lian *et al.* 2018, Adedeji *et al.* 2019). MD is distributed worldwide with annual economic losses estimated to be $1-2 billion dollars (Morrow and Fehler 2004,

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Nair 2018). Marek's disease virus is transmitted horizontally by direct or indirect contact between chickens mainly through the airborne route (Davidson & Borenshtain 2002, Schat and Nair 2013). MDV-1 and MDV-2 are contagious, but HVT does not spread easily between infected chickens early in life (Islam *et al.* 2007). Natural infection of MDV is through inhalation of viral infected epithelial cells of the keratinized layer of feather follicles (Baigent and Davison 2004). Feathers/dander with MDV are infectious in contaminated poultry dust/house for several months (Denesvre *et al.* 2013). Clinical presentation of MD may vary in chickens with lymphoma or paralysis syndromes, but few lesions are specific to MD (Gimeno and Pandiri 2013). In affected flocks, birds appear unthrifty with ruffled feathers and stunted growth, other non-specific signs include emaciation, paleness, anorexia, and diarrhea especially in birds with chronic disease (Schat and Nair 2013). MDV can induce immunosuppression resulting in affected chickens succumbing to secondary infections or other pathogens which may mask the virus (Haq *et al.* 2013, Gimeno and Schat 2018). The gross lesions commonly observed in the classical and acute cases of MD are enlargement of the peripheral nerves, formation of lymphoma in various organs and tissues (Payne 1985, Schat and Nair 2013). In the field, diagnosis can be complicated because of similarities of MD or co-infection with other viral neoplastic diseases of poultry such as avian leukosis (AL) and reticuloendotheliosis (RE) (Nair *et al.* 2020). Hence, confirmatory diagnosis is a multi-step process, involving a combination of flock history, clinical signs, gross, histopathologic findings and nucleic acid detection (Zelnik 2004, Gimeno and Pandiri 2013). For molecular assay such as polymerase chain reaction (PCR), detection of MDV meq gene is important, it is the gene responsible for oncogenicity of MDV which is present in less and very virulence strains of the virus (Davidson 2020). However, in resource limited countries like Nigeria, confirmatory diagnosis of MD is usually a challenge because of poor access to laboratory facilities by poultry veterinarians. Control and prevention of MD is based on a combination of biosecurity and vaccination. All MD vaccines are live vaccines which can be classified as 1st generation vaccines i.e HVT a MDV-3, 2nd generation vaccines, a combination of HVT and SB-1 a MDV-2, and finally 3rd generation vaccines an MDV-1 isolate called Rispen-CVI998 which is the gold standard MD vaccine (Atkins *et al.* 2013, Ralapanawe *et al.* 2016, Schat 2016). In recent years combination of MDV-1 Rispen-CVI998 and HVT stored in liquid nitrogen is popular and effective (Diaz 2014, Schat 2016). With 180 million chickens reared on extensive or free-range (46%), semi-intensive (33%) and intensive/commercial (21%) husbandry systems, majority of the commercial poultry farms are layer stock for egg production (FAO 2019). Most of the hatcheries in Nigeria are concentrated in the South-Western part of the country, where 80% of the day old chicks (DOC) are hatched and transported to the rest of country (Akinwumi *et al.* 2010, Jwander *et al.* 2015). The hatcheries routinely vaccinate against MD using either HVT or a combination of MDV Rispens-CVI988 and HVT. Since the first report of MD in Nigeria in 1962, several outbreaks of MD have reported with increasing prevalence of the disease in poultry farms (Hill and Davis 1962, Owoade *et al.* 2008, Okwor and Eze 2011, Jwander *et al.* 2015, Sani *et al.* 2017). Inasmuch MD is not a reportable disease, veterinary clinics in Nigeria regularly records cases of the disease in farms. Hence the impact of MD on poultry production in Nigeria is unknown and coupled with the fact that diagnosis on the field is not supported by laboratory confirmation. There is a paucity of data on the MD outbreaks in Nigeria and description of the epidemiological features are not documented, particularly in relations to the possible role of poultry husbandry systems as drivers of the disease. Hence, MD poses a constant threat to poultry farms in Nigeria. This study presents the molecular and pathological investigations of MD outbreaks in poultry farms in Plateau State, Nigeria.

Materials and methods

Study area

Plateau State is located in North Central Nigeria with 17 Local government areas (LGA) and population of 3,500,000 people. The study area covers five Local Government Areas (LGA) of Plateau State; Jos-North, Jos-South, Jos-East, Bassa and Bakin Ladi (Figure 1). Most of the poultry farms in Plateau State are located in the above mentioned LGAs. Plateau State is situated approximately on latitude 9.60 N, 10. 20 N and longitude 8.50 E, 9.10 E with a more temperate climate than the rest of Nigeria. The State is best suited for poultry production with average monthly temperatures of 21 °C-25 °C that sometimes drops as low as 11 °C. Livestock production particularly poultry farming is a major economic activity in Plateau State with over 3,000 poultry farms. The poultry production system in Plateau State are small-holder backyard and commercial farms with population of 50-50,000 chickens per farm (Maduka *et al.* 2015). A few poultry hatcheries are also located in Plateau State which supplies day old chicks (DOC) mainly to the northern parts of the country (Akinwuni *et al.* 2010). Majority of the poultry farmers in Plateau State keep layers or are egg producers, while it is a common practice also for farmers to keep multi-type and species of poultry like broilers, layers and turkeys within the same premises or poultry pen.

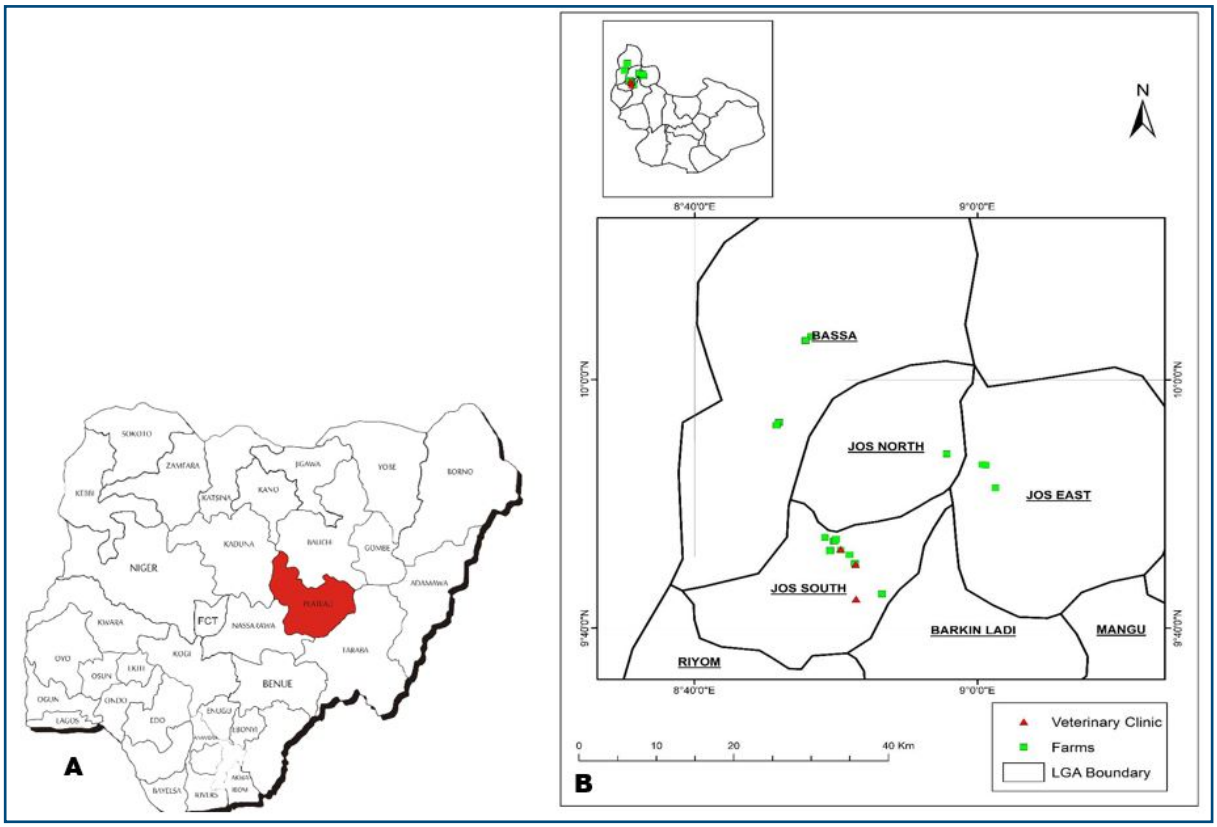

Figure 1. A. *Map of Nigeria showing Plateau State.* **B.** *Map of Plateau State showing the study area, Veterinary clinics and farms were samples were collected.*

Case history, gross pathologic findings, and sample collection

Samples were collected from chicken carcasses submitted to four veterinary clinics located in Jos North (1) and Jos South (3) LGAs (Figure 1) of Plateau State Nigeria from April 2013 to August 2014. The farms that submitted sick dead chickens for necropsy were from Jos-North, Jos-South, Jos-East, Bassa and Bakin Ladi. The case definition was clinical diagnosis of MD based on history, onset of disease before ≤ 14 weeks of age with neoplastic/tumourous lesions in visceral organs and gross lesions on peripheral nerves. The age, clinical signs, vaccination history and gross lesions observed were recorded. Tumours of the liver, spleen, lungs, ovaries, heart, intestine, skin and kidney observed during necropsy were collected. Samples from different dead chickens but from the same flock/farm were pooled together and gross lesions of chickens from the same farm were also recorded together. Samples were collected in two parts, one part in sample bottles for conventional PCR. The second part of the samples were placed in 10% buffered formalin and stored at appropriate conditions at Viral Research Division, National Veterinary Research Institute, Vom, Nigeria until used.

Investigation of Marek's disease outbreaks on poultry farms

Visit to selected poultry farms with clinically diagnosed cases of MD was carried out based on consent and availability of the farmers. The farms visited were located in Jos-North, Jos-South, Jos-East, Bassa LGA (Figure 1B). During farm visit, history was recorded including age, type of bird, source of birds, population, date of onset, vaccination history, mortality rate and egg production history. Necropsy was carried out on dead chickens on the farms and samples collected. The geographic co-ordinates of veterinary clinics/ hospitals and some of the affected farms visited were recorded.

Histopathology

Necropsy was performed, and histological processing was done as previously described (Schat and Nair 2013, Akanbi *et al.* 2020). Briefly, tumourous visceral organs of clinically diagnosed cases of MD namely heart, lung, liver, spleen, were collected at necropsy. The organs were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 µm. They are thereafter mounted on clean glass slides and stained with hematoxylin and eosin

(H&E) stains for microscopic examination using low and high powered field of Carl Zeiss binocular microscope.

Polymerase Chain reaction

Tumourous tissue samples were homogenized and DNA extracted using QIAamp DNA mini kit (Qiagen, Hilden Germany) according to manufacturer's protocol. Conventional PCR protocol targeting the meq gene which is the oncogene of MDV-1 using the following primer sequences forward 5'-TTCCCTCTTCTGCCCTCC-3', Reverse 5' TCCTGTTCGGGATCCTCG-3' with the expected amplified PCR product of 200 bp (Woz´niakowski *et al.* 2013). Briefly, the PCR was carried out in a 25 µl reaction mixture containing 20 pmol of each primer, 12.5 µl (Thermo Scientific Dream Taq®) Green PCR master mix 2X) and DNA template 2 µl. The thermo cycling conditions include initial denaturation at 94 °C for 3 minutes followed by 35 cycles each consisting of 90 s at 94 °C, 1 min at 60 °C and 2 min at 72 °C, and final extension of 72 °C for 7 min. The positive controls used was (virulent MDV serotype 1) provided by Dr. Aly Fadly of the Avian Diseases and Oncology Laboratory, East Lansing, MI, USA).

Results

Clinical signs and gross pathologic findings

One hundred and thirty six chicken (136) carcasses were examined from 110 farms consisting of 90 layer farms and 20 broiler farms during necropsy. The farms were located in Jos North (12), Jos East (4) Jos South (88), Bassa (5), Bakin Ladi (1). Farm records revealed onset of the disease was from 3-14 weeks of age in layer farms and 6-11 weeks in broilers farms (Table I). Also 5.6% (n = 5) of layer farms with clinically diagnosed cases of MD had 2-3 flocks of different ages affected by the disease on the same farm (Table I). Records showed that 16.6% (n = 15) of layer farms revaccinated their chickens using HVT FC-126 strain vaccine once. While 11.1% (n = 10) revaccinated with HVT FC-126 strain vaccine twice between 3-21 days of age, irrespective of the vaccination history from the hatchery. Another 2.2% (n = 2) of layer farms revaccinated with combined MDV-1 Rispens-CVI988 and HVT FC-126 strain vaccine (Table I). There were no history of vaccination against MD in all the broiler farms. Common clinical signs observed were stunted and uneven growth within the flocks, undeveloped combs, paralysis of the legs (Figure 2a, Table I), anorexia and diarrhea. The common gross lesions observed during necropsy were cachexia, (Figure 2c) hepatomegaly with lymphoma (53.6%, n = 59) which were several times the normal size, diffuse, nodular or both, with grey or white discoloration (Table I, Figure 2d). Splenomegaly with lymphoma (56.4%, n = 62) (Figure 2e), heart with multiple nodular tumours (12.7%, n = 14) (Figure 2f), and tumour of the lungs (5.5% n = 6). The proventriculus was thickened and firm on palpation with prominent glands (1.7%, n = 16), prominent sciatic nerves (15.5% n = 17), skin tumours (2.7%, n = 3) and weight loss/severe emaciation with tumour on visceral organ (s) (33.6%, n = 37) (Table I, Figure 2f). Other gross lesions observed were tumours of the intestine (1.8%, n = 2), muscles (1.8%, n = 2) and ovaries (3.6%, n = 4). Also observed was atrophy of the spleen (9.1%, n = 10).

Outcome of field investigation on twenty poultry farms clinically diagnosed with Marek's disease

Farm visit to twenty (20) poultry farms revealed (Figure 1), one of the farms had two flocks and two other farms had three flocks all affected by MD. All

Table I. *Summary of history and gross pathologic findings of chickens clinically diagnosed as cases of Marek's disease in Plateau State, Nigeria.*

History and clinical signs	Layer (N=90)	Broiler (N=20)	
Age at onset of disease	3-14 weeks	6-11 weeks	
History of Marek's disease	7 (6.4%)		
Repeat MD Vaccination at Farm			
HVT once	15 (16.6%)	-	
HVT twice	10 (11.1%)	-	
Rispens +HVT	2 (2.2%)	-	
Gross pathologic findings	**Layer (N=90)**	**Broiler (N=20)**	**Total (N=110)**
Hepatomegaly with lymphoma	50 (55.5%)	9 (36%)	59 (53.6%)
Splenomegaly with lymphoma	54 (60.0%)	8 (32%)	62 (56.4%)
Heart with lymphoma	9 (10.0%)	5 (20%)	14 (12.7%)
Lungs tumours	4 (4.4%)	2 (8%)	6 (5.5%)
Skin tumours	-	3 (15%)	3 (2.7%)
Prominent/enlarged sciatic nerve	15 (16.7%)	2 (8%)	17 (15.5%)
Enlarged Provetriculus	16 (17.7%)	-	16 (14.5%)
Weight loss/emaciation with tumour in visceral organ(s)	37 (41.1%)	-	37 (33.6%
Intestinal tumours	2 (2.2%)	-	2 (1.8%)
Tumours of the muscles	2 (2.2%)	-	2 (1.8%)
Lymphoma of the ovaries	4 (4.4%)	-	4 (3.6%)
Underdeveloped ovaries @18 weeks >	8 (8.8%)	-	8 (7.2%)
Atrophy of the spleen	10 (11.11%)	-	10 (9.1%)
Lymphoma of the Bursa	-	-	-

farms visited had birds on deep litter management system. The populations of chickens on the farms visited were 800-10,000 chickens. Based on farm records 60% (n = 12) had onset of the MD at 5-8 weeks of age of the chickens. The chickens were stunted with uneven growth within flocks of the same age (Figure 2a). The average mortality rate was 20%-60% which was over a period of several weeks due to the prolong nature of MD. All the farms visited were layer farms and egg production performance by flocks affected by MD ranged between 36% and 80%, with 20% (n = 4) of the farms at 80% egg production performance and for the rest it was 36%-75%. In addition, 20% (n = 4) of the farms visited had previous history of MD, while the rest did not indicate any previous history of MD. Flock history also showed that 80% (n = 16) of the farms visited repeated vaccination against MD at least once before 21 days of age using HVT vaccine, however some of the farmers vaccinated twice using HVT vaccine. Ninety percent (90% n = 18) of the farms sourced replacement stock from Southwest Nigeria and the birds are transported over 12-14 hours by road to Plateau State. Other husbandry practices observed include keeping of multi-age flock in the same premises and lack of all-in, all-out management system or fallowing of farms. MD was confirmed on the farms based on history, clinical signs, histopathology and results of conventional PCR. There was clustering of farms visited in Bassa, Jos South and Jos East LGAs (Figure B).

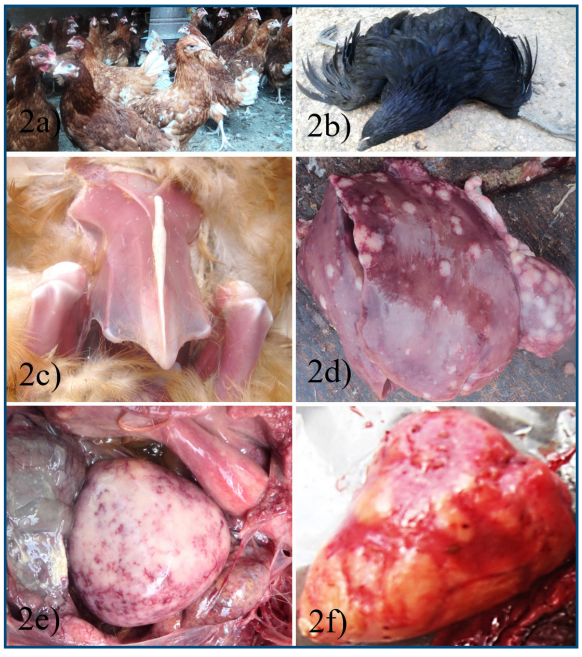

Figure 2. A. *Twenty five (25) week old layers farm diagnosed with Marek's disease, note stunted and uneven growth, only few birds had developed comb.* **B.** *Fifteen (15) week old pullet with classical leg paralysis or 'hurdle jumper position' as result of Marek's disease.* **C.** *Carcass of a layer chicken with prominent kneel bone as a result of severe emaciation.* **D.** *The liver of broiler chicken with hepatomegaly and diffuse lymphoma.* **E.** *Severe splenomegaly in layer chicken.* **F.** *Heart of a 9 week old broiler chicken with multifocal lymphoma nodules.*

Histopathology results

Microscopically, lesions observed in tumours or lymphoma of visceral organs were mainly proliferating lymphocytes, lymphoblasts and macrophages admixed with some inflammatory polymorphonuclear cells. In the heart (Figure 3a), severe diffuse lymphocytic (lymphocytes and lymphoblast) infiltration with myocarditis and vasculitis. Microscopic lesions in the spleen were periarteriolar lymphoid cellular accumulation, vasculitis with lymphoblast and; macrophages infiltration (Figure 3b). While in the liver, there was bridging lymphohistioplasmacytic cellular infiltration with multifocal to diffuse hepatocellular necrosis and destruction of hepatic cords (Figure 3c-d).

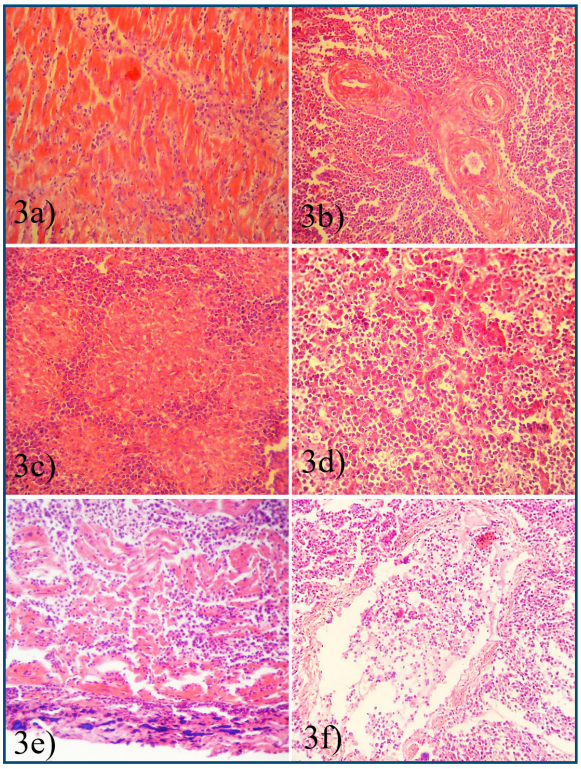

Figure 3. A. *Heart, severe, diffuse, lymphohistioplasmacytic myocarditis. H&E X 100.* **B.** *Spleen, diffuse infiltration of lymphohistioplasmacytic cells, occasionally forming nodules. H&E X 100.* **C.** *Liver, mid-zonal hepatocellular necrosis with bridging lymphohistioplasmacytic cellular infiltration. H&E X 100.* **D.** *Liver, multifocal to diffuse hepatocellular necrosis and destruction of hepatic cords with lymphohistioplasmacytic cellular infiltration in inter-hepatic cord and sinusoidal spaces. H&E X 400.* **E.** *Intestine, severe, diffuse, lymphohistioplasmacytic infiltration, H&E X 100.* **F.** *Lung, Bronchiopneumonia, with lymphohistoplasmacytic accumulation in large pulmonary airway, H&E X 100.*

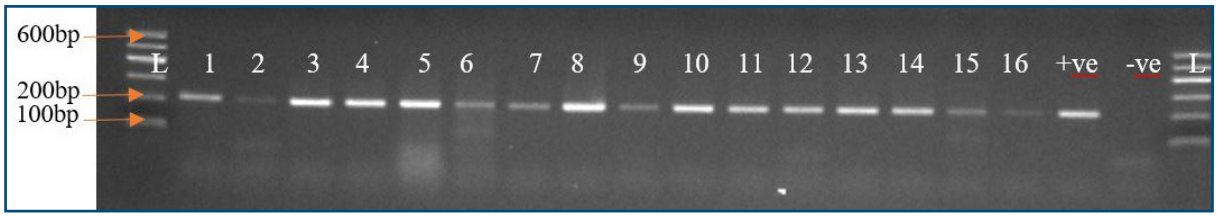

Figure 4. *Agar gel electrophoresis of PCR product of virulent MDV-1 strain.* Lanes 1- 16 are the visceral and feathers samples collected from clinically diagnosed cases of Marek's disease. The positive samples were amplified at 200bp. L = A 100 bp DNA marker (Qiagen®). +ve = Positive control. -ve = Negative control.

Polymerase chain reaction results

The samples analyzed were from broilers (18.2% n = 20), and layers (81.8%, n = 90). Of the tumorous samples analyzed, meq gene of MDV-1 was detected in 68.2% (75/110) comprising 55.0% (n = 11/20) of broiler samples and 71.1% (n = 64/90) of layer samples.

Discussion

It is estimated that over 70 percent of Nigerians directly or indirectly depend on the poultry industry for their livelihood, it is the most commercialized sector of the agricultural industry in Nigeria with a net worth of USD 1.7 billion per year (FAO 2019). However, growth of the poultry sector is seriously hampered by several poultry diseases including MD. Previous reports have suggested that Nigeria is one of the poultry producing countries in the world with increasing prevalence of MD, despite routine vaccination poultry with MD vaccines (Gimeno 2004, Dunn and Gimeno 2013). But, there are no reports that investigated MD in vaccinated poultry flocks in Nigeria. In this study, MD outbreaks were confirmed in vaccinated and unvaccinated poultry farms in Plateau State, North-Central Nigeria based on history, clinical signs, gross lesions, histopathology and detection of meq gene of MDV-1 by PCR. All chicken carcasses sampled in this study had tumour or lymphoma lesions in at least one visceral organs i.e. liver, spleen, heart, kidneys and lungs (Figure 3, Table I). Cases of visceral tumours or lymphoma in chickens < 14 weeks was used as criteria for sample collection. Due to the similarities of MD and other neoplastic diseases of chicken such as AL and RE, age is an important criteria for differentiation and diagnosis of these diseases in the field (Schat and Nair 2013). MDV-1 is considered the cause of tumours of visceral organs in chickens < 14 weeks of age, whereas AL and RE cause tumour/lymphoma in chickens older than 14 weeks of age (Gimeno and Pandiri 2013). The gross lesions in this study were consistent with what has been observed with MD in other reports (Schat and Nair 2013). The most common gross lesions observed in this report were enlarged liver with lymphoma and enlarged spleen with lymphoma in dead chickens at necropsy. Lymphoma of the visceral organs in MD is usually several times the normal size similar to the findings in the study (Nair 2018). Lesions on the peripheral nerves are considered pathognomonic for MD, were observed in 15.5% of dead chickens examined in this study. Visceral tumours or lymphoma can occur without corresponding gross peripheral nerve lesions in MD cases as previously reported and observed in this study (Table I) (Schat and Nair 2013). Thus the absence of lesions on nerves cannot be used as an exclusion criteria for MD clinical diagnosis (Nair *et al.* 2020). Another finding was severe emaciation with visceral organ tumours in 33.6% carcasses examined at necropsy. Weight loss, emaciation and chronic wasting are observed in prolong course of MD due to starvation or dehydration (Schat and Nair 2013, McPherson and Delany 2016). Histopathologic results revealed infiltration of lymphocytes, lymphoblasts and macrophages in visceral organs samples collected. These are characteristic microscopic lesions of MD as previously described and confirmed the MD in the samples analyzed (Adedeji *et al.* 2019, Nair *et al.* 2020). The virulent MDV-1 was detected in 68.2% of the tumourous samples collected from 110 poultry farms. Detection of the virulent MDV-1 in samples alongside gross lesions and histopathologic lesions confirmed MD in poultry farms in Plateau State, Nigeria. MD was confirmed in both broilers and vaccinated layer farms in this study. MD outbreaks were recorded in layer flocks despite vaccination against MD at the hatchery and revaccination by poultry farmers. Whilst, broiler type chickens are not routinely vaccinated against MD in Nigeria even at the hatchery, because broilers are not kept beyond 10 weeks to reduce of production cost. Broilers are commonly kept alongside layers in Nigeria which exposes them to virulent MDV-1 (Jwander *et al.* 2015, Maduka *et al.* 2016). Despite the successes of MD vaccines, it has been suggested that the advent of MD vaccine drives MDV field viruses towards greater virulence, nonetheless little evidence supports this theory since vv MDV strains were first described (Schat *et al.* 2016, Reddy *et al.* 2017). Suggested

reasons for failure of vaccines to protect against MD, particularly in some African countries includes; poorly administered vaccines, the wrong type of vaccine, poor biosecurity practices, early chick exposure to pathogenic MDV and contaminated MD vaccines (Morrow and Fehler 2004, Gimeno 2008, Shittu *et al.* 2019). Poor biosecurity and management practices such as keeping of multiage flocks and not fallowing farms leads to high contamination which were observed in this report. These husbandry practices in poultry farms in Nigeria exposes chicks early to virulent MDV-1 and may negate the merit of vaccination. In addition, these unwholesome practices are also drivers of not only MD but other economically important poultry diseases in Nigeria. Albeit, revaccination of chickens with MD vaccines have been reported to be beneficial and it is a widely practiced protocol (Wu *et al.* 2009). But, it appears not to be beneficial based on findings from this study, because 11.1%- 16.6% of farms vaccinated or revaccinated with HVT still experienced outbreaks of MD. Likewise, sourcing of DOC from long distances which are transported by road, thereafter vaccinated after a few days may contribute to failure of the vaccines to protect against MD. The major economic burden of MD is attributed to both direct losses from chicken morbidities, mortalities and egg production losses, whilst indirect losses result from the use of expensive MD vaccines (Rozins *et al.* 2019). The twenty farms visited experienced 20%-60% mortality rates in their poultry flocks, although this may have exacerbated by secondary pathogens due to the immunosuppressive nature of MD. Even so, these mortalities were directly or indirectly attributed to MD and they occur over a period of several weeks due to the prolong course of MD in some flocks or farms. In addition, there was also clustering of farms confirmed with MD in this study (Figure 1). In order to control MD, biosecurity practices must be enhanced particularly a massive change in husbandry practices. In as much as vaccination against MD is important, it can only be effective when augmented with good biosecurity measures. Further studies should conducted on the economic impact of MD in Nigeria and MDV strains circulating in poultry flocks should be characterized.

Conclusions

This study reports a concise molecular, pathologic and epidemiologic features of MD in vaccinated and unvaccinated in poultry farms in Nigeria. MD was confirmed in vaccinated layer and unvaccinated broilers farms where samples were collected. In order to mitigate economic losses associated with MD, prompt diagnosis, implementation of biosecurity measures and proper vaccination are recommended.

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