Comparison of the immune response between local manufactured and commercial inactivated Newcastle Disease Virus vaccine in a challenge trail with field isolated Newcastle Disease Virus

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Summary

Velogenic Newcastle Disease Virus was isolated from broiler chickens in Northern Iraq. An inactivated vaccine was manufactured locally using as seed virus ELD50/ml10⁹ and then compared with commercial inactivated vaccine in an experimental study which included 120 broiler chicks divided into three groups (G1 unvaccinated control, G2 for commercial vaccine and G3 for local vaccine). The chicks were injected subcutaneously at 3 days old followed by booster Lasota live vaccine eye drop. Indirect ELISA technique was used to estimate the antibody titer from the collected sera of chicks at age 7, 17 and 27 days (pre-challenge) and challenged at 31 days old with the same virus. The results indicated that there were significant differences (P<0.05) between vaccinated group G2 and G3 at 27th day old and showed a high antibody level with high protection percentage compared with the control. G1 which shown no survival, 100% mortality and severe histopathological lesions, while in G2 and G3 was 43% and 87% respectively. Post-challenge antibody titers of survival chicks showed in G3 significantly over the G2 with less severe histopathological lesions. This study concluded that vaccine failure could occur due to factors of the immune status of the host, improper storage of vaccine, improper vaccination and variant pathogenic virus strain. More epidemiological surveillances are required to decide the actual impact of the disease in poultry farms and matching the vaccines.

Keywords: Newcastle Disease Virus, Challenge test, Inactivated vaccine, Histopathology.

Introduction

Newcastle disease (ND) is one of the most important diseases that affect birds, in particular chickens. The epizootic nature of the disease has caused severe economic losses in the poultry farms worldwide (1). The Newcastle disease virus (NDV) strains pathogenicity can be classified into three pathotypes (velogenic, mesogenic and lentogenic) on the basis of the severity of disease in chickens. Severity is determined by in vivo pathogenicity test parameters, including the mean death time in chicken embryos and the intra-cerebral pathogenicity index in day-old chicks (2). A lethal infection by a velogenic NDV pathotype results in high mortality in chickens. The spread of NDV in chickens routinely vaccinated with NDV vaccines derived from known strains such as Clone 30 or La Sota. Many researchers have reported that the viruses isolated in Korea and China belong to the VIIId sub-genotype of genotype VII in poultry in spite of excessive vaccination programs (3 and 4). They suggested that these VIIId isolates are antigenically distinct from the currently available NDV vaccine strains. Alternatively, it is possible that the vaccines are not sufficiently immunogenic to prevent the spread of NDV (5). Some researchers concluded that vaccine produced from virus isolated from previous local NDV outbreak revealed high level of protection (5 and 6). The research was designed to prepare an inactivated vaccine from recently local isolates of NDV and study the efficacy of the vaccine and their protections capacity against the infection with significant level of antibodies to reduce mortality rate.

Materials and Methods

Local isolated velogenic NDV from poultry flocks was used as seed of virus, with titer of...
10^6 HA, the inactivation of virus achieved with formalin at a final concentration of 1/1000 were done according to method of Palaya (1991) (7). Efficacy of inactivation of the viruses test: Conducted according to method described by (8) in ECE 10 day old injection with 0.2 ml of the fluid (containing the inactivated virus by formalin) then the harvested embryonic fluid after 5 days and repeating the previous process twice to make sure of the inactivation of the viruses. Then, allantoic fluid was collected, preserved in the refrigerator (4°C). Inactivated ND virus was mixed with cofactor oil (Oil Adjuvant), the watery part was prepared (Liquid Phase) by adding 0.01% of 10% merthiolate as preservative material to the inactivated allantoic fluid, then mixing well on a magnetic stirrer for two hours. At the same time add the oil phase “Incomplete Freund’s adjuvant as water-in-oil emulsion” (7). Physical properties color, Viscosity test, stability of vaccine: were done according to (9). Ten chicks aged five days were immunized with inactivated vaccine subcutaneously and observed for 35 days and clinical sign and gross lesion were recorded to ensure safety of vaccine. One hundred twenty broiler chicks (Ross 308 Breeders) one day old were divided into 3 groups 40 chicks of each in separated boxes. All groups except control were vaccinated with ND Clone 30 (Intervet®) at 1 day old, at 3 days old the chicks in the second group were vaccinated with 0.5 ml of commercial inactivated ND, the third groups vaccinated with 0.5 ml of field isolate inactivated vaccine subcutaneously followed with Lasota vaccine at 10 days old via ocular route and control left without vaccines. Experimental chickens were monitored by collecting serum at 7, 17 and 27 days old pre-challenge and in 41 days old 10 days post-challenge. Blood samples collected and serum were separated to evaluate the antibody titer then stored at -20°C until use. Antibody titre against NDV in serum was determined by Indirect ELISA technique using ELIZA Kit (Symbiotic kit). Seven chicks taken from each group at 31 days old were housed separately and brood properly and inoculated intra-ocular and intra-nasal with 0.5 ml Local isolated velogenic ND virus contain 10^9 (ELD50)/bird, clinical sign and gross lesion of ND of sick and died birds were recorded, then organ samples (lung as a site of infection) were collected for histopathological study (10 and 11).

Results and Discussion

The result of safety test revealed no specific symptoms related to ND which confirms the safety of vaccine. This finding agreed with (6) noted that inoculation double dose of inactivated (killed) vaccine via subcutaneous route back of the neck for safety ensuring did not produce side effects. The evaluation of immune response was determined (Table, 1) at 7, 17 and 27 days-old chicks pre-challenge the result showed significantly differences in mean±SE titer at 27days-old in vaccinated group (G2 and G3) were 1008±193 and 2816±614 respectively similar finding with (12), also titer produced after immunization with inactivated vaccine produce higher titer after 27 days old, while decline mean±SE antibody titer in non-vaccinated control group (G1) was 296±16.69.

<table>
<thead>
<tr>
<th>Groups</th>
<th>vaccines</th>
<th>mean ±SE Titer in 7 day old</th>
<th>mean ±SE Titer in 17 day old</th>
<th>mean ±SE Titer in 27 day old</th>
<th>mean ±SE Titer in 41 day old (10pc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1</td>
<td>Unvaccinated Control</td>
<td>4251±1078</td>
<td>906±33 a</td>
<td>296±16 b</td>
<td>-</td>
</tr>
<tr>
<td>Group2</td>
<td>Commercial Vaccine</td>
<td>4251±1078</td>
<td>844±153 a</td>
<td>1008±193 b</td>
<td>2106±133 a</td>
</tr>
<tr>
<td>Group3</td>
<td>Experimental Vaccine</td>
<td>4251±1078</td>
<td>1145±219 a</td>
<td>2816±614 a</td>
<td>2986±417 a</td>
</tr>
</tbody>
</table>

Small letter indicates significant different P<0.05 in days of age

The mean±SE antibody titer of G3 immunized with experimental vaccine 10^9 EID50/ml show titer significantly over the G2 immunized with commercial vaccine. This result agreed with (13) who suggested that administrating inactivated vaccine with live vaccine produce higher immunity and for longer period same as Hooper which concluded that inactivated vaccine contained high titer of seed virus and produced higher
The mean antibody titers were determined and observed. On 41 day old (10 days post-challenge) these values increased significantly and were (2106±133 and 2986±417 in (G2 and G3) respectively (Table, 1). The result of histopathological sections, the main changes of lung tissue showed severe pulmonary congestion with heterophils in the lumen of blood vessels and fibrin networks deposition in the interstitial tissue in control group (Fig. 2 and 3) and G2 (commercial vaccine) (Fig. 4 and 5). The lung showed severe lesion with severe congested blood vessels fibrin networks deposition in the interstitial tissue and edema, compared with G3 (experimental vaccine) showed only mild congested blood vessels and antrum lung and mild congested dilated sinusoids (Fig. 6 and 7). This study is in agreement with severe lesions observed in birds after experimental with NDV by (21).
The result showed that the commercial vaccine fail to prevent ND clinical sign and gross lesion the same as proved by (22). It has been observed that available ND vaccines fail to protect against morbidity and mortality caused by new variants NDV. Also (23) suggest that NDV variants may be evolved in poultry as a result of suboptimal vaccination (23). OIE proved that regardless of genotype variants circulating NDV strains, all NDV isolates belong to the same serotype. If the vaccination is given correctly, ND vaccines prepared with any NDV should protect poultry from clinical disease and mortality in the event of a virulent challenge (24).

**Reference**

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مقارنة الاستجابة المناعية بين لقاح مرض النيوكاسل المقتول المزود محلياً مع التجاري بالتحدي مع فيروس مرض النيوكاسل العشبي في الحقل

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الخلاصة

عُزل في هذه الدراسة عينة ضعيفة من فايروس مرض النيوكاسل من مزارع دجاج اللحم في شمال العراق، خضِر لقاح المقتول المحلي من عزلة فايروس مرض النيوكاسل تم استخدام 9 ELD50/ml كبرى كمية لقاح المقتول. وُفرت 10 لقاحات للدراسة التجريبية. ضمت 120 من أفراخ دجاج اللحم وقسمت إلى ثلاث مجموعات: المجموعة الأولية هي مجموعة السيطرة غير المقمِّرة في حين نفخت المجموعة الثانية باللقاح التجاري المقتول والمجموعة الثالثة باللقاح المقتول المحلي. حققت الأفراخ في عمر 3 أيام تحت الجلد فضلاً عن الجرعة المنشطة للفيروس الحي عن طريق العين. استخدمت اختبار الألابيز غير المباشر لتقييم وحساب مياء المناعة عن طريق جمع المصل من المجاميع من نافذة ثانية بعد تزويج الدجاجة 17 يوماً في عمر 31 يوم تم تحدي نفس الفايروس. وقد تبين وجود اختلافات معنوية للمجموعتين الثانية والثالثة في عمر 27 يوم والثاني 27% على التوالي. كما أظهرت النتائج أن اختبار التحدي قد أزداد في الدجاج الحي وقد توقعت المجموعة الثالثة عن طريق عن أعراض مرضية مرغوبة أقل شدة من المجموعة الثانية. وخلص النتائج إلى أن فشل اللقاح يحدث بسبب عدد الأسباب الإيام الخاطئ للتحدي. والتحكم في بيئة فيروس النيوكاسل وسائل الفيروس مسببة للأمراض. المزيد من التحال عن الدم لتحديد الأثر الفعلي للمرض في مزارع الدجاج ومتابعتها.

الكلمات المفتاحية: فيروس النيوكاسل، اختبار التحدي، اللقاح المقتول، الفحوصات النسيجية المرضية.