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Evaluation of Serum Antibody Titers Against Newcastle Disease in Broiler Poultry in Maputo and Matola Regions, Mozambique

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Abstract: Newcastle disease (ND) is very infectious, greatly contagious and fatal viral disease of poultry and other birds. Biosecurity and efficient vaccination are two important tools to control the disease. Although broiler poultry is regularly vaccinated, ND outbreaks are yearly reported in Mozambique, which may indicate poor immunization. To test this hypothesis, the objective of the study was to evaluate the antibody titers against ND in commercial unvaccinated day old chicks and vaccinated adult chicken broilers raised in small farms in Matola and Maputo regions. Serum samples were collected from unvaccinated broiler day-old chicks (n = 250) and vaccinated adult broiler chicken (n = 300). Serum samples were analyzed by a commercial indirect ELISA. Our findings demonstrated that one day old chicks presented high protective antibodies titers and good and low coefficient of variation (CV), which suggested optimal maternal immunity transfer. Only 23.6% of broiler serum sample had protective antibody titers (GMT \geq 1000) and more than 2/3 were seronegative (GMT = 0). Additionally, as little as 13.3% of analyzed flocks was protected against ND. Taken together, this data suggest that the evaluated adult broiler poultry population were susceptible to ND, which may explain the yearly ND outbreaks reported in those regions.

Key words: Newcastle disease, ELISA, serology, chicken broiler, Mozambique

INTRODUCTION

Newcastle disease (ND) is extremely infectious, highly contagious and fatal viral disease of poultry and other birds, characterized by respiratory, digestive and nervous symptoms (Mishra *et al.*, 2000). It is one of the most important diseases of poultry due to devastating consequences of ND virus infections on infected birds, with flock morbidity and mortality rating up to 100%. Moreover, it may cause negative economic impact owing trading restrictions and embargoes placed on areas and countries where ND outbreak have occurred. Efficient application of vaccines and rigorous biosecurity are 2 important ways to control the disease (Spradbrow, 1990, 1993/4).

Although vaccination is commonly used to control ND in poultry industry, ND outbreaks are yearly reported in Mozambique, specially affecting adult broiler chickens from small farmers (MINAG, 2012). We hypothesize that the occurrence of these outbreaks may be, among other reasons, related to deficient immunization of the birds. In fact, the type of vaccines used and vaccination protocols differ among different farmers countrywide.

While studying humoral immunity profile through serologic evaluation has been shown as important tool to evaluate vaccination programs (Brentano *et al.*, 2000), this technical instrument is rarely used in Mozambique. Humoral response is determined by detection of

different classes of immunoglobulins against ND in serum and secretions (Souza *et al.*, 1999; Al-Garib *et al.*, 2003).

The purpose of the work reported in this paper is to evaluate the antibody titers against ND in commercial unvaccinated 1-d-old chicks and vaccinated adult chicken broilers.

MATERIALS AND METHODS

1-d-old chicks: A total number of 250 samples were collected from non-vaccinated 1-d-old chicks purchased from 5 local commercial hatcheries (A, B, C, D and E). These chicks were mainly of Cobb500 and Ross breeds.

Adult chicken broilers: Three hundred ND vaccinated adult broiler chicken blood samples were collected from 30 flocks. Because we see ND outbreaks from age of 30 days onwards, only chickens between 30 to 40 days of age were included in the study.

Table 1: Maximum and minimum GMT, CV and Protection rate in 1-d-old chicks from 5 analyzed hatcheries

Hatchery	GMT (min and max)	CV (%)	Protection rate (%)
A	2151-7029	35.4	100
B	1659-5620	31.6	100
C	1555-5895	49.5	100
D	1446-4778	25.4	100
E	1544-4879	51.7	100

Table 2: Proportion of protected or unprotected flocks, GMTs and CVs

	Flocks	GMTs (Min-Max)	GMTs (Medium)	CV (Min-Max)	CV (Medium)
Protected flocks	13.3% (4)	1323-9970	3157	34-187	104.3
Unprotected flocks	86.7% (26)	0-969	130	36-316	159.8

Serologic analysis: Serum was separated from blood samples by centrifugation at 15,000 x g for 3 min and stored at -80°C until the day of analysis. Commercial ELISA kit (Synbiotics Co., San Diego, CA) was used to determine the titer of mAb (IgG/IgY) against ND. The ELISA kit was used according to manufacturer's protocol using an automated microplate reader (EL x 800, BIO-TEK Instruments Inc., Winooski, VT). The geometric mean titer (GMT) in each individual sample was also calculated according to manufacturer's instructions. All serum samples were read against positive and negative control antisera provided by the kit and used in each run. Individuals with GMTs of 1000 and above (Sales *et al.*, 2007) were considered immune against ND. Similarly and according to ELISA kit manufacturer instructions, flocks with GMT = 1800 and CV = 45 were considered adequately protected.

RESULTS

Table 1 summarizes the GMT, CV for mAb against ND in unvaccinated broiler chicks from 5 local hatcheries. The GMTs obtained reached the protection level and varied slightly among hatcheries analyzed and ranged from 1446 to 7029. Its distribution was 10, 80 and 10% for GMTs of 1000 to 2000, 2001 to 5000 and >5000 (data not shown), respectively. The CV varied from 25.4 to 51.7%.

The ELISA results in vaccinated adult broiler chickens from 30 flocks demonstrated that only 23.6% of analyzed samples had protective antibody titers (GMT \geq 1000) and more than 2/3 had not antibody titers at all (GMT = 0) (data not shown). In line with these findings, only 13.3% of analyzed flocks was successfully vaccinated and its CV varied from 34 to 187 (Table 2).

DISCUSSION

Measuring maternal antibodies in 1-d-old chicks is an important tool to evaluate the vertical transfer of maternal immunity and its uniformity. The CV is used frequently to evaluate poultry humoral immune response and vaccination programs. Good CVs, between 30-50%, show that flock immunization was succeeded by generating uniform antibody titers (Opengart, 2003).

The GMT and CV obtained in 1-d-old chicks from Maputo and Matola regions suggest that there was optimal maternal immunity transfer (Romero *et al.*, 1989; Meszaros *et al.*, 1992; Ribeiro *et al.*, 2000; Opengart, 2003).

Monitoring serologic antibodies against diseases in poultry industry is a useful instrument to evaluate and adjust vaccination programs (Brentano *et al.*, 2000). The

overall population with protective antibody titers in the study regions was below 70%, indicating that vaccinated broiler birds did not have protective immunity against Newcastle disease. This is in line with the epidemic theory, which suggests that if 70% of the population is immune, the disease outbreak is unlikely to occur because there are not enough susceptible individuals to propagate an epidemic (Thrusfield, 1995; Young *et al.*, 2002). In agreement with these findings, our study also found that only 23.6% of adult broiler flocks were successfully vaccinated against ND. Moreover, more than 2/3 of serum samples had not antibody titers at all (GMT = 0) (data not shown). These findings are in accordance with previous published report that recorded unprotected and low levels of antibody titers against ND in broiler birds of old ages (Sales *et al.*, 2007).

The broiler poultry industry in Mozambique is still dominated by small farmers, who receive very little input from veterinarians and other relevant technicians. Moreover, very few are being trained on biosecurity and poultry immunization. Poor protection of broiler chicken against ND seen in this study might have been due to inappropriate methods of vaccination, poor vaccine quality, vaccine failure, unsuitable vaccination schedule or vaccination technique and immunosuppressive diseases. Poor manufacturing practices of vaccine standards, lack of adequate storage facilities, application of expired vaccines and inadequate vaccine handling during transportation and continuous treatment with antibiotics during vaccination have been also reported as the other possible causes for the vaccine failure in developing countries (Tariq, 1999; Sil *et al.*, 2002; Vui *et al.*, 2002). However, to address the exact causes of vaccine failure in Mozambique, more research is warranted.

Conclusion: Our serological study has shown that, unlike what was found in 1-d-old chicks, the adult broiler birds analyzed were susceptible to ND, which may explain the yearly reported ND outbreaks throughout the country. The obtained results reinforce the need of efficient immunization programs to avoid losses related to ND outbreaks.

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