NEWCASTLE DISEASE VACCINATION PROGRAMS FOR BROILERS, LAYERS AND MEAT BREEDER CHICKENS

Greg Underwood¹ and Clive Jackson²

¹BIOPROPERTIES Pty Ltd, 36 Charter St., Ringwood, VIC 3134
²Biological Technology Transfer Pty Ltd, 2 Victory Avenue, Camden, NSW 2570

Introduction

All Australian states currently (or will shortly in 2005), require commercial poultry to follow mandatory vaccination programs as described in a set of Standard Operating Procedures (SOPs) developed by the Newcastle Disease (ND) Management Group (NDMG) and endorsed by the respective state government authorities. Those states that previously adopted the SOPs in 2003 allowed a transition period for ND vaccination during which time live ND V4 vaccination could be administered to existing flocks prior to the use of a live plus inactivated (killed) ND vaccination program for new layer and breeder flocks.

During the transition period satisfactory serology results were achieved with spray vaccination of day-old broiler chickens, where it had been shown that maternal antibody (Mab) was only in the order of 2³ at the time (Wells, 2002). Wells went further to suggest that as antibody (Ab) prevents disease, but not infection, by ‘seeding’ a flock with day-old vaccination, the vaccine virus would replicate when Mab levels had subsided below a certain permissive threshold in the flock. It was found that there was little difference in the seroconversion profile between flocks vaccinated at 1-day of age by spray compared to the control flocks vaccinated at 16 days of age, well after Mab had diminished below detectable levels at 12 days of age. Similar findings were reported after the introduction of ND vaccination in Victoria (Rubite, 2003). Following the introduction of hyperimmunisation (live plus inactivated vaccination) of meat breeder flocks, broiler Mab levels have been boosted as high as 2⁷ (S. Rubite Pers. Comm.). In addition, more recent reports indicate that the government SOP serology standards are not always being achieved, particularly in broilers, following vaccination according to the recommended procedures (Underwood et al 2004; Arzey 2005).

This paper aims to review the effect of Mab on the development of an active serum Ab response, highlight some concerns with the efficacy of the ND vaccination programme currently adopted by the broiler industry, and to suggest ways to meet the current national requirements more consistently which may include variation from the programs currently prescribed in the SOPs.

Development of Vaxsafe® ND Vaccine (living)

Newcastle disease (ND) virus, strain V4, was first isolated at the Queensland Veterinary Research Institute in 1966 (Simmons 1967). A low passage isolate was transferred to CSIRO in 1966 and stored in liquid nitrogen. A vial of the original isolate was supplied to BIOPROPERTIES Pty Ltd (BPL) in 2003, and subsequently passaged in SPF chickens during the development of a seed lot. BPL has since completed an extensive testing programme in conjunction with AAHL to develop and register (approved February 2005) a commercial vaccine, Vaxsafe® ND Vaccine (living), as closely related to the original V4 isolate as possible.

Over the past two years, BPL has undertaken a series of laboratory and field studies on the safety and efficacy of Vaxsafe® ND Vaccine. These studies used the existing registered live ND V4 vaccine as a
benchmark in many of the trials. Whilst the trials were designed toward satisfying regulatory requirements, they also touched on many of the variables that can impact on the success or failure of live ND V4 vaccine relative to the NDMG SOP requirements. In addition, BPL has undertaken field trials in layer pullets and meat breeding chickens to extend the claims for the safety and efficacy of Vaxsafe® ND Vaccine.

**Effect of Mab on the development of an active serum Ab response**

During the development of Vaxsafe® ND Vaccine, a series of laboratory efficacy studies were conducted in Mab negative and positive chickens to assess the immunogenicity of the vaccine. When Mab negative chickens were vaccinated at 14-days of age (doa), active Ab was induced that exceeded $2^4$ haemagglutination inhibition (HI) units within 14 days after vaccination (Figure 1). However, when Mab positive commercial broiler chickens were vaccinated in the presence of high levels ($\geq 2^3$ HI units) of Mab, the active Ab response was significantly suppressed as described in the following three experimental reports.

**Experiment 1** was carried out using 1-doa broiler chickens with an average of $2^{4.6}$ HI units of MAb. Age/Mab-matched groups were vaccinated at 1, 7, 12, and 17-doa (Mab levels were $2^{4.6}, 2^{4.5}, 2^{3.8}$ and $2^{2.2}$ HI units, respectively). The group vaccinated at 1-doa was re-vaccinated at 17-doa. It was found that chickens vaccinated at 7-doa exceeded the threshold of $2^3$ HI units before chickens vaccinated at 12-doa, and the 7-doa-vaccinated group had numerically higher (p>0.05) titres compared to chickens vaccinated at 12-doa at each subsequent sample interval through to 42-doa (Figure 2). Secondly, at 17-doa, Mab had declined below $2^3$ HI units, and the group vaccinated at this time developed a significant (p<0.05) Ab response by 28-doa. Additionally, the Ab level at 28-doa was significantly higher in the group vaccinated at 17-doa compared to the groups vaccinated at 7 or 12-doa. Interestingly, the group vaccinated at 1-doa had an Ab level that was not significantly different (p>0.05) from the unvaccinated group at 17-doa, however following vaccination of the two groups, the Ab response was significantly suppressed compared to the naive age/Mab-matched group (Figure 3). Finally, the mean Ab titre of chickens vaccinated at 12-doa was significantly higher (p>0.05) than an age/Mab-matched group vaccinated with an existing registered live ND V4 vaccine (Figure 4).

**Experiment 2** involved vaccination of 1-doa broiler chickens (mean Mab of $2^{6.15}$ HI units) and the Ab level was measured at weekly intervals thereafter to 35 doa. Unvaccinated ‘in-contact’ control chicks were housed together with vaccinates. It was found that the Ab response in vaccinates was suppressed and remained at approximately $2^3$ HI units throughout the study (Figure 5). There was no difference between the serum Ab response in vaccinates and in-contact controls.

**Experiment 3** was carried out under field conditions and involved over 2.2 million broilers. All birds were vaccinated at 10 days of age in accord with the NDMG recommended SOPs. It was found that the average Mab level on the day of vaccination was $2^{2.1}$ HI units, and at 33-35 days of age (first pick-up) the average Ab titre was $2^{4.5}$ HI units in flocks vaccinated with Vaxsafe® ND Vaccine, compared to $2^{3.5}$ HI units in flocks vaccinated with another registered live ND V4 vaccine (Figure 6). In addition, over 90% of broiler flocks exceeded protective HI antibody levels at 35 doa compared to only 57% for the control product. Additionally, all production parameters recorded were not significantly different (p>0.05) in flocks given Vaxsafe® ND Vaccine compared to those of flocks given the control product.

**Vaxsafe® ND Vaccine as a live primer in a hyperimmunisation programme**

To evaluate the priming effect of Vaxsafe® ND Vaccine prior to administration of an inactivated vaccine, six meat breeder flocks, each of 30,000 to 46,000 birds, were vaccinated with one dose of either Vaxsafe® ND or another live ND V4 vaccine at 3 to 4 weeks of age via the drinking water. On most farms only one
shed of birds was vaccinated with the other ND V4 vaccine. The birds were subsequently injected with an inactivated ND V4 vaccine at 16-18 weeks of age. Serum samples were collected and tested for ND HI antibody responses at pre- and post-live vaccination and pre- and post-inactivated vaccine administration. Primary active antibody response to both live vaccines was similar (2^4 to 2^7 at 6-8 weeks through to 14–18 week of age). Following injection of inactivated vaccine, titres of 2^9 to 2^11 were found in the two flocks tested at 18-22 weeks of age. However, by 29 weeks of age, the active antibody titres of two flocks tested at that age had fallen to between 2^7 and 2^8.

**Assessment of alternative live (strain V4) ND vaccination programmes**

**Meat Breeders**

As mentioned previously, during the transitional phase of the ND eradication program long-lived birds (greater than 18 weeks of age) were required to be vaccinated with live vaccine only. One organisation in Queensland compared the Ab response profile after vaccination with ND V4 live (Fort Dodge Animal Health) at 3 weeks of age (woa), 18 woa, and every 6 weeks thereafter against the Ab response in flocks on a hyperimmunisation programme consisting of a live ND V4 at 3 woa followed by an inactivated at 16 woa (Figure 7). It was found that the peak Ab response after hyperimmunisation was approximately 10^8 at 35 woa, falling to approximately 2^6.5 by 54 woa. The peak Ab response on a continuous live programme (every 6 weeks) was approximately 2^6.5 between 35-42 woa. The mean flock titres exceeded 2^5 at all sample intervals. It is too early to conclude the Ab profile of the flocks on a repeat live programme (every 8 weeks) however early indications are that the peak Ab response is equivalent to the 6-weekly programme (approximately 2^6.5) and titres exceed 2^5 through to 35 woa. Overall, the repeat live programme induced a peak HI antibody response that was significantly lower than the hyperimmunisation programme, however serum Ab titres remained above 2^5.0 through to 54 weeks of age.

**Commercial layers**

During the development of Vaxsafe® ND Vaccine, it was apparent that re-vaccination could further boost serum antibody levels (data not shown). Therefore, BPL participated in a field trial following approval by the Queensland state government, to assess the effect of ‘repeat live’ vaccination in commercial layer flocks. One flock was vaccinated twice in rearing (9 and 12 weeks of age) with Vaxsafe® ND, before separation at 16 week into two different housing systems (Barn Lay and conventional cages). These flocks were re-vaccinated at 8-10 week intervals thereafter (18, 26, 34, 42, 50 wks). A third flock, reared separately, was vaccinated with the existing registered live ND vaccine at 9 weeks, followed by an inactivated vaccine (hyperimmunised) at 12 weeks of age. This flock was transferred into cages at 16 weeks of age and not vaccinated further during production. Each flock was bled on the day of vaccination and again 3 weeks later to strategically monitor the ‘peaks and troughs’ of Ab titre throughout the life of the flock.

The hyperimmunised flock showed a rapid rise in ND HI titre after vaccination, peaking at log 2^7.6 at 18 weeks of age (Figure 8). Antibody levels fell thereafter and were below the SOP standard at 34 weeks of age. In contrast, the flocks on the repeat live program only achieved a pre-lay titre of about 2^4 and maintained a titre between 2^4 and 2^5 until 42 weeks. From that age, the birds in cages on both the repeat live program and the hyperimmunisation programme showed a further increase in HI titre to 2^5.5. In contrast, the HI titre of the barn lay birds on the repeat live program maintained a mean titre of between 2^4 and 2^4.5 until 50 weeks of age.
Discussion and recommendations

Vaxsafe® ND Vaccine was registered by the APVMA for use in broilers in February 2005. This vaccine, developed from a seed stored since 1966, underwent extensive laboratory investigation and is as close to the original isolate in genetic and biological character as possible. During the development of Vaxsafe® ND Vaccine in pre-registration trials, the conditions for safety and efficacy for product registration were met, however it was noted that high levels of maternally-derived Ab suppressed the active Ab response following vaccination.

The influence of Mab on ND V4 replication was reported by Westbury et al (1984). In that study it was found that Mab levels $\geq 2^{2.6}$ significantly suppressed the Ab response following vaccination. Those findings are consistent with the present report where Mab levels > $2^3$ consistently suppressed Ab response, but Mab levels < $2^{2.3}$ had no deleterious effect. The threshold for Mab interference has been shown to vary between vaccines of different pathotype. For example, Westbury et al (1984) showed that V4 replication was suppressed by lower Mab levels compared to Hitchner B1 virus, which was in turn suppressed at lower Mab levels compared to La Sota virus. In the present report, Vaxsafe® ND Vaccine induced an active Ab response earlier than the existing registered ND V4 vaccine when each was administered to age- and Mab-matched hatch mates. It is possible therefore, that the difference in active Ab response observed under field conditions between the two products (1.0 Log$_2$ at 33-35 doa), was due to a varying pathogenicity of the two local vaccine products.

One of the most significant changes in the conditions of broiler vaccination since ND vaccination was introduced is the ND Mab status at hatch, due to the adoption of hyperimmunisation strategies in meat breeder flocks. Prior to the introduction of hyperimmunisation programmes, meat breeders were largely free of endemic field virus and in turn had little if any ND Ab. Wells (2002) described mean broiler Mab levels of $2^3$ when broiler vaccination was first introduced in NSW, and in turn, found that vaccination at 1-day old could induce satisfactory levels of active antibody response. As hyperimmunisation was adopted, the industry moved from placing broilers with little or no Mab, to a background of high Mab levels, at times exceeding $2^6$. It has recently been reported that vaccination responses are poor, and at times fail to meet the specified SOP targets under the current vaccination regimes. This is particularly a problem where flocks have tested Ab negative at thinning, but when re-tested 14 days later, have titres exceeding $2^7$, suggesting exposure to wild-type virus on the vehicles and equipment that move from farm-to-farm during the pick-up procedure.

At present, both V4 vaccines are being administered at day-old in the hatchery as an off-label practice. This practice departs from the preferred national SOP vaccination procedures. In the present report it was demonstrated that vaccination in the face of high Mab levels ($> 2^5$) can still induce an active Ab response following vaccination, however it does not have the same kinetic pattern, or reach the same amplitude, as the vaccination of naive chickens. One of the concerns therefore, particularly if broilers are vaccinated at 1-doa in the face of high levels of Mab, is that vaccine virus replication will be suppressed. Whilst, theoretically the shed will be seeded with vaccine virus (Wells, 2002), active replication will be delayed until the Mab level has declined below a certain threshold, with the exact threshold dependent upon the pathogenicity of the virus. Any strategy that separates the time of administration from the time of an active immune response leaves open the opportunity for wild-type virus entry/replication before vaccine virus replication. Such a strategy could theoretically therefore, be selecting for evolution of virulence of wild-type viruses. Wild type viruses with higher intrinsic pathogenicity will replicate in the presence of higher levels of Mab while vaccine viruses will not. This is of particular concern on large broiler complexes or where litter is re-used, as early exposure is more likely. While ND has rarely been reported
internationally to evolve toward virulence, this pathway has been reported within Australia and appears to be an ongoing feature in the local industry. Therefore strategies to avoid or reduce selection pressure on field viruses should be of paramount importance. There are several ways to avoid this theoretical selection pressure:

(a) Firstly, consistent with earlier reports (Westbury et al 1984), vaccination with ND V4 should be delayed until the ND Mab level has fallen below $2^{2.6}$. Given the current meat breeder hyperimmunisation programme, vaccination of broiler flocks may need to be delayed until 12-17 days of age (or later vaccination for younger donor flocks). For example, flocks with an average Mab of $2^7$ should NOT be vaccinated before 14 days (allowing an average Mab half-life of 3.5 days for broilers). Indeed this approach is preferable on relatively isolated sites, where early exposure to wild-type virus is less likely (single age, single batch litter), and should ensure the development of a solid active Ab response within 2 weeks and before thinning.

(b) A second strategy would be to amend the current SOPs to allow meat breeder flocks to be re-vaccinated throughout lay with live vaccine instead of hyperimmunisation with inactivated vaccine. This would reduce the Mab levels transferred to broilers and therefore enhance the (serological) response to early vaccination. It has been demonstrated that in meat breeders active Ab levels can be maintained at or above $2^5$, and thereby not compromise the health status of the flock or target titres specified in the existing SOPs. Indeed there is the theoretical benefit of regular induction of local immunity at the epithelial surface with a repeat live programme that will provide a greater suppression of wild-type virus replication if the flock were exposed, compared to inactivated vaccination which induces poor local immunity.

(c) The third alternative is broiler re-vaccination. The lack of Ab response after vaccination of broilers with high Mab levels is not a local phenomenon. In the absence of adequate humoral antibody responses from day-old vaccination, dependence on local antibody and cell-mediated immunity for life-long protection in broilers is contrary to accepted recommendations (MAFF 1974). These programs, and indeed virtually all other international territories where ND vaccination is routine, normally recommend a second vaccination at about 18-21 days of age to stimulate high levels of neutralising antibody. At this time, revaccination of broilers would be unpalatable for the Australian industry due to the added cost involved.

In summary, the NDMG should consider whether the current recommendations in the SOPs meet the overall objectives of the eradication program, and further, if the off-label practice routinely used by the broiler industry is further compromising the success of the programme. The control of endemic virulent and precursor viruses in Australia following the 1998-2002 outbreaks is partly dependent on the capability of the ND V4 vaccine out-competing those viruses. The delayed and lower humoral response following vaccination with ND V4 observed in the present studies, suggests the possibility that field viruses more virulent than V4 may have a greater opportunity for replication in these flocks if they are able to replicate in the presence of higher levels of Mab than the V4-strain vaccine. Although there is evidence that ND V4 vaccine will reduce the excretion of precursor viruses, the level and frequency of challenge from such viruses is not well understood. Consequently the continued application of sub-optimal programs could well encourage the evolution of further precursor or virulent endemic strains.

In the interim, a number of alternate programs could be considered including:
- Delay primary vaccination until mean flock ND Mab levels fall below $2^3$.
- Reduce ND Mab levels in broiler progeny by limiting the administration of inactivated ND vaccines and substituting a repeat live program.

If hyperimmunisation of parents and day-old spray vaccination of broilers is to continue, the Australian industry may be faced with the prospect of broiler re-vaccination at about 18-21 days of age, to achieve the NDMG-endorsed target titres consistently.
References


Groves, P. (2003) Serological response of Meat Breeders to vaccination with live and killed Newcastle Disease Vaccines Proc. February Scientific Meeting of AVPA, University of Sydney, pg26


Acknowledgements

The authors wish to thank Dr. Rod Jenner for providing data on the vaccination of meat breeder flocks with live ND V4 vaccine only. They also wish to thank Ms. Mary Harney & Kirsten Gooding for assistance with the preparation of this manuscript.
Figure 1 - ND Antibody Response of Mab Negative Broilers vaccinated with or in-contact with chickens vaccinated with Vaxsafe® ND Vaccine

Figure 2 - ND Antibody Response of Mab positive broilers following vaccination with Vaxsafe® ND Vaccine at 7, 12 or 17 days of age

Figure 3 – ND antibody response in broilers after vaccination with Vaxsafe® ND Vaccine at 17 days of age only, or at 1 and 17 days of age (repeat vaccination).

Figure 4 – Comparison of the ND antibody response in paired broilers given either Vaxsafe® ND Vaccine or an existing registered ND V4 live vaccine at 12 days of age.
**Figure 5** – ND antibody response following vaccination of Mab positive 1-day-old broiler chickens with Vaxsafe® ND Vaccine together with the ND antibody response of in-contact chickens.

**Figure 6** – Comparison of the ND antibody response of Mab positive broiler chickens given Vaxsafe® ND Vaccine and another registered ND V4 vaccine (Vacc A) under field conditions.

Notes: Vaxsafe® ND and Vacc A administered (oral) to commercial maternal antibody positive broiler chickens at 10 days of age. The field trial involved 2.2 million broilers across 2 states of Australia. Columns with different superscript are significantly (p<0.05) different. Difference in mean serum antibody level between Vaxsafe® ND and Vacc A at 33-35 days of age was 1.0 Log 2 (p=0.06). 91% of flocks were positive at 33-35 days after vaccination with Vaxsafe® ND (57% positive after vaccination with the control).

**Figure 7** – ND Ab response in meat breeders: comparison of the Ab profile between hyperimmunisation and two different repeat live programmes.

Notes: L3K16 = live ND V4 at 3 weeks followed by inactivated at 16 weeks of age (woa); L3L18L+6 = live ND V4 vaccine at 3, 18 and every 6 weeks thereafter; L3L18L+8 = live ND V4 at 3, 18 and every 8 weeks thereafter (note: this group is in progress with the oldest sheds 38 woa). X-axis is the mean blood test age (note: actual test age within ±3 weeks of the mean). Plotted values represent the mean HI titre ±SEM. Broken line at 2.5 HI units indicates minimum acceptable HI titre according to SOP requirements.

**Figure 8** – ND HI Ab response of layer chickens vaccinated every 8-10 weeks with Vaxsafe® ND Vaccine, or with a hyperimmunisation (L+ K) program.

Notes: L = live V4 vaccine; K = inactivated (killed) vaccine; Barn = barn-lay housing (floor reared); cages = caged housing (wire floors). L+ K = hyperimmunisation with live (existing registered V4 vaccine) at 8 weeks of age followed by inactivated at 12 weeks of age. Live vaccine only groups vaccinated with Vaxsafe® ND three times in rearing, followed by re-vaccination at 8-10 weekly intervals after 16 weeks of age. Each point represents the flock mean (16 samples) HI titre (Log2) ±SEM.