THE USE OF EMBRYONATED CHICKEN EGGS WITH MATERNAL ANTIBODY TO NEWCASTLE DISEASE VIRUS FOR THE PRODUCTION OF LA SOTA STRAIN VIRAL ANTIGEN

J. ADAMU and S. S. ABAH

Department of Veterinary Microbiology Faculty of Veterinary Medicine Ahmadu Bello University, Zaria

ABSTRACT

Embryonated eggs from Specific Pathogen Free (SPF) flocks are used ideally in the production of Newcastle disease vaccine. However, in developing countries where SPF flocks are not available, embryonated eggs from healthy chicken flocks are utilized. The present study assessed the use of embryonated eggs having maternal antibodies to Newcastle disease virus (NDV) for the propagation of NDV La Sota vaccine strain. Log_2 hemagglutination inhibition (HI) antibody titers to NDV in sera of 18 Isa White breeder chickens were determined. Subsequently within one week, 60 eggs were collected from the breeder hens and incubated for 12 days. Maternal antibodies to NDV in the 12-day-old embrayonated eggs were determined from the egg yolk and allantoic fluid. Two fertile eggs each from five hens were then inoculated with 0.1 or 0.2 mL of La Sota vaccine reconstituted in PBS with pH of 7.4 (200 doses/mL). In addition, three eggs from different hens were inoculated with 0.1 mL of the La Sota vaccine. Log_2 hemagglutination (HA) NDV titers in allantoic fluid were determined from the eggs three days post inoculation. The geometric mean of HI log_2 antibody titers to NDV detected in sera, yolk and allantoic fluid of eggs were, 7.37, 7.09 and 1.93, respectively. There was no statistically significant difference ($p \square 0.05$) between NDV HA log_2 titers in allantoic fluid harvested following inoculation with 0.1 (between 9 log_2 to 11 log_2) and 0.2 (between 8 log_2 to 12 log_2) mL La Sota vaccine. There was no correlation ($r = -0.28$) between antibodies to NDV in sera of breeders and the viral titers obtained in allantoic fluid of their embryonated eggs post inoculation with La Sota vaccine. The results of this study indicate that embryonated eggs with maternal antibodies to NDV could be used for propagation of NDV to yield high viral titers. Hence, it is recommended that breeder flocks raised for the purpose of producing eggs intended for NDV vaccine production could be duly vaccinated against field NDV infection.

INTRODUCTION

Embryonated eggs are used for isolating NDV, producing NDV vaccines and antigens for use in serological tests. Maternal antibodies to NDV are encountered in embryonated chicken eggs from hens raised under field conditions sequel to their exposure to field NDV infections. Isolation and pathogenicity characterization of NDV requires SPF chicken eggs devoid of maternal antibody to NDV. In countries where embryonated eggs from an SPF flock are not available it is practical to use embryonated eggs from a healthy local flock for NDV vaccine production (Grimes, 2002). It has been documented that the presence of maternal antibody would slightly lower the NDV titers obtained from eggs (Yachida *et al.*, 1979). Also, a higher 50 % protective dose of a modified-live NDV *in ovo* vaccine is required in maternal antibody-positive embryos (Ahmad and Sharma, 1992). However, presence of maternal antibody to NDV in embryonated eggs did not influence the protection ability of live virus vaccines administered *in ovo* to protect chickens against ND (Ahmad and Sharma, 1992; Okwor *et al.*, 2014). Presently, the production of NDV La Sota vaccine and NDV antigen in Nigeria involved the use of embryonated eggs that are not from SPF chickens. It is difficult to obtain embryonated eggs that have very low or free from maternal antibody to NDV from breeder flocks that are not SPF. The aim of this study was to assess the effect of maternal antibody to NDV in embryonated eggs on the titer of NDV La Sota strain antigen production.

MATERIALS AND METHODS

Breeders: A flock of 2-year-old Isa white parent stock (P2) comprised of four cocks and fourteen hens served as the source of sera and eggs for this project. These breeders were reared solely for supply of embryonated eggs to the Virology Laboratory Department of Veterinary Microbiology Ahmadu Bello University, Zaria.

Newcastle disease vaccine: Two vials of Newcastle disease vaccine La Sota produced by the National Veterinary Research Institute Vom, were purchased from a commercial source. Each vial of 200 doses was reconstituted with 1mL of PBS (pH 7.4). The HA titers for each vial of the La Sota vaccine were determined.

Sera: Sera were obtained from the breeder cocks (1 - 4) and hens (5 - 18). About 1 - 2 mL of blood was collected from the wing vein of each bird using 2 mL disposable syringe. Sera were extracted after overnight blood coagulation at room temperature, and stored in vials at -25°C until tested.

Embryonated eggs: A total of 60 eggs were collected from the breeder hens within one week from the day their sera were obtained. Where possible, the eggs were labeled with the identity of the laying hen. The eggs were stored in a room with temperatures ranging between 20 - 22°C prior to incubation. The eggs were placed in an embryonated egg incubator (OVA-Easy Advance®, Brinsea product Ltd, UK) after disinfection with 75 % ethanol. The incubator temperature and humidity were 37.5°C and 55 to 60 percent respectively, with automatic turning every 2 hours.

Experimental design: Sera were obtained from the Isa White breeders to determine their HI antibody titers to NDV. Subsequently, within one week, embryonated eggs laid by the breeder hens were obtained and incubated for eight days and then candled for removal of infertile and dead embryos. At 12 days post incubation, the embryonated eggs were divided into three groups. The first group comprised of ten eggs that were inoculated with 0.1 mL (200doses/mL) of La Sota vaccine (Egg Number: 5, 6, 7, 8, 11, 13, 14, 15, and 18). The second group comprised of six eggs that were inoculated with 0.2 mL (200doses/mL) of La Sota vaccine (Egg Number: 6, 11, 13, 15, 16, and18). The third group comprised of six eggs that were removed to obtain yolk and allantoic fluid tested for presence of maternal antibodies to NDV (Egg Number: 14, 16, A, B, C and D). Allantioc fluids were harvested at three days post inoculation with La Sota vaccine and their HA NDV titers were determined. To examine for possible influence of genetic variation among the embryos, 15 chicks were hatched from another set of eggs to determine the extent of their phenotypic differences.

Statistical analysis: The geometric mean of HI $\log_2$ antibody titers to NDV detected in sera, yolk and allantoic fluid of embryonated eggs were calculated. Wilcoxon signed ranks test was used to determine association between NDV titers obtained in allantoic fluid using 0.1 or 0.2 mL of La Sota strain inoculum. Spearman's correlation was used to determine relationship between antibody production in the breeder hen and NDV titer from egg after inoculation of 0.1 mL La Sota strain of NDV. Values of $p \leq 0.05$ were considered as significant at 95 % confidence interval using SPSS version 16.0 (SPSS Inc. Chicago, Illinois. USA).

RESULTS AND DISCUSSIONS

The NDV $\log_2$ HI titers obtained from the sera of 18 Isa white breeders ranged between 6 - 8 $\log_2$ for cocks and 4 - 10 $\log_2$ for hens. The geometric mean NDV $\log_2$ HI titer was 7.37. Egg yolk and allantoic fluid were successfully separated from four out of six 12-day-old embryonated eggs. Generally, antibodies to major viruses affecting poultry that are being detected in hens' sera, egg yolk and day-old chicks have similar titers and are well correlated (Boudaoud and Mamache, 2012). For NDV antibodies, HI titers in yolk are usually higher than in the corresponding hen's sera (Raj *et al.*, 2009). This comparison could not be made in the present study due to lack of enough samples of egg yolk from identified hens. However, the geometric mean NDV antibody titers in sera and yolk were similar in agreement with earlier studies. The maternal antibodies to NDV $\log_2$ HI titers obtained from the yolk and allantoic fluid of four Isa white 12-day-old embryonated eggs ranged from 5 - 9 $\log_2$ and 0 - 7 $\log_2$ with geometric means of 7.09 and1.93, respectively. There was no statistically significant difference (p 0.05) between NDV $\log_2$ HA titers in allantoic fluid harvested following inoculation with 0.1 (ranged from 9 - 11 $\log_2$) and 0.2 (ranged from 8 - 12 $\log_2$) mL La Sota vaccine (Table 1). Moderate antibody levels could be detected in the amniotic and allantoic fluids from the 12th to the 18th days of incubation (Bencina *et al.*, 2005). The present study demonstrated low mean NDV antibodies in allantoic fluids at the 12th day of incubation which

seem not to have interfered much with propagation of the La Sota vaccine strain. However, there is the need for further investigation to ascertain the genetic stability of La Sota vaccine strain, when obtained from embryonated eggs containing some level of maternal antibodies to NDV. There was no significant correlation ($r = -0.28$) between antibodies to NDV in sera of eight breeder hens and the viral titers obtained in allantoic fluid of their embryonated eggs three days post inoculation with 0.1 mL (200 doses/mL) La Sota vaccine (Table 2). This confirm that maternal antibody to NDV had no significant influence on the amount of NDV production in 12-day-old embryonated eggs and by extension the effect might be lower in 9 to 11-day-old embryos. Newcastle disease virus was not detected in the allantoic fluid of one embryonated egg (number 14). This finding may be due to error during inoculation or other reasons which could not be explained. However, maternal antibody to NDV in egg yolk was shown to be influenced by genetic factors (Hamal *et al*., 2006). Embryos used in the present study showed diverse genetic differences as observed phenotypically after hatching the chicks (Plate I). Breeder flocks vaccinated or exposed to field NDV strain should be checked for their potential of still being used for La-Sota vaccine antigen production.

Conclusion

In spite of phenotypic and possible genetic variability in the Isa White breeder hens' embryonated eggs with maternal antibody to NDV, high La Sota vaccine virus antigen titers up to $11 Log_2$ were obtained. Doubling the dose of La Sota strain inoculum did not yield significant increase in NDV antigen obtained from the embryonated eggs with maternal antibody to NDV.

REFERENCES

Ahmad, J., and Sharma, J.M. (1992). Evaluation of a modified-live virus vaccine administered in ovo to protect chickens against Newcastle disease. *Am. J. Vet. Res.* 53(11):1999-2004.

Bencina, D., Narat, M., Bidovec, A. and Zorman-Rojs, O. (2005). Transfer of maternal immunoglobulins and antibodies to Mycoplasma gallisepticum and Mycoplasma synoviae to the allantoic and amniotic fluid of chicken embryos. *Avian Pathology*, 34 (6): 463-472.

Boudaoud, A. and Mamache, B. (2012). Use of egg yolk antibodies to predict optimal age of vaccination against infectious bursal disease (IBD) in broilers. *Inter J Poult Sci*, 11 (2): 138-142.

Grimes, S.E. (2002). Incubation of fertile eggs. In: *A basic laboratory manual for the small-scale production and testing of I-2 Newcastle disease vaccine*. FAO Regional Office for Asia and the Pacific publication 2002/22, Pp. 19.

Hamal, K.R., Burgess, S.C., Pevzner, I.Y. and Erf, G.F. (2006). Marternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. *Poult Sci*, 85: 1364-1372.

Okwor, G.O., El-Yuguda, A. and Baba, S.S. (2014). Profile of maternally derived antibody in broiler chicks and *in-ovo* vaccination of chick embryo against Newcastle disease. *World J vaccines*, 4:72-80.

Raj, N., Poulose, P., Surya, P.S., Ravishankar, C. and Sebastian, M. (2009). Comparison of antibody titres of Newcastle disease virus in randomly collected sera and egg yolk of layers. *Journal of Veterinary and Animal Sciences*, 40: 20-21.

Yachida, S., Aoyama, S., Takahashi, N., Iritani, Y. and Katagiri, K. (1979). Effect of Incubation temperature or maternal antibody on virus growth and mortality of embryonated chicken eggs inoculated with the B1 strain of lentogenic Newcastle disease virus. *Avian Dis*, 24 (4):983-988.

Table 1: Log_2 hemagglutination (HA) titers of Newcastle disease virus (NDV) in allantoic fluid at three days post inoculation of 12-day-old Isa White embryonated eggs with La Sota vaccine

Egg number	Log_2 HA titer of NDV La Sota strain	
	0.1 mL inoculum (20 doses)	0.2 mL inoculum (40 doses)
6	11	10
11	11	8
13	11	12
15	10	11
18	9	11
Z = – 0.137	P = 0.45	

Table 2: Log_2 hemagglutination inhibition (HI) antibodies to Newcastle disease virus (NDV) in Isa White breeders and hemagglutination (HA) titers of La Sota strain propagated in their eggs

Bird number	Log_2 HI NDV antibodies	Log_2 HA titer of La Sota
5	7	11
6	7	11
7	8	11
11	6	11
13	6	11
14	7	0
15	8	10
18	9	9
r = – 0.28	P = 0.503	

Plate I: Isa White breeder Parent Stock and chickens hatched from their eggs