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Persistence of Passively Administered Antibodies and Their Role in Protection Against Newcastle Disease Virus Challenge

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Abstract: The persistence of passively administered antibodies (Abs) in the chicken sera and their role in the protection against Newcastle disease virus (NDV) challenge were determined in this study. Haemagglutination inhibition (HI) test was employed to measure the Ab titres in chicks sera. Clinical signs were observed and moralities were recorded, after challenge with the virulent virus, to determine the protection levels offered. High levels of Abs were detected in sera of chicks received single dose of yolk extracts (YE), derived from immune chickens, and remained for more than seven weeks after injection. Much higher levels of Abs were detected in sera of chicks received booster dose of the YE as compared to that received single dose. Following challenge, 90% and total protection were obtained in chicks received single and booster dose of the YE respectively.

Key words: NDV, passive immunity, antibody, protection

Introduction

Newcastle disease (ND) is regarded throughout the world as one of the most important diseases of poultry (Murphy *et al.*, 1999). It is included as an Office Internationale des Epizooties (OIE) list A disease (Office Internationale des Epizooties, 2001). This is not only due to its devastating effect on poultry industry with the flock mortality rates up to 100% (Gordon and Jordan, 1982), but also the economic impact that may ensue due to the severe nature of the disease and associated consequences (Aldous and Alexander, 2001). It is caused by a virus (*avian paramyxovirus* 1) that belongs to the genus *rubulavirus* of the sub-family *paramyxovirinae* of the family *Paramyxoviridae* (Rima *et al.*, 1995).

Antibodies to Newcastle disease virus (NDV) can pass via the egg yolk to the chicken progeny. The levels of these maternal antibodies, in day-old chicks, directly related to their titres in the parent (Heller *et al.*, 1977). Although these maternal Abs are protective to chicks against virulent viruses but rapid and gradual decay in their levels is proved problem (Allan .*et al.*, 1978). Passive immunization against the disease via administration of immune sera (Umino *et al.*, 1987; Umino *et al.*, 1990; Reynold and Maraqa, 2000) or egg yolk extracts (Stone *et al.*, 1994) to the chickens had been previously studied. Various levels of protection in these passively immunized chicks against virulent virus challenge were reported.

The present study investigated the duration of acquired passive immunity following administration of yolk extract (Y.E.) into the chicks. The protective potential of this immunity is also investigated.

Materials and Methods

Chicks: Sixty, one-day old, While Hissex broiler chicks were obtained from the Arab Company for Agricultural production and Processing (Khartoum, Sudan).

Chicken eggs: Eggs were obtained from Arab Company for Agricultural production and Processing (Khartoum, Sudan). They were laid by parent stock which vaccinated with the live attenuated ND vaccine containing La Sota strain of the virus at 2, 4, 6 and 8 weeks of age. The parent chickens were known to contain high titre of serum Abs and consequently high levels of these Abs are expected in their egg yolk.

Newcastle disease virus (NDV): The Herts 33/56 strain of NDV was used in this study to challenge the immunized virus. The virus was obtained from the Central Veterinary Research Laboratory (CVRL) (Khartoum, Sudan). The EID50 of the virus was determined before used.

Preparation of yolk extracts: Egg yolk extracts were prepared essentially as described by Stone *et al.* (1994). The egg yolk was separated carefully from albumen in a petridish and the content was thoroughly mixed with syringe. The yolk was then mixed with normal saline at the rate of 1: 4 (v/v) and centrifuged at 3000 rpm for 15 minutes. The supernatant was collected and tested for NDV antibodies by hemagglutination inhibition (HI) test. Only yolk extracts that yielded high antibodies were used in this study.

Chicks inoculation: Chicks were inoculated with the YE subcutaneously (s/c) using 1 ml syringe. Blood was sampled from the wing vein.

Haemagglutination inhibition (HI): The HI test was carried out according to Abdalla *et al.* (1999). Two-fold serial dilutions of serum samples were made with normal saline in microtitre plates. Volumes of 0.05 ml of a known NDV antigen (PHI DooRn ND HI antigen I ml 3089 Intervet, Holland) containing 4 haemagglutinating units were added in each well of the plate. Three rows of wells were left as controls: the first row contained a known NDV antiserum (positive control), the second row contained NDV antigen (negative control) and the third row contained normal saline with RBCs (reagent control). The plate was shaken by a titerteck plate shaker and left for 30 minutes at room temperature before the addition of 0.05 ml of chicken RBCs to each well. The plate was then rotated and left for 20 minutes or till a pattern of HA appeared. Haemagglutination inhibition titres were expressed as the reciprocal of the highest dilution that cause 50% inhibition of agglutination (Allan *et al.* 1978). The base two logarithmic titre was calculated.

Experimental design: The chicks were divided into three groups (G1, G2 and G3) with 20 chicks per group. When they were 28 day old, Each bird in G1 received 1 ml of normal saline whereas birds in G2 and G3 received I ml of YE. When chickens were 40 days old, birds in G1 and G2 received $10^{7.5}$ EID_{50} Herts strain of NDV whereas birds in G3 received the booster dose of YE. When chickens were 55 day old, only birds in G3 received $10^{7.5}$ EID_{50} Herts strain of NDV. Blood was sampled from each group at 28, 40, 55, 58, 62 and 71 days of age before sera were tested for NDV antibodies by HI test.

Statistics: The statistical significance of differences between groups of data was determined using the two-tailed Student's unpaired t-test.

Results

The Ab levels as detected by HI in chicks immunized against NDV are demonstrated in Table 1. When chicks were 40 days old (twelve days following the first inoculum), no increase, but slight decline, in Ab levels was observed in chicks in G1 while significant ($p < 0.05$) increase noted in chicks in G2 and G3.

Table 1: Antibody titres in chicks passively immunized against NDV challenge

Chicken age (day)	Immunication of chicks		
	G1 (Control)	G2 (Single dose)	G3 (Booster dose)
28	4.20±0.16	4.60±0.15	4.30±0.19
40	4.00±0.13	5.50±0.14	5.80±0.18
55	5.80±0.15	8.00±0.14	10.60±0.15
58	7.60±0.13	8.80±0.15	11.80±0.16
62	8.00±0.13	11.20±0.13	11.20±0.17
71	8.00±0.17	9.00±0.12	10.90±0.15

*HI Ab titre (geometric mean ± S. D.) n = 8

Following challenge of immunized chicks in all groups, an obvious increase in Ab titres was also observed. Mortalities and classical ND signs were only recorded among chicks in G1 and G2. Number of 8 and 2 birds died in G1 and G2 respectively. 90% and total protection are obtained in chicks received single dose (G2) and those received double dose (G3) respectively.

Discussion

Acquired passive immunity has the advantage of quick appearance of the absorbed antibodies in the blood. Thus can be administered to birds even during the incubation period of a particular disease to provide a quick protection level against the disease agent. In the present study, emphasis was placed upon passive immunization of chickens and its advantage in protection against virulent NDV hit. The results of this study showed that there was an obvious rise of NDV antibodies following passive immunization of chicks with Y.E. High levels of antibody were demonstrable in chickens blood after 12 days post inoculation of Y.E. This proved in disagreement with Stone *et al.* (1992) and Abdel Rahman and Chu (1993) who reported that HI Ab titres reached the maximum levels 1 to 4 days after yolk inoculation to the chicks. This discrepancy in the findings could be attributed to the variations in the dose of the YE that birds received in either experiment.

In the present study, the Ab levels in chicks sera was observed high for about 7 weeks following primary immunization. Previously, Heller *et al.* (1977) reported similar observations for the natural passive Abs against NDV passed from the laying hen to the eggs. They confirmed the persistence of passive Abs in the egg yolk obtained from eggs of chickens hyperimmunized with the virus and maintained up to the 91st day of their age. This proved

the efficiency of acquired passive immunity as it simulates the natural passive immunity.
Total protection against challenge with a virulent strain of NDV, which was 100 per cent fatal for the off spring of non-vaccinated, was obtained in this study when chicks was boostered with passive administration of antiserum against the whole virion. Similar findings were published by Umino *et al.* (1987) who observed total protection in chicks passively immunized by sera prepared against the whole virion but not against some viral proteins. More supportive data to this finding was reported by Umino *et al.* (1990) when they confirmed that even combination of monoclonal antibodies against some NDV proteins having neutralizing activity will not provide total protection when passively administered as the whole virion did.
The passively administered Abs against NDV, derived form the egg yolk of vaccinated hens, remained high for prolonged periods and to a protective level is our conclusion to this presentation.

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