

*Full Length Research Paper*

# Isolation time of brooding chicks play an important role in the control of Marek's disease

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This work reported the use of a combination of measures for the control of an outbreak of Marek's disease (MD). Post- introduction vaccinations of young chickens in a farm that had an outbreak over a period of six years did not yield good result. The disease was therefore controlled by brooding new birds in isolated pens far away from the other one, use of biosecurity measures and brooding birds during the rainy season when the environment was damp with minimal dust in the air. Result showed marked reduction of infection during the first one year of trial. By the second and third years of trial, no infection was detected in the birds. This study therefore recommends isolation brooding, biosecurity measures, brooding during the rainy period and completely avoiding brooding during the harmattan period as good methods of controlling cases of MD outbreaks in Nigeria.

**Key words:** Marek's disease, isolation brooding, vaccination, biosecurity measures, young chickens, Nigeria.

## INTRODUCTION

Marek's disease (MD) is attributed to a renowned veterinarian Dr. Joseph Marek, who in 1907 described the disease as polyneuritis during a research endeavour in four adult paralysed cockerels (*Gallus domesticus*) at Royal Hungarian Veterinary School Budapest (Goyal et al., 2008). MD is one of the commonest causes of economic loss in the development of poultry industries of many countries (Baigent et al., 2006). The estimated annual global loss to the disease is about \$2 billion (Smith et al., 2011). The disease is a lymphoproliferative disease of the domestic chicken in which mononuclear infiltration of the visceral organs as well as infiltration and demyelination of the peripheral nerves are common features (Payne, 1999; Osterrieder et al., 2006). It is caused by a cell associated alpha herpesvirus classified in the family Herpesviridae and Marek's disease virus (MDV) is the prototype of the group designated as serotype I (Calnek, 2001; Singh et al., 2012). MD manifests in chickens of about 4weeks of age, but clinical disease is most common in chickens between 12 and 24

weeks, though older chickens may be affected (Payne, 1985).

MD is a ubiquitous infection of poultry throughout the world and outbreaks in farm resulting in significant losses are very common (Payne, 1985). Prior to the use of vaccine, it constituted a serious economic threat to the poultry industry in the world and it still causes great economic losses in areas where vaccinations are not routinely practiced (Schat, 1998; Lobago and Woldemeskel, 2004). The variability of syndromes and types of MD, its wider host range and the propensity of the virus to evolve in time have created a great impact on the diagnosis and control of the disease (Witter, 1997). MD has been described in Nigeria and it constitutes a problem to poultry production (Okwor and Eze, 2011). Nigeria is a tropical country with two successive seasons annually. These are the rainy season that occurs between March and October and the dry season that occurs between November and February. The rainy season is associated with rains, damp and humid atmosphere and minimal dust

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**Table 1.** Experimental protocol.

Year I	Isolation brooding of a total of 1800 during rainy periods with strict biosecurity measures
End of Year I	Random serum sample collection (50) for AGID test
Year II	Isolation brooding of a total of 1500 during rainy periods with strict biosecurity measures
End of Year I	Random serum sample collection (50) for AGID test
Year I	Isolation Brooding of a total of 1800 during rainy periods with strict biosecurity measures
End of Year I	Random serum sample collection (50) for AGID test

while the dry season is associated with dry environment, dust and wind especially during the hamattan period. Poultry wastes are drier and easily suspended in the air and could constitute an important mode of spread of airborne infections. Control strategy for MD requires an understanding of the epidemiology of the disease especially as it relates to virus shedding and spread (Atkins et al., 2011). MDV is an airborne pathogen with infection occurring via inhalation and virus shedding occurs by infected feather follicles epithelium (Islam et al., 2008; Atkins et al., 2011). Transmission of MD is mostly by inhalation of infectious dander and poultry dust. The follicular cells of the feathers are the most important source of infection and are responsible for the infectivity of dander, poultry house dust and litter; the virus particles in these materials are able to survive for up to one year or more at room temperature (Payne, 1999). The resulting dust and dander from dead stratified cells and moulted feathers can remain in the environment and act as reservoirs of infection for chickens (Atkins et al., 2011). The infection tends to persist in commercial farms for long periods and spreads from one batch of birds to another through horizontal transmission, (Anderson et al., 1998). Control of MD can be possible through reduction and restriction of dust circulation within the farm, since transmission of MD is mostly by infectious dander circulating in poultry dust and the environment. This work therefore reported the use of isolation brooding and brooding during periods of low atmospheric dust concentrations to control measures to outbreaks of MD.

## **MATERIALS AND METHODS**

### **Case history/Outbreak**

Persistent outbreaks of a disease were reported in a farm located in Nsukka, South East Nigeria. The birds were reared on deep litter system. The first outbreak was among a batch of 300 brown shaver pullets from Zartech Hartchery Ibadan at the age of 12 weeks. The disease was diagnosed to be MD by clinical signs and post mortem lesions and later confirmed by agar-gel precipitation test as described by Sharma (1998). The disease has lingered in the farm for six years as attempts to control it by vaccination of subsequent batches of chicks introduced into the farm only did not yield the desired result as the birds still shed the virus and infected

subsequent batches. The vaccine used was the commercially available cell-free freeze-dried Herpesvirus of turkeys (HVT) vaccine. A single dose of the vaccine was administered to each bird subcutaneously in the thigh muscle. The disease presented both acute and classical conditions and occurred in other batches of pullets (batches varied between 300 and 1800 birds) reared in the farm within the period. The age incidences in all cases were between 10 and 14 weeks. The severity of the disease varied among the batches with some of the breeds showing better resistance than others.

Outbreaks were noticed to be more severe and more generalized in birds reared during the dry harmattan period than in those reared during the rainy season. A combination of isolation of the brooder birds reared away from the older virus shedder birds and timing of introduction of the brooder birds during the rainy season were tried as measures to control and possibly eradicate the disease.

### **Experimental design**

The study covered the period of May 2009 to April 2011. The isolation brooding and laboratory studies were carried out as shown in Table 1.

### **Isolation brooding**

The first control strategy against the disease was done using isolation brooding. Here, the brooding house which was fairly located away from other pens that housed older productive birds was demarcated with wood and palm front fencing. The windows of the brooding house facing the direction of these other pens were closed permanently. Entry into the brooding premises was restricted to the attendants who must disinfect themselves, thier clothings and foot wears before entry. Strict biosecurity measures were applied both during brooding and upon introduction of the birds into the pens. A combination of ISOL<sup>®</sup> and IZAL<sup>®</sup> were used to disinfect the brooding premises on a regular basis. Other biosecurity measures were washing of hands and feet on arrival, changing into the farm apron and foot wears, matching of leg in foot dip before entering the farm.

The birds were left in the brooding house from day-old to 24 weeks (6 months) before they were transferred to their laying pen. The fencing was replaced with new ones each time a new batch of birds was introduced.

### **Timing of brooding**

In addition to the isolation brooding and biosecurity measures mentioned earlier, the pullets were introduced between March and

**Table 2.** Clinical parameters as observed during an effort to control an outbreak of MD in a farm.

Year	No. of birds introduced	No. of birds showing clinical signs of MD (25-90 weeks)	Percentage morbidity resulting from MD
1	1800	83	4.6
2	1500	0	0
3	1800	0	0

**Table 3.** Laboratory findings as observed during an effort to control an outbreak of MD in a farm.

Year	No. of birds introduced	No. of samples examined	No. positive	No. negative	Percentage seropositive
1	1800	50	8	42	16
2	1500	50	1	49	2
3	1800	50	0	50	0

September. This coincided with the period of rain when the environment was relatively damp and dusts were minimal in the air. No bird was introduced between October and February.

#### **Clinical examination and sample collection**

The birds were observed on a daily basis for clinical signs of MD. Serum samples were collected and used in agar-gel immunodiffusion test. Blood was collected randomly from the birds.

#### **Agar gel immunodiffusion test (AGID)**

The conventional MD diagnosis was done following that described by Sharma (1998). Briefly, feather tips and follicle epithelium from a flock already confirmed to have MD using specific immune serum was used to prepare the antigen. Agar gels were prepared in petri dishes to a thickness of 2 to 3 mm and holes were cut in the agar using templates with a centre well and 6 wells spaced at equal distances around the centre well. The antigen was placed in the centre well and the test serum samples were placed in other wells and incubated for 24 h at 37°C. Formation of a precipitin line showed positive reaction. When two positive samples were placed in adjacent wells, a continuous line of identity was formed.

## **RESULTS**

During the first year, out of a total of 1800 pullets that were procured at day old and reared up to 24 weeks of age, none showed clinical disease during the brooding and rearing period. When they were introduced into laying pen after the isolation period, 83 birds were observed to have clinical signs of MD between 25 and 90 weeks of age giving 4.6% (Table 2). Figure 1 shows classical clinical sign (paralysis of limb and wing) in layers seen during one of the experiment. At the end of the first year of study, out of the 50 samples collected, 8 were positive while 42 were negative giving 16% seropositive (Table 3).

During the second year of study, the 1500 pullets introduced did not show clinical signs of Marek's during

the brooding and rearing periods. When introduced at 24 weeks into the laying pen, no birds was observed with clinical signs of MD, thus giving 0% morbidity from MD (Table 2). At the end of this year, out of the 50 serum samples collected, one sample was positive while 49 samples were negative thus giving 2% seropositive (Table 3).

During the third year of studies, out of a total of 1800 pullets that were procured at day old and reared up to 24 weeks of age, none showed clinical disease during the brooding and rearing period. When introduced at 24 weeks into the laying pen, no birds was observed with clinical signs of MD, thus giving 0% morbidity from MD (Table 2). Serum samples collected from this last batch of layers showed no detectable antibodies against MDV giving 0% seropositive (Table 3).

## **DISCUSSION**

Among the factors that have been associated with host resistance to MD are genetic constitutions, prior infection or vaccination with avirulent herpesvirus strains, and old age (Anderson et al., 1971; Crittenden et al., 1972). The result of the aforementioned trial showed an effective control of infection by a combination of the practices mentioned earlier. These are in agreement with the report of Payne (1999) who observed that isolation of growing chicks from sources of infections, use of genetically resistance strains and vaccination are good measures for the control of MD. Vertical or transovarian transmission is unimportant in MD (Vietitz, 1987). The first strategy or method is keeping birds away as much as possible from sources of infection since horizontal transmission through infectious dander and dusts are the most important means of spread and transmission of the virus (Anderson et al., 1998). The second strategy was also tried or aimed at rearing the birds when it is damp as there is less dust in



**Figure 1.** Paralysis of limb and wing in classical Marek's disease.

the poultry farms during the raining seasons. This will minimize the quantity of virus being transmitted through aerosol.

Anderson et al. (1971) has used filtered air and positive pressure houses in the control of MD. MD is spread mostly by inhalation of infectious dander carried in poultry dusts and litter and this being a ubiquitous agent can survive in the dust and remain infective for months (Witter, 1998; Calnek and Witter, 1997). Many birds also act as carriers shedding the virus in the environment (Payne, 1985). Moreover, vaccination greatly reduces clinical disease but not persistent infection by MD virus. The vaccine viruses are also carried through the life of the fowl and are continually shed which results in the ubiquitous presence of the virus (Office International des Epizooties (OIE), 2010).

Experiments by many scientists have shown age-related resistance to MD as is observed in older birds being refractory to infection with increased resistance to tumor formation (Witter and Gimeno, 2006; Anderson et al., 1971). Witter et al. (1973) demonstrated that birds 20 to 22 weeks of age and free of prior infection, were substantially more resistant to mortality and tumor

induction caused by exposure to the virus than one day old chicks. It therefore means that keeping young bird away from sources of infection up to when they become older could be a good way of controlling MD. As shown in this work, the birds were isolated from infectious sources in the farm and also reared when there was less dust circulating in the air. It yielded good result within three years and therefore supported the claim that MD can be controlled by isolation brooding. This delay of contact was done up to 24 weeks in other birds to make sure that the birds were mature enough to resist the infection.

It should be noted that most hatcheries in Nigeria do not vaccinate day old chicks against MD. Normally, birds are vaccinated at day old in hatcheries in order to protect them before resistance to MD is developed. Attempts to eradicate MD in this poultry farm by post-exposure vaccination did not yield the desired result; because it did not prevent the shedding of the virus and transmission of infection. Pre-exposure vaccinated chickens may also shed the virus and transmit infection, although tumors and deaths will be reduced. Vaccinated birds can be viraemic and can persistently transmit the virus (Hlozanek et al., 1977). MD vaccines especially Serotype 1 vaccine

strains (Rispens) need special storage facilities (liquid nitrogen) and this can only be maintained mainly by hatcheries and research institutes. Hatcheries are in better positions to vaccinate day old chicks before exposure to the field virus.

## Conclusion

Isolation brooding, biosecurity measures and brooding during periods with low proportion of dusts in the air is an effective way of controlling MD in cases of outbreaks. Brooding completely outside the farm may even be a better way of control. The use of concrete fencing in place of wood and palm fronts may enhance control by isolation brooding. Efforts to procure vaccinated birds from hatcheries will help in the control of the disease. Moreover, in infected farms, brooding of birds during the hamattan period should be discouraged.

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