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DETECTION AND SEROTYPING OF MAREKS DISEASE VIRUS IN DISEASED CHICKENS IN ABEOKTA

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ABSTRACT

Outbreaks of Marek's disease (MD) in vaccinated flocks still occur sporadically and lead to economic losses. This study reports the detection of serotypes 1 and 3 Marek's disease virus using molecular techniques in pullets showing clinical signs of the disease. An enzyme-linked immunosorbent assay (ELISA) was applied to evaluate the antibody response of chicks and the diseased pullets from the same parent stock against Marek's disease (MD) at 4 and 21weeks of age respectively. Despite vaccination at day old, antibody titre in the chicks was negative while it was highly variable in the diseased pullets. From the results, the susceptibility of previously vaccinated birds to the pathogenic strain of MDV indicates inappropriate vaccination at day old and a call for concern in the poultry industry. It is therefore recommended that serology be carried out on birds to determine their immune status before and after MD vaccination.

INTRODUCTION

Marek's disease (MD) is a highly contagious lympho-proliferative disease of chickens. The causative virus is cell associated and belongs to the subfamily Alphaherpesvirinae, family Herpesviridae (Witter and Schat, 2003, Osterrieder and Vautherot, 2004). MD viruses have been subdivided into three serotypes: oncogenic strains and their attenuated variants are the members of serotype 1; nonpathogenic strains from chickens are serotype 2; and nonpathogenic herpesvirus of turkey origin belongs to serotype 3. Despite the production of commercial vaccines from all three serotypes of the virus (Witter and Schat, 2003), there has been report of the outbreak of MD, which has been said to be able to cause a loss of approximately $1 billion (Spencer *et al.*, 1976; Okada *et al.*, 1977; Vallejo *et al.*, 1998; Yonash *et al.*, 1999) in any outbreak per year, even in vaccinated flocks. Recent evidence has shown that the inability of existing live vaccines to prevent replication is the major factor for driving the virulence of the virus (Peter, 2012).

MATERIALS AND METHODS

Case history

A flock of 1015 twenty one (21) weeks old *Bovans nera* layer birds were reported to be anorexic and dull with mortality ranging between 2 and 4 daily. The daily egg production of the flock had also stopped increasing and remained stagnant between 25 and 30%. Vaccines which had been administered to the birds included Newcastle disease (HB-1) and Marek's disease (HVT) vaccines at day old, Infectious bursal disease vaccine at 15 and 21 days of age, fowl pox vaccine at six

weeks old and a combination of Newcastle disease (Komarov strain) and egg drop syndrome vaccine at eighteen weeks of age.

Clinical and necropsy examination

Clinical observation in sick birds during the study was noted. A macroscopic post mortem examination was conducted on the dead birds during the study period. The abnormality or lesion observed on a number of body organs and/or tissues was noted.

Sample collection

Tissue sample collection

Tissue samples for detection of MDV were collected from the liver during post-mortem examination of dead birds. These were stored at -20^{0}C until they were screened using the polymerase chain reaction.

Blood sample collection

A total of 24 blood samples were collected randomly from birds from the affected flock while 20 blood samples were collected from 4weeks old birds from the same parent stock on the same breeder farm. Blood was collected through the jugular vein into sterile bijou bottles and was allowed to clot on the bench for 1 hour at room temperature after which haemoglobin free sera were formed. These sera were transferred into 1.5mL eppendorf tubes and stored at –20^{0}C until used.

Detection of MDV antibody

The MDV antibody in the serum samples were detected using the ELISA method as described by Adeniran and Oyejide (1995).
Briefly, fifty microlitres (containing 50 doses) of reconstituted MDV antigen in coating buffer was dispensed into each well of the microtitre plate. The plates were then incubated at room temperature for 1h with occasional agitation and thereafter incubated for 24h at 4°C. Plates were then flipped empty and wells washed three times (5min soaking) with the washing buffer. After which the plates were stamped on a dry paper towel to make sure all the wells were properly dried.

Dilutions of the positive, negative and test sera were made in sterile U-bottom microtitre plates using the dilution buffer and 50µL of each sera dilution was added into the wells in duplicate. After 1hour of incubation, the plates were flipped empty washed thrice (5min soaking) and stamped dry on paper towel. The Horse Radish Peroxidase (HRP) conjugate was added at 50µL per well, allowed to incubate for 20min at room temperature and the wash cycle was repeated 3times (5min soaking) and dried. Chromogen/substrate (50 µL per well) was then added at room temperature for color development. Finally, the reaction was stopped with 50 µL of 0.1M H_2SO_4 after 10minutes. The optical density (OD) was measured at 492nm wavelength using a Sunrise® (Touch screen model) ELISA plate reader.

The following interpretations were made for optical density results:

Upper limit of negativity (ULN) was taken as the mean OD value of negative control sera (0.833nm). Any serum with OD value greater than ULN (0.833nm) is regarded as containing antibody. The higher the OD value, the higher the quantity (level) of antibody. Optical density readings ranged from negative (0.400-0.800 nm), strong (0.835-1.000 nm) to very strong (1.000-1.116 nm).

Detection of MDV DNA

Procedure for DNA Isolation and Purification

Isolation of MDV DNA from samples and

its purification was done using Qiagen RNA minikit (QIAGEN GmbH, Germany), following the manufacturers procedure.

The polymerase chain reaction for MDV was carried out by adding 2.5μl of DNA to 22.5μl of PCR mix containing 17.2μl of distilled water, 2.5μl of 10X PCR buffer, 2.0μl of $MgCl_2$ (50mM), 0.5μl of dNTP (10mM), 0.1μl of forward and reverse primer for all MDV serotypes or primers specific for the 3 serotypes of MDV and 0.1μl of taq polymerase (5μ/μl).

Polymerase Chain Reaction (PCR)

The PCR reactions was carried out using the following cycling conditions; initial denaturation at 94^0C for 5min, 35 cycles of amplification at 94^0C for 30 sec, 55^0C for 30 sec and 72^0C for 1min and final extension at 72^0C for 10min. The PCR product sizes were visualized by UV illumination in 2% agarose gel stained with ethidium bromide as compared to the 1kb+ size (invitrogen). The positive specimens were detected with band at 491bp for all MD serotypes, 446bp for serotype 1, 327bp for serotype 2 and 316bp for serotype 3.

RESULT

Post mortem examination

Post-mortem examination carried out showed the liver was grossly enlarged with presence of focal nodules. Miliary nodules were also present throughout the entire length of the intestine, surface of the lungs and on the heart and kidney surfaces. Sciatic nerve was not seen to be enlarged.

Marek's disease Virus detection

Tissue samples collected for Marek's disease virus detection in the laboratory were positive for the virus. This samples were positive for MDV (491bp) generally, MDV1 (446bp) and MDV3 (316bp) as shown in Figure 1.

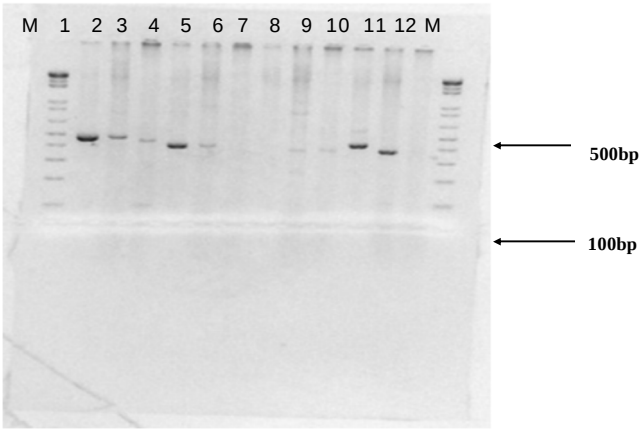


Figure 1: Agarose electrophoresis for the detection of genome segment of serotypes 1, 2 and 3 of Marek's disease virus. PCR products were separated on ethidium bromide stained 2% agarose gel. Wells 1, 2, 3 contains MDV PCR product (491bp), wells 4, 5, 6, contains MDV 1 PCR product (446bp), wells 7, 8, 9 contains MDV 2 (327bp) PCR product and wells 10, 11, 12 contains MDV 3 PCR product (316bp).

Marek's disease antibody detection

The chickens for this study had been previously vaccinated with HVT vaccine at day old in the hatchery. Result from the twenty four chickens tested from the affected flock showed the level of antibody in sera from the birds ranged from being negative to very strong (figure 2). The result from 4weeks old birds on the same farm from the same parent stock with the adult birds showed the level of antibody to be zero (figure 3). This is despite the HVT vaccine that had been administered at day old in the hatchery.

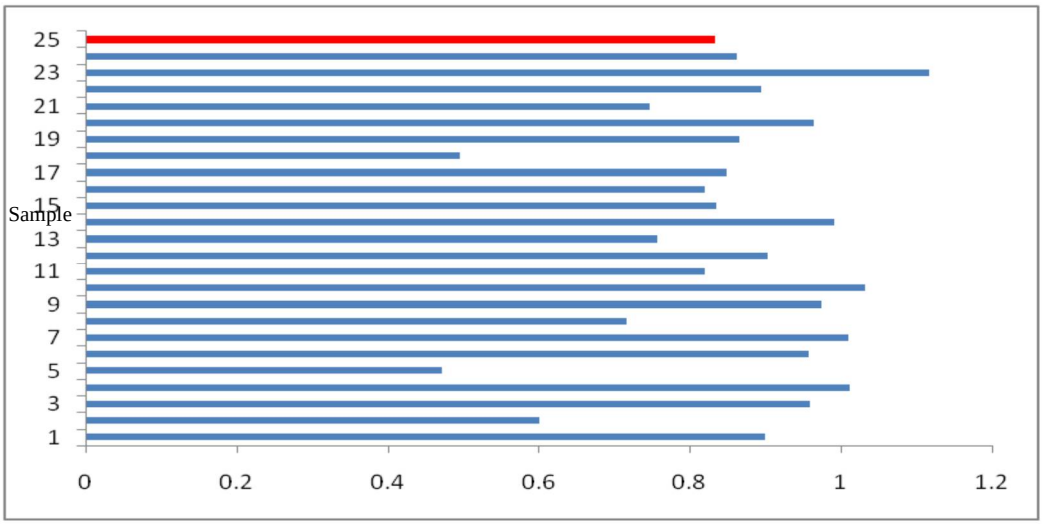
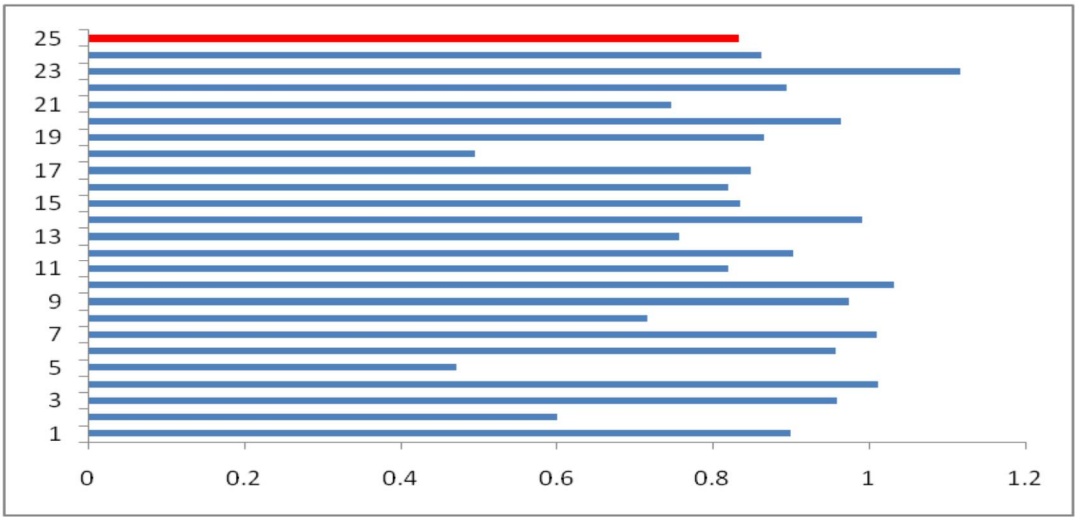


Figure 2: Chart showing MD antibody titre of individual 24 weeks old bird and negative control Samples 1 to 24 are field samples and 25 is negative control OD= Optical density



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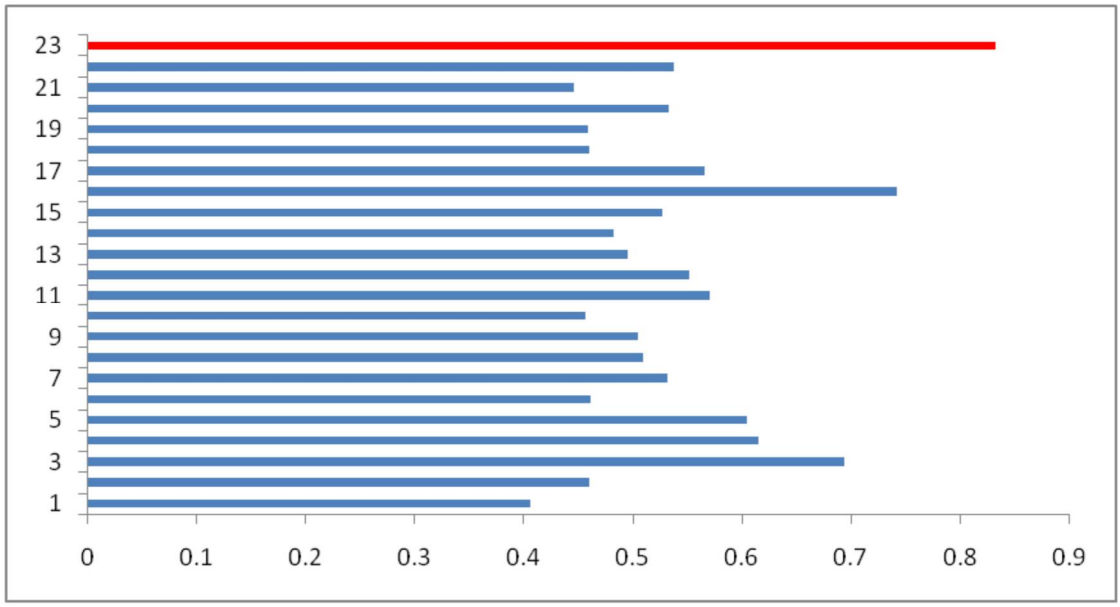

Figure 3: Chart showing MD antibody titre of individual 4 weeks old bird and negative control Samples 1 to 22 are field samples and sample 23 is negative control OD= Optical density

DISCUSSION

Molecular techniques have been used for the detection of Marek's disease in some poultry flocks in Nigeria (Owoade and Oni, 2008), were a 491bp amplicon common for all the serotypes of MDV and specific to the highly conserved region of the gB MDV gene was amplified. In this study however, aside amplifying the gB MDV gene, three pairs of internal primers were used to synthesize serotype-specific PCR amplicon, (gB MDV-1 F and gB MDV-1 R, gB MDV-2 F and gB MDV-2 R, gB MDV-3 F and gB MDV-3 R), which are specific to the hypervariable region of gB MDV gene (Owoade and Oni, 2008).

Results from this study indicate the presence of serotypes 1 and 3 of Marek's disease virus in the sampled flocks. These serotypes are the pathogenic Marek's disease virus and the Herpes virus of turkey respectively. The presence of the pathogenic strain is witnessed with the rate of morbidity and mortality on the farm. The birds were negative for the serotype 2 (apathogenic strain) of Marek's disease virus.

Some studies have showed that there could be great variability of incidence of MD in houses on the same site and even between pens within a house and that this was related to factors occurring during the first 8 weeks of life (Davison, 2004). It was discovered that viruses of variable pathogenicity were present, ranging from apathogenic, through mildly pathogenic to the very pathogenic in each group of chickens (Davison, 2004). This may be responsible for the birds in the other pen house not infected despite being on the same location.

It was also found that the use of HVT vaccine alone for vaccination at day old did not provide good protection against these strains but a better immunity was conferred when

HVT was combined with CVI988/Rispens vaccine (Schat and Nair, 2008). The birds on this farm were vaccinated with HVT at day 1 which would be the reason why the birds became infected.

Studies have also showed that vaccination with HVT at 1 day of age become more effective with increasing time between vaccination and challenge (Peter and Venugopal, 2012). Since the birds were vaccinated at day 1 and were 24weeks old at the time of infection, it indicated that some factors like poor nutrition and secondary bacterial or viral infection might have suppressed the immunity of the birds or that the vaccine was not potent when it was administered.

Despite the widespread use of vaccines, MD outbreaks still occur in different countries (Baigent et al. 2006). Factors such as improper storage or administration of Marek's disease vaccine, presence of maternal antibodies in the chicks, suppression of immune system by other pathogens or stress, and emergence of vv or vv+ MDV in the field have been said to be responsible for many outbreaks of MD (Baigent et al. 2006). In this case, the factor responsible for this outbreak would not be ascertain but it's most likely due to administration of a poor vaccine to the birds at day 1 as the other 4week old birds were antibody negative. It is therefore recommended that serology be carried out on birds to determine their immune status before and after MD vaccination.

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