

IMMUNE RESPONSE TO THE SIMULTANEOUS VACCINATION OF DAY-OLD CHICKENS WITH LIVE AND INACTIVATED OIL-BASED NEWCASTLE DISEASE VACCINES

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ABSTRACT

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The immune response, as measured by the haemagglutination-inhibition test, to the simultaneous administration of live Hitchner B1 and 2 commercially available, inactivated, oil-based, emulsified, Newcastle disease vaccines at day-old is described. The response was monitored from day-old to 18 weeks, when the birds were challenged with a standardized virulent virus. It was found that the haemagglutination-inhibition titre fell below $\log_2 5$ when the chicks were 10 weeks of age. Challenge at 18 weeks yielded a mortality rate of 25% in the groups receiving both live Hitchner B1 and an oil-based vaccine in comparison with 94% in the group receiving Hitchner B1 alone.

Simultaneous application of live and oil-based vaccines at day-old is conclusively insufficient to maintain adequate protection until 18 weeks and it is recommended that a booster vaccine be administered at 10 weeks.

INTRODUCTION

The economic threat of mortality and loss of production from Newcastle disease has resulted in continuous efforts being made to develop vaccination programmes that are effective, cheap and labour-saving, and that produce minimal side-effects.

To achieve these aims, considerable attention has been given in recent years to the use of inactivated, emulsified, oil-based vaccines. Such vaccines have proved very successful as boosters of immunity in previously vaccinated laying hens (Levy & Zakay-Rones, 1973; Zantiga 1974; Box & Furminger, 1975), but have yielded a very poor response when administered alone to day-old chickens (Zakay-Rones & Levy, 1973; Allen, 1974; Bennejean, Guittet, Picault, Bouquet, Devaux, Gaundry & Moreau, 1978).

A great improvement in immune response, as measured by the haemagglutination-inhibition (HI) test, has been reported when the oil-based vaccine is accompanied by a simultaneous administration of live Hitchner B1 vaccine at day-old (Warden, Furminger & Robertson, 1975). Robertson, Warden & Furminger (1976) found that the antibody titre remained at high levels for 16-20 weeks without further boosters. If boosted with another oil-based vaccine at 16 weeks, the geometric mean titre (GMT) at 69 weeks, without further boosters, was $\log_2 9.1$ (Robertson, Warden & Kerr, 1978). Bennejean *et al.* (1978) concluded that satisfactory protection after simultaneous live and oil-adjuvant vaccines administered at day-old lasted up to at least 11 weeks.

Since none of the available literature describes in detail the HI titre levels after 11 weeks, it was decided to investigate the variations in immunity, as measured by the HI test, on a weekly basis from day-old until 18 weeks. It was hoped thus to get an indication of the variation in immune status of a replacement flock after live Hitchner B1 and inactivated oil-based vaccines were administered simultaneously at day-old.

MATERIALS AND METHODS

Birds

Three hundred and forty Ross tint chicks were obtained from a commercial breeder. The parent flock had been vaccinated for Newcastle disease with a commercial La Sota eye-drop at 6 days, a La Sota aerosol at 18 days and a commercial, inactivated, oil-emulsion, Newcastle disease vaccine at 6 and 18 weeks. The maternal immunity of these chicks could thus be regarded as reasonably representative of those sold commercially.

Live Hitchner B1 vaccine

A commercial Hitchner B1⁽¹⁾ vaccine containing $10^{6.3}$ ELD₅₀ per dose was used.

Inactivated oil-emulsion vaccines

Two commercially available oil-emulsion vaccines (Newcadin⁽²⁾ and Broiler plus⁽³⁾) were used.

Grouping

Three hundred and forty birds were divided into 3 groups which received:

Group A: one dose of Hitchner B1 by the intra-ocular route at day-old.

Group B: one dose of Hitchner B1 by the intra-ocular route plus the manufacturer's recommended dose of Newcadin emulsion at day-old.

Group C: one dose of Hitchner B1 by the intra-ocular route plus the manufacturer's recommended dose of Broiler plus at day-old.

Serum samples

Ten birds from each group were bled from the brachial vein each week. The blood was allowed to coagulate and the serum removed.

Haemagglutination-inhibition test

A standardized microtitre HI test, using 4 haemagglutinating units and serial twofold dilutions of serum, was used.

The results were recorded after 60 min as the \log_2 of the reciprocal of the serum dilution, giving 50% inhibition of haemagglutination, as described by Coetzee (1980).

Challenge

All the birds were challenged at 18 weeks, using the velogenic "Steyn 79" isolate. A challenge dose of 10^3 ELD₅₀ per 0.2 ml was administered intratracheally, as described by Coetzee (1980). A group of 20 non-vaccinated SPF birds served as a negative control.

The birds were observed for 3 weeks after challenge.

RESULTS

The geometric mean titre (GMT) and the maximum and minimum levels obtained over the 18-week pre-challenge and the post-challenge levels are shown in Fig. 1. For the purposes of this trial, HI titres of 3 or below were regarded as negative and given the same basal value.

⁽¹⁾ Salsbury (S A) Veterinary (Pty) Ltd

⁽²⁾ Evans Medical

⁽³⁾ Philips-Duphar B.V.

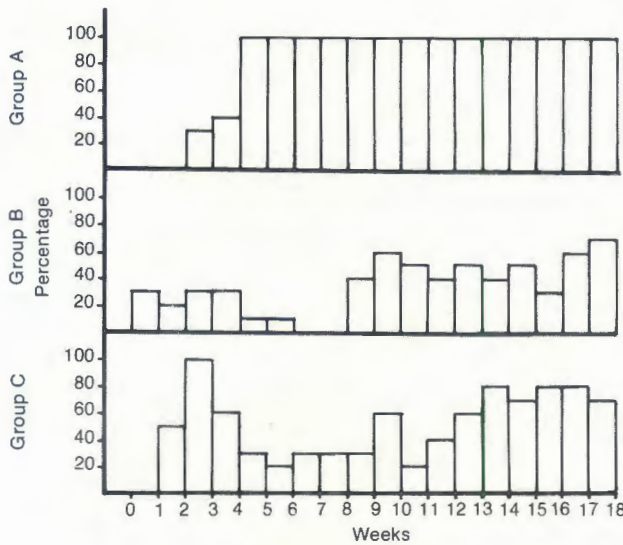


FIG. 1 The geometric mean haemagglutination-inhibition titres and the maxima and minima obtained in 3 groups of chickens subjected to different Newcastle disease vaccination programmes

The level of maternal antibodies was determined at day-old as 6,5.

Group A

The HI titres dropped from a GMT of 6,5 at day-old to below 3 by 3 weeks. Levels remained low for the duration of the trial.

Group B

The HI GMT dropped from 6,5 at day-old to 4,5 by 3 weeks, after which it rose to a maximum of 6,9 by 8 weeks followed by a decline to below 5 by 10 weeks. Levels remained below 5 for the duration of the pre-challenge period except at 16 weeks, when a rise to 5,3 was recorded.

Group C

From a GMT of 6,5 the titre dropped to below 3 by 3 weeks, followed by a rapid rise to above 5 from 6-11 weeks. A drop in titre to 3,4 was noted at 10 weeks, but was found to be statistically insignificant. Titres dropped below 5 between 11-12 weeks and thereafter remained low for the remainder of the trial period, except between 17-18 weeks, when a slight rise was recorded. With the exception of Weeks 7 and 8 in Group B, a variable proportion of the titres remained below $\log_2 5$ for the entire trial period (Fig. 2).

During Weeks 2-4 the percentage of birds showing HI titres below $\log_2 5$ in Groups B and C varied from 20-100%. After 10 weeks this percentage varied from 20-80%, but showed a gradual upward tendency with time.

The spread of HI titres in Groups B and C varied from 2-5 \log_2 units, with a majority of the values showing a 3-4 \log_2 unit spread. After challenge this variation was reduced to a difference of 1 \log_2 unit.

The results of the 18-week challenge are detailed in Table 1.

For the purpose of this trial, birds showing nervous symptoms were included as a portion of the total mortality, since such birds are unlikely to survive under commercial conditions. In addition, nervous symptoms were recorded as such only at the end of the challenge period. Birds showing nervous symptoms and succumbing before the expiry of the 3-weeks challenge period were recorded as mortalities.

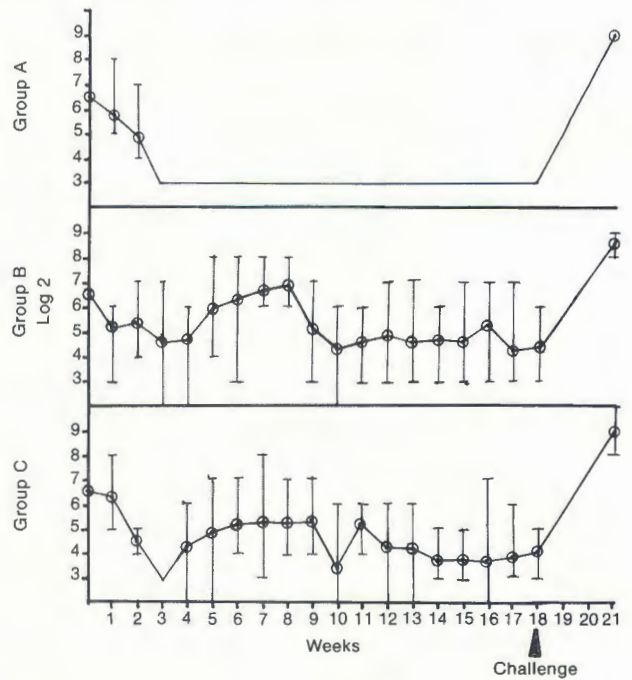


FIG. 2 Histogram of the percentage of birds in 3 groups of chickens subjected to different Newcastle disease vaccination programmes, showing haemagglutination-inhibition titres below $\log_2 5$

TABLE 1 Results of 3 groups of birds subjected to different vaccine programmes and challenged with a standardized virulent Newcastle disease virus strain at 18 weeks

	Group A	Group B	Group C	Unvaccinated Group
No. in trial	108	105	120	20
Mortality	100	17	24	20
Nervous symptoms	1	11	5	0
Total mortality	101	28	29	20
Total survivors	7	77	91	0
% mortality	94	27	24	100
% mortality due to nervous symptoms	1	39	17	0
% survival	6	73	76	0

Statistical analysis

A statistical analysis was performed by subtracting the observations of the control group from the individual observations in Groups B and C for each week. The transformed observations were then analysed by the non-parametric method of Kruskal-Wallis. Differences at the 5% level of significance between the different weeks were calculated by Dunn's method.

The results correlate closely with direct inspection of GMT titres, except that in Group B the rise in titre between 11-13 weeks and in Group C the drop in titre at 10 weeks were not significant. The rise in titre at 16 weeks in Group B was significant but inexplicable.

DISCUSSION

Phillips (1973) has stated that, since HI titres of $\log_2 5$ and higher will protect birds against mortality and that titres of $\log_2 7$ and higher will protect against a drop in egg production, the HI titre of rearing flocks should be at least 5 and that of in-lay flocks at least 7.

In this trial the HI GMT dropped below 5 by 10 weeks in Group B and between 11-12 weeks in Group C, after which the titres could be regarded as indicative of a subimmune status in a percentage of the birds.

The danger of "marginally vaccinated" or sub-immune flocks has been pointed out by Beard (1975), where such flocks act as symptomless amplifiers and shedders of virus and thus perpetuate the disease within the flock and provide a potential dangerous reservoir for other populations.

In addition, subimmunity is a cause for concern in so far as field cases seem to indicate that there is a higher incidence of nervous symptoms in subimmune birds. The reasons for this are not clear but may involve differential tropism within the challenge virus strain or the retardation of the pathogenesis for a sufficient period to allow the development of nervous symptoms. The pathogenesis of some other paramyxoviruses is known to include a hypersensitivity component (e.g. measles, canine distemper) and it is interesting to speculate that this is also the case in Newcastle disease.

The fact that challenge with a standard virulent virus at 18 weeks caused approximately 25% mortality in both Groups B and C, of which a variable proportion showed nervous symptoms, is a further indication of the sub-immune status of the birds at this age.

It would appear that birds receiving live Hitchner B1 and oil-emulsion vaccines simultaneously at day-old require a booster with either a live or inactivated vaccine at 10-11 weeks.

Such a booster would appear even more advisable if one takes the spread factor of the HI titres into account. For almost the entire period of this trial a percentage of the birds in both Groups B and C showed HI titres of below $\log_2 5$. These values were higher between Weeks 2-4 (20-100%) and after Week 10 (20-80%) and give an indication of the percentage of birds in each group possessing subimmune status. Further trials on the susceptibility to challenge are required to determine the degree of susceptibility between Weeks 2-4.

Just prior to challenge the percentage of birds showing HI titres of $\log_2 5$ and below was 70% in both Groups B and C. However, the mortality was only 27% in Group B and 24% in Group C. Such lack of complete correlation between HI titres and challenge raises the question of the usefulness of the HI test as a quantitative measure of immunity. The HI test, being a test for humoral immunity, ignores both local and cell-mediated responses, both of which make important contributions to protection (Zakay-Rones, Levy & Spira, 1971; 1972; Timms & Alexander, 1977; Spanoghe, Peeters, Cotlear, Devos & Viaene, 1977).

Although it is true that a low or negative HI titre may not be totally correlated with protective immunity, a high titre is highly correlated with protection (Beard, 1975; Phillips, 1973; Coetzee, 1980). Flocks monitored and maintained at high HI levels by booster vaccination when levels drop below a standard minimum are thus less at risk than those whose immune status is marginal or unknown. The HI test as a monitor of flock immune status thus remains a useful diagnostic aid.

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