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Overcoming the susceptibility gap between maternal antibody disappearance and auto-antibody production

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ABSTRACT

In the first 10–14 days of a chick's life, protection is conferred by maternal antibodies. Further broiler protection is achieved by active vaccination. However, the high level of maternal antibodies interferes with the induction of an effective immune response by vaccination at a young age. As a result, there is a gap between the reduction in protective maternal antibodies and elevation of self-produced antibodies following active vaccination. The major aim of this study was to test an approach consisting of passive and active vaccination to overcome this gap and to provide continuous resistance to infectious viral diseases during the broiler's growth period. Newcastle disease virus (NDV), which is one of the world's most prevalent infectious diseases of poultry, was tested as a model. Following subcutaneous injection of 18 hemagglutination-inhibiting (HI) units of anti-NDV immunoglobulin Y per 1-day-old chick, protective $\log_2$ antibody titers above 4 could be detected to at least 17 days of age. The combination of passive immunization on day 1 of age with attenuated live vaccination on day 10 led to high protective titers throughout the entire growth period, up to 41 days of age. Moreover, the HI titers in the group of birds immunized with the combined vaccination were significantly more homogeneous than those in the group vaccinated only with live virus. Thus, full protection against NDV of all broilers in flock during their entire growth period was achieved by a vaccination regime that combines passive immunization and live vaccination.

1. Introduction

Infectious diseases constitute a major problem in poultry breeding. For viral diseases, the main solution is prevention by vaccination [1]. In its first days of life, the chick is protected by maternal antibodies [2,3]. However, the half life of maternal antibodies in chicken is relatively short [4]. For the main infectious viruses, full protection of chicks by maternal antibodies is conferred for 10–14 days, after which those protective antibodies are significantly reduced and chickens become susceptible [2].

Continuous protection of broilers is achieved by active vaccination. However, a high level of maternal antibodies interferes with vaccination efficacy at 1 day of age [5]. Maternal antibodies have been found to interfere with the production of an efficient immune response, including inhibition of B and T helper cells and of antigen processing, and activation of T suppressor cells, as shown in gnotobiotic pigs [6]. The gap between the reduction in protective maternal antibodies and elevation in self-produced antibodies following active vaccination is the most sensitive period to viruses [7]. A partial solution is vaccination with live partially virulent viruses that induce an immune response despite the maternal antibodies. However, such a vaccine has negative effects on the vaccinated birds, e.g. damage to the immune system by vaccines for immunosuppressive viruses such as infectious bursal disease virus (IBDV) [8] or weight loss by vaccination against Newcastle disease virus (NDV) [9].

Newcastle disease is one of the most prevalent infectious diseases of poultry. It is distributed worldwide and can cause large economic losses for poultry breeders. The disease is caused by NDV, which infects over 240 species of birds and spreads primarily through direct contact between infected and healthy individuals. NDV is a paramyxovirus—an enveloped, nonsegmented,

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negative-sense RNA virus. All NDVs are considered to belong to one serotype which is divided into two classes [10]. Vaccination programs against NDV differ among countries, from no vaccination to three to four vaccines during the chicken's growth period [11]. The aim of vaccinating against NDV is to stimulate neutralizing antibodies. Based on the correlation between hemagglutination inhibition (HI) test and neutralizing antibody titers and protection, a HI $\log_2$ titer ≥ 4 is considered protective [12].

Avian embryos and neonates acquire passive immunity through the transfer of maternal immunoglobulin Y (IgY) from the egg yolk to the serum. Antigen-specific IgY can be extracted on a large scale from eggs laid by chickens immunized with selected antigens. Therefore, the use of IgY for passive immunization has been extensively studied, demonstrating its effectiveness at preventing or treating infectious diseases, especially those of the intestinal tract, caused by various pathogens [13].

In the last decades, due to massive genetic selection, broiler growth rate has increased and their growing period has been reduced to 35–40 days [14,15]. Vaccination programs are aimed at protecting birds during this period.

The major aim of this study was to establish a vaccination protocol to provide continuous resistance to viral infectious diseases at a young age, thereby overcoming the gap between the decrease in maternal antibodies and the increase in auto-antibodies produced in response to active vaccination.

2. Materials and methods

2.1. Determination of anti-NDV antibody levels in field flocks

Anti-NDV antibody levels in the sera of 50 Leghorn layers (Yavne Hatchery Kvuzat Yavne, Israel), yolk immunoglobulin (IgY) extracted from 50 eggs, and sera from 50 chicks (1 day of age) all from the same commercial flock, were determined by HI test, performed according to the OIE (World Organisation for Animal Health) Terrestrial Manual 2012 [12]. Briefly, serial twofold dilutions of sera or egg yolk IgY were incubated with four hemagglutination units (HAU) of inactivated NDV commercial vaccine at room temperature for 30 min. The four HAU were prepared on the day of each assay. Chicken erythrocytes (1% in PBS) were added and HI was scored after incubation for 30 min at room temperature. The HI titer is the reciprocal of the highest serum dilution that completely inhibited agglutination. Negative and positive controls were included in each assay.

2.2. Antibody production and purification

Vaccination regime to obtain high and long-lasting antibody titers against NDV in egg yolk was as follows: 5-month-old laying Leghorn layers that were vaccinated prior to the experiment as recommended by the Israeli Ministry of Agriculture from day 1 to 3 months, were additionally vaccinated in our experiment with inactivated NDV at 3-week intervals between the first, second and third vaccinations, and at a 2-month interval between the third and fourth vaccinations. Eggs were collected 2 weeks after every vaccination. Blood was drawn 2 weeks after the third and fourth vaccinations and 4 months after the fourth vaccination. All sera and egg-extracted IgY from all experiments was kept at −20 °C until examination.

Eggs were kept at 4 °C for up to 10 days before IgY extraction. Briefly, double distilled water (DDW) was added to egg yolks (9:1 v/v) and the solution was incubated for 30 min at room temperature (RT). pH was adjusted to 5.2 with 10% acetic acid and the solution was incubated for 2.5 h at RT. The solution was then filtered through Whatman filter paper until the fluid became transparent. Ammonium sulfate (40%, Sigma, Belgium) was slowly added with gentle mixing for 3 h at 4 °C. The solution was then centrifuged at 15,500*g* for 20 min and the pellet was collected, weighed and resuspended in 40% ammonium sulfate (1:1 w/v). Before each experiment, solution was centrifuged at 15,500*g* for 20 min. The pellet was resuspended in DDW and dialyzed overnight against PBS in 3.5-kD cassettes (Thermo, USA). The titers of specific antibodies were determined by HI test.

2.3. Determination of IgY administration route

Broiler chicks in all the experiments of this study were from commercial breeder flocks, carrying high level of antibodies against NDV. During the experiments broilers were raised in isolated rooms with free supply of food and water.

One-day-old broiler chicks were administered 18 HI units of anti-NDV IgY by eye dropper (ED), intramuscularly (IM) or subcutaneously (SC). Each group was consisted of 15 birds. Blood was drawn every 4–6 days for 27 days after vaccination. The presence of specific anti-NDV antibodies was examined by HI test.

2.4. Vaccination of broilers

2.4.1. Passive immunization of broilers

Broiler chicks were injected SC with 15 or 18 HI units of anti-NDV IgY in 200 μl PBS at 1 or 10 days of age. Each group was consisted of 10 birds. Blood was drawn 1 week after passive immunization and every 4 days thereafter.

2.4.2. Combination of passive immunization and vaccination with inactivated NDV

Broilers were injected SC with 18 HI units of anti-NDV IgY in 200 μl PBS at 1 or 10 days of age, and vaccinated IM with inactivated NDV vaccine at 10 days of age, according to the manufacturer instructions for commercial birds. Each group was consisted of 10 birds. Blood was drawn 1 week after vaccination and every 4 days thereafter.

2.4.3. Combination of passive immunization and vaccination with live NDV

Broiler chicks were injected SC with 18 HI units of anti-NDV IgY in 200 μl PBS at 1 day of age. At 1 and/or 10 days of age, the chicks were ED vaccinated with live NDV commercial vaccine, according to the manufacturer instructions for commercial birds. Each group was consisted of 15 birds. Blood was drawn once a week.

2.4.4. Ethics

All animal studies were approved by the Animal Care and Use Committee of Israel.

2.5. Statistics

All results were analyzed by one-way ANOVA with the Newman–Keuls method. The analyses were performed with Graphpad Prism 5 software for Windows.

3. Results

3.1. Determination of anti-NDV antibody levels in field flocks

The rate of transfer of specific anti-NDV antibodies from layer to egg yolk and then to the hatched broiler chick was determined by HI test in 50 representatives at each stage. Forty-eight out of fifty layers had HI titers of 8–10 (average 9). In egg yolks, the HI titers were in the range of 6–12 (average 8.5), and in 1-day-old broiler chicks, serum HI titers were between 5 and 10 (average 7.5).