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Comparison of humoral immune response and challenge protection of live oil adjuvanted, aqueous live and killed adjuvanted Newcastle disease (LaSota strain) vaccines in broiler chicks

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ABSTRACT

Efficacy of live-in-oil Newcastle disease (ND) vaccine (half and full antigenic dose) was compared with aqueous live and killed-in-oil ND vaccines using the ND virus LaSota strain in broiler chicks. Broiler chicks (7-day-old) were either vaccinated against ND with aqueous live, killed-in-oil, live-in-oil with half and with full antigen dose vaccines or were inoculated only with the oil adjuvant or kept as non-vaccinated controls (20 birds/group). Serum samples collected at weekly interval from day 1 to day 42 of their age were titrated for the presence of NDV antibodies by HI. The titres were higher in all the vaccinated groups than controls from 1 week after vaccination onward. The titres were higher in both live-in-oil with half and full antigen dose vaccines than aqueous live and killed-in-oil on day 28 and 35 post vaccination. Challenge with a velogenic field NDV (day 14 post vaccination) to 10 birds of each group gave higher protection (70–90%) in vaccinated chicks than controls. It is concluded that live-in-oil ND vaccine (half and full antigenic dose) gave higher titre over 35 days post vaccination than aqueous live and killed-in-oil ND vaccines in broiler chicks.

Key words: Broiler chicks, LaSota strain, Live-in-oil vaccine, Newcastle disease, Oil emulsion

The only method to control the Newcastle disease is prophylactic vaccination coupled with biosecurity measures. Live and killed vaccines with various regimens are used for the control of this disease but none of the procedure is entirely successful in preventing the disease. Although live vaccines are generally inexpensive, easy to administer and give high titres yet have short lived immunity, a problem that can be overcome by adjuvanting with oil (Peleg *et al.* 1993, Samina *et al.* 1999). In addition to its biological advantages, the method of preparation of live-in-oil vaccines saves the expenses on cold storage and shipment necessary for conventional killed-in-oil vaccines (Peleg *et al.* 1993).

The present study was designed to compare live-in-oil ND vaccine with aqueous live ND vaccine and formalin killed oil adjuvanted ND vaccine using LaSota strain.

MATERIALS AND METHODS

Commercial lyophilized vaccine (LaSota strain), reconstituted in 50ml phosphate buffered saline was used as the aqueous live ND vaccine without adjuvant. Day-old broiler chicks (120) were divided into 6 groups of 20 birds each. At the age of 7 days, birds of groups A, B, C and D were vaccinated subcutaneously 0.5 ml aqueous live, formalin killed-in-oil, live-in-oil with half antigen dose and live-in-oil with full antigen dose NDV vaccines, respectively, while the group E was inoculated only with the oil adjuvant (control 1) and the group F was kept as non-vaccinated (control 2).

The water phase (tween–80) and oil phase (span–80) surfactants were added to reduce the surface activity of aqueous phase and oil to produce a stable emulsion (Stone 1988). Two live-in-oil vaccines containing half antigen dose and full antigen dose, respectively, were prepared just before use (Table 1).

The killed-in-oil vaccine was prepared using oil emulsion. Reconstituted vaccine (5 ml) was mixed with equal volume of 0.2% formalin to attain the final concentration of 0.1% (Buxton and Fraser 1977) and was inactivated for 24 h at 37°C (Table 1). The residual infectivity of the inactivated inoculum was tested in 9-day-old embryonated eggs via allantoic cavity route, and the allantoic fluid was tested by spot HA test (Allan *et al.* 1978).

Sterility of the killed-in-oil vaccine was tested in thioglycolate broth and blood agar for 72 h at 37°C (Mahboob

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Table 1. Preparation and physical properties of various Newcastle disease (LaSota strain) vaccines

Vaccine	Reconstituted[1] ND vaccine (ml)	NS (ml)	Formalin 0.2% (ml)	Oil emulsion (ml)			Total volume (ml)	Dose per 0.5ml	Emulsion type	Visosity (sec)	Stability (weeks)		
				Paraffi oil	Span 80	Tween 80					4°C	37°C	RT[5]
Aqueous Live	5	45	–	–	–	–	50	One	Aqueous	–	–	–	–
Killed-in-oil	5	–	5	36	3	1	50	One	W/O	7	> 9	4	> 9
Live-in-oil[2]	2.5	7.5	–	36	3	1	50	Half	W/O	7	> 9	4	> 9
Live-in-oil[3]	5	5	–	36	3	1	50	One	W/O	7	> 9	4	> 9

[1]Commercial lyophilized vaccinal LaSota strain (1000 doses) reconstituted in 50ml phosphate buffered saline, [2]half antigen dose, [3]full antigen dose, [4]water-in-oil, [5]room temperature.

Table 2. Humoral immune response of 4 types of Newcastle disease vaccine and controls in broiler chicks during 2 weeks before and 5 weeks after vaccination

Vaccine	HA antibody titres against New Castle disease (mean±SD) Day of titre evaluation with reference to vaccination (Day 0)						
	–7	0	7	14	21	28	35
Aqueous live	36.8±1.4	18.4±1.4	48.5±1.5 ^{a}	84.5±1.5 ^{a}	64±1^{a}	55.8±1.4^{a}	48.5±1.5^{a}
Killed-in-oil	42.2±1.5	18.4±1.4	36.8±1.4 ^{a}	55.8±1.4 ^{a}	64±1.6^{a}	64±1.6^{ab}	55.7±1.8^{a}
Live-in-oil (half antigen dose)	36.8±1.4	18.4±1.4	48.5±1.5 ^{a}	84.5±1.5 ^{a}	128±1.6^{ab}	168.9±1.5^{b}	168.9±1.5^{b}
Live-in-oil (full antigen dose)	36.8±1.4	18.4±1.4	55.8±1.4 ^{a}	97±1.5 ^{a}	147±1.4^{b}	194±1.5^{b}	194±1.5^{b}
Adjuvant inoculation (control 1)	42.2±1.5	18.4±1.4	12.1±1.5 ^{b}	12.1±1.5 ^{b}	12.1±1.5^{c}	9.2±1.4^{c}	8±1^{c}
Non-vaccinated (control 2)	42.2±1.5	21.1±1.5	12.1±1.5 ^{b}	12.1±1.5 ^{b}	10.6±1.5^{c}	8±1^{c}	8 ± 1 ^{c}

The values with different superscripts in the same column differ (P<0.05): vaccine was done in chicks at 7 days of age.

et al. 1996). Safety of the vaccine was checked in broiler chick (Alexander 2004).

All the vaccines were homogenized manually by vigorous shaking for 15 min and the appearance, viscosity and stability were recorded as per Stone *et al.* (1978). Stability of the vaccines was observed at 37°C, 4°C and at room temperature. Blood samples were taken on weekly interval (day 0–42) from 5 randomly selected birds of each group. Sera were separated and titrated for the presence of ND antibodies by haemagglutination inhibition test (Allan *et al.* 1978).

Birds (10) of each group were exposed to challenge protection test at the age of 21 days by injecting intra muscular velogenic NDV (Filed isolate having EID_{50} $10^{7.16}$), in a single dose (0.1 ml). Mortality was recorded for 10 days post challenge. Supernatant of triturated spleen and liver from all dead birds was inoculated in 9–day-old embryonated eggs via allantoic cavity. Allantoic fluid was harvested, and ND virus was confirmed by HA and HI tests.

Analysis of variance technique using Randomized Complete Block Design and Tukey test were applied to record statistical differences among the groups. Mortality rate was compared by Chi square test (Steel and Torie 1984).

RESULTS AND DISCUSSION

All the vaccines had clear appearance. Stability of these vaccines at 3 different temperatures and their viscosity were similar.

Humoral immune response of 4 types of vaccine and controls in broiler chicks is presented in Table 2. The mean titres did not differ between the 6 groups at day 1 and 7 (pre vaccination period) but the titres were higher (P<0.05) in all the vaccinated groups than controls from 1 week after vaccination onward. The titres did not differ between the 4 vaccine groups over 2 weeks post vaccination (P>0.05). The titres continued to rise in live-in-oil vaccine (half and full antigenic dose) groups up to day 28 post vaccination, and were higher (P<0.05) than aqueous live and formalin killed-in-oil on day 28 and 35 post vaccination.

Protection rate of broiler chicks after 14 days post vaccination was 90% with live in oil (full dose), 80% with live in oil (half dose) and aqueous live, 70% with killed in oil, and 10% for both the controls. Protection rate did not differ among 4 types of vaccine, however it was significantly

higher than the controls ($P<0.05$).

Water-in-oil emulsion to prepare oil adjuvanted vaccine has the advantage that it resists drying, is difficult to wash away, and is less corrosive. The most important finding in the present study was that the titres of the live-in-oil vaccines continued to rise over a longer period post vaccination as compared to killed-in-oil vaccine and aqueous live vaccine. According to Peleg *et al.* (1993) the advantage of live-in-oil vaccine might be due to escape of infective virus from the trapping oil environment and thus initiation of infection and replication of the virus in various tissues and organs of the body. This triggering of the immune system by live virus at a very early stage of immunization is assumed to be an effective event. It manifests itself later on when followed by boosters of the remaining virus which is killed in the oil and released from it continuously at a certain rate, behaving then as a killed-in-oil vaccine. By virtue of the higher level of immunity obtained with live-in-oil vaccine, the duration of the immunity would be extended, a feature so important in the broiler industry. It might be expected that a single dose of vaccine in one-day-old broilers would be sufficient for the entire period before marketing. The live-in-oil ND vaccines were found 30–50 times more effective in efficacy than either the same vaccines reconstituted in water or killed-in-oil vaccines when used in chicken of various ages and breeds (Peleg *et al.* 1993). In addition to its biological advantage, the method of preparation of live-in-oil vaccine saves the expensive space of cold storage and shipment necessary for conventional killed in oil vaccines. Roy and Venugopalan (1998) reported that live oil adjuvanted vaccine (LaSota strain) provided 100% protective immunity for 11 weeks in White Leghorn chickens, while non-adjuvanted vaccine fell below 100% protection by 6 weeks after vaccination. Similarly, Samina *et al.* (1999) observed that the live-in-oil ND+IBD vaccine gave better protection in broiler chicks against challenge with IBD virus and showed higher antibody level to ND virus as compared to killed in oil adjuvanted ND+IBD vaccine. The present study supports the earlier findings that live-oil adjuvanted vaccines provide better protection against challenge for a longer period of time as compared to aqueous live and killed oil adjuvanted vaccines. However the protection rate and titres of live-in-oil half and full antigen dose were similar in the present study, indicating that the half antigen dose is as reliable as the full one in addition to economy in the use of antigen.

Although the protection level did not differ significant among the groups inoculated with 4 types of vaccine, the protection rate was highest in live-in-oil with full antigen dose when challenged with the velogenic NDV having EID_{50} $10^{7.16}$. On the basis of humoral immune response the live-in-oil vaccines proved to be better than both the aqueous live commercial vaccine and the killed-in-oil vaccine.

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