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*Full Length Research Paper*

# Safety and efficacy studies of Newcastle Disease vaccines in very young African local ecotype chicks and in commercial pullets

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Two Good Clinical Practice studies are described. Firstly, the safety and efficacy of live, attenuated LaSota and I-2 Newcastle Disease (ND) vaccines and inactivated, adjuvanted ITA-NEW ND vaccine were evaluated in eight-day old local ecotype chicks. For all vaccines safety and efficacy were satisfactory and serological titres exceeded the putative protective level of  $\geq 2^3$  before 14 days post-vaccination. The vaccinated groups displayed no significant differences. The data suggest that these vaccines are effective in very young village poultry. Secondly, in 35 day-old ISA-Brown pullets, MSD a 10x field dose formulation of Clone 30 vaccine, was compared to I-2 after a heat-stress test approximating to local conditions of delivery and use (24h, 32.3°C, in the dark). By 14 days post-vaccination, the heated MSD vaccine and heated I-2 titres exceeded  $2^3$  but the response of the heated MSD group was significantly higher than the heated I-2 group. Non-heated MSD induced a very rapid and higher response than those induced by the heated vaccines, as by 7 days post-vaccination, a  $2^3$  titre was reached and exceeded (GMT 4.0). The 10x normal field dose approach to conferring thermotolerance to live vaccines appears to be a simple, cheap and pragmatic method for use in hot climates.

**Keywords:** Newcastle Disease vaccines, agricultural development, thermotolerance, LaSota, I-2, ITA-NEW, Clone 30.

## INTRODUCTION

Approximately 70% of Africa's chickens, comprising some 1.5 billion birds (FAO 2007), are multi-age village or backyard chickens of local ecotypes with unknown genetics (Sector 4, FAO 2007). These birds act as food

security and also may provide cash income for poor rural households especially women, so enabling the purchase of household and other extra items such as children's school accessories (Alexander et al., 2004; Perry et al., 2002; Peters et al., 2012a, b;). The most serious disease threat to these village chickens is Newcastle Disease (ND). This is due to its endemicity and mortality rates that can approach 100%. In addition to its severe impact on

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the rural poor, ND impacts national economies due to trade restrictions and embargoes (FAO/IAEA 2004, Alexander and Senne, 2008; GALVmed, PANVAC and IIAM Conference, 2009). ND can be controlled and prevented by routine vaccination using live attenuated or inactivated vaccines (OIE, 2013) but in the developing world there is insufficient routine husbandry or disease control for village chickens (Alexander and Senne, 2008; Tirunesh and Gerima, 2016). In reality, livestock health is often overlooked despite it being a powerful socio-economic driver (<http://www.onehealthinitiative.com>; One Health Initiative, The World Bank, 2010).

GALVmed (Global Alliance for Livestock Veterinary Medicines; [www.galvmed.org](http://www.galvmed.org)) is a not-for-profit organisation which aims to improve socio-economic conditions by enhancing the availability of livestock medicines in markets that are unattractive to the global animal health industry. An important component of this aim is to develop technical partnerships with regional veterinary laboratories and here ND was identified as a priority disease for GALVmed to work on. This was because despite several vaccines being available there were few data obtained under controlled conditions suitable for registration dossiers in two vital areas: vaccine safety and efficacy in very young backyard chicks and vaccine thermotolerance which is a major need for effective ND and other veterinary vaccines in hot climates to reduce dependence upon a 'cold chain'.

One ND vaccine that has been employed in the field uses the I-2 strain which is naturally attenuated (sequencing indicated its avirulent status:  $^{112}\text{R-K-Q-G-R-}\downarrow\text{-L-I-G}^{119}$ ; Wambura et al. 2007) and is also naturally thermotolerant (Australian Centre for International Agricultural Research, ACIAR; 2002). The I-2 master seed was made freely available to developing countries by ACIAR but it was not formally registered in these countries. GALVmed was asked to take pro-poor action by obtaining Good Clinical Practice (GCP) standard data (EC Europa 1991, VICH 2000) and preparing an I-2 Registration Dossier that would be placed into AU-PANVAC ownership. For the thermotolerance investigations, some commercial ND vaccines were available but their properties under controlled conditions suitable for registration were unclear. However, a 10x formulation of Clone 30 ND vaccine did become available for study. So two completely separate studies to evaluate available vaccine candidates were implemented to GCP standard, one for their safety and efficacy in very young chicks and the other to evaluate some vaccines for their thermotolerance.

## MATERIALS AND METHODS

### Studies

Two separate GCP standard studies were conducted with the collaborating laboratories receiving appropriate training from GALVmed before commencement. The first

study used 8-day old backyard chicks and evaluated the ability of three ND vaccine formulations to induce protective antibodies. The second study used 35 day commercial birds and was carried out to assess protection against challenge from two live, attenuated vaccines that had been pre-heated (see below) to simulate conditions of transport and field use, before reconstitution, titration and vaccination. The parallel group design flows for the studies are shown in Figure 1A and 1B.

### Birds

The first study was carried out at Sokoine University of Agriculture (SUA), Sokoine, Tanzania and used backyard (i.e. unknown genetics) male and female day-old chicks sourced from a local commercial hatchery's minimum disease layer flock that was negative for ND virus antibody. For study 2 carried out at the Kenya Veterinary Vaccine Production Institute (KEVEVAPI), Nairobi, Kenya, new-born ISA-Brown female chicks (Sigma Ltd, Kenya) were reared in an on-site Brooder House for 35 days to allow decay of any maternal ND antibodies. All birds were given feed and water *ad libitum* throughout the studies.

### Randomisation

After confirmation of seronegativity (i.e. undetectable ND antibodies; see Haemagglutination Inhibition below), birds in both studies were fitted with numbered leg rings (Roxan Developments Ltd, Selkirk, Scotland). The rings had earlier been randomly allocated to treatments on a blinded basis. For study 1, seven day old chicks were randomly allocated into four treatment groups of 50 per group. For study 2, 33 day old pullets were randomly allocated to three treatment groups of 50 birds per group. All birds were observed frequently throughout both studies for general health (EC Europa, 1991). More detailed clinical examinations were carried out around the time of vaccination (Figure 1A and 1B).

### Vaccines, vaccination and heat treatment

The vaccines in study 1 (see Table) were a commercial, locally available, attenuated LaSota vaccine (V1.1; Shafit Labs Ltd, Israel) a locally produced I-2 vaccine originally from ACIAR master seed (V1.2; Central Veterinary Laboratory, CVL, Temeke, Tanzania) and an adjuvanted, inactivated, thermotolerant vaccine (V1.3; ITA-NEW; Laprovet, France).

In study 2, the commercial ND Clone 30 vaccine supplied at 10 times the registered dose (MSD vaccine, MSD, The Netherlands) was used as a control (V2.1; see Table)

Figure 1 A. Study 1 parallel flow study design (N.B. post-treatment days are grouped by daily activity; D, Study Day).

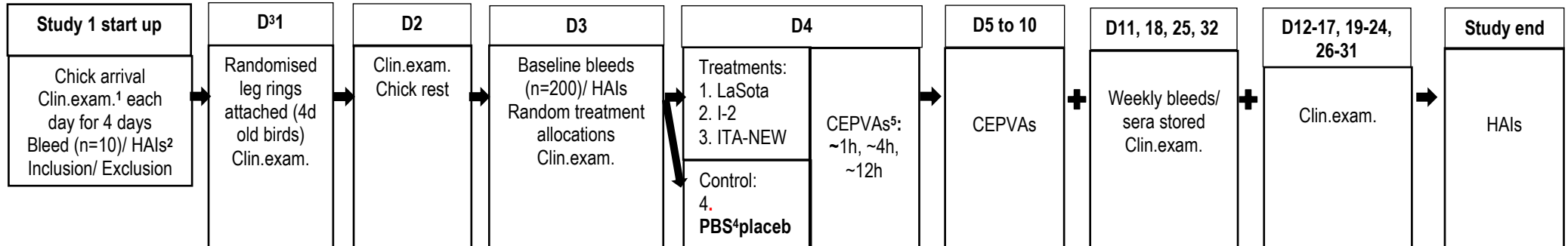
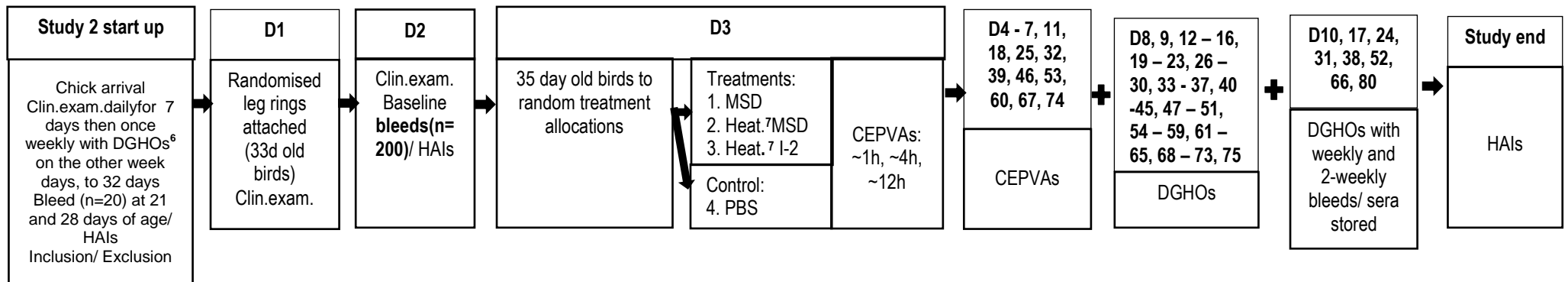


Figure 1B. Study 2 parallel flow study design (N.B. post-treatment days are grouped by daily activity; D, Study Day).



1, Clinical examination; 2, Haemagglutination Inhibition titres; 3, Study Day; 4, Phosphate buffered saline, pH 7.4; 5, Clinical examinations post-vaccination; 6, Daily General Health Observations; 7, Heated (24h at 32.3°C in the dark).

and when pre-heated (V2.2) as below. Another I-2 vaccine also from ACIAR master seed (KEVEVAPI) was used when pre-heated (V2.3) as below. All vaccines

were titrated in duplicate before use and were administered within two hours of reconstitution. In study 1 all vaccines were used without prior heat treatment. In

study 2 some MSD vials and all the I-2 vials were heat-stressed in the dark for 24h at 32.3°C (Avt temperature logger; Comark, England) before reconstitution and use.

**Table.** Details of vaccines used in the two studies.

Study	Site	Vaccine <sup>1</sup>	Vaccine identity	Vaccine manufacturer	Number of birds	Age at vaccination (days)	Volume and route of vaccination <sup>1</sup>	Active component per vial <sup>2</sup>
1	SUA	V1.1	LaSota strain	Shafit Labs, Israel	50	8	20µl IO	≥10 <sup>8</sup> EID <sub>50</sub>
		V1.2	I-2	CVL, Temeke, Tanzania	50	8	20µl IO	≥10 <sup>8</sup> EID <sub>50</sub>
		V1.3	ITA-NEW	Laprovect, France	50	8	200µl IM	≥10 <sup>8</sup> EID <sub>50</sub>
		V1.4	PBS control	PBS	50	8	20µl IO	Not applicable
2	KEVAVAP I	V2.1	Non-heated Clone 30	MSD	50	35	50µl IO	≥10 <sup>9</sup> EID <sub>50</sub>
		V2.2	Heated <sup>3</sup> Clone 30	MSD	50	35	50µl IO	10 <sup>6</sup> EID <sub>50</sub>
		V2.3	Heated <sup>3</sup> I-2	KEVAVAPI	50	35	50µl IO	10 <sup>6</sup> EID <sub>50</sub>
		V2.4	PBS control	KEVAVAPI	50	35	50µl IO	NA

1 = IO, intra-ocular; IM, intra-muscular

2 = vaccines were reconstituted and administered according to the manufacturer's instructions. They were titrated before administration.

3 = 24h at 32.3°C in the dark before reconstitution and titration; titre drops of 3 log and 2 log for MSD and I-2 respectively to 10<sup>6</sup> EID<sub>50</sub> per vial for both were noted.

## Sampling

Before study 1 started, 10 four day old chicks were randomly selected, sacrificed and their blood sampled to confirm naïve serological status to NDV. Study birds were then bled (jugular vein) for baseline values on study day 3, i.e. at randomisation and 24h before treatment, and then post-treatment bled weekly to 28 days (Figure 1A). In study 2, when the birds were 21 days old, 20 birds were randomly selected and bled from the ulnar vein to confirm naïve serological status to ND virus. This was repeated a week later with 20 different birds. Finally all birds were bled for baseline values 24h after randomisation (day 2; i.e. 24h before treatment), then after treatment bled weekly from weeks 1 to 5, then bled at two-weekly intervals from 7 weeks to 11 weeks (Figure 1B).

## Haemagglutination Inhibition (HAI) titres

In both studies, post-treatment sera were stored at -20°C and analyses carried out only after the end of the in-life phases to reduce variation. NDV antibody titres were determined by haemagglutination inhibition (HAI) in study 1 according to Allan & Gough (1974) and in study 2 according to ACIAR (2002). Both studies used 25µl containing 4 haemagglutinating units of ND strain V4 antigen. Fresh red blood cells were collected from NDV-free birds, pooled and

thoroughly washed as described in the methods cited above. HAI tests always incorporated External Quality Assurance, EQA, samples.

## Statistical analysis

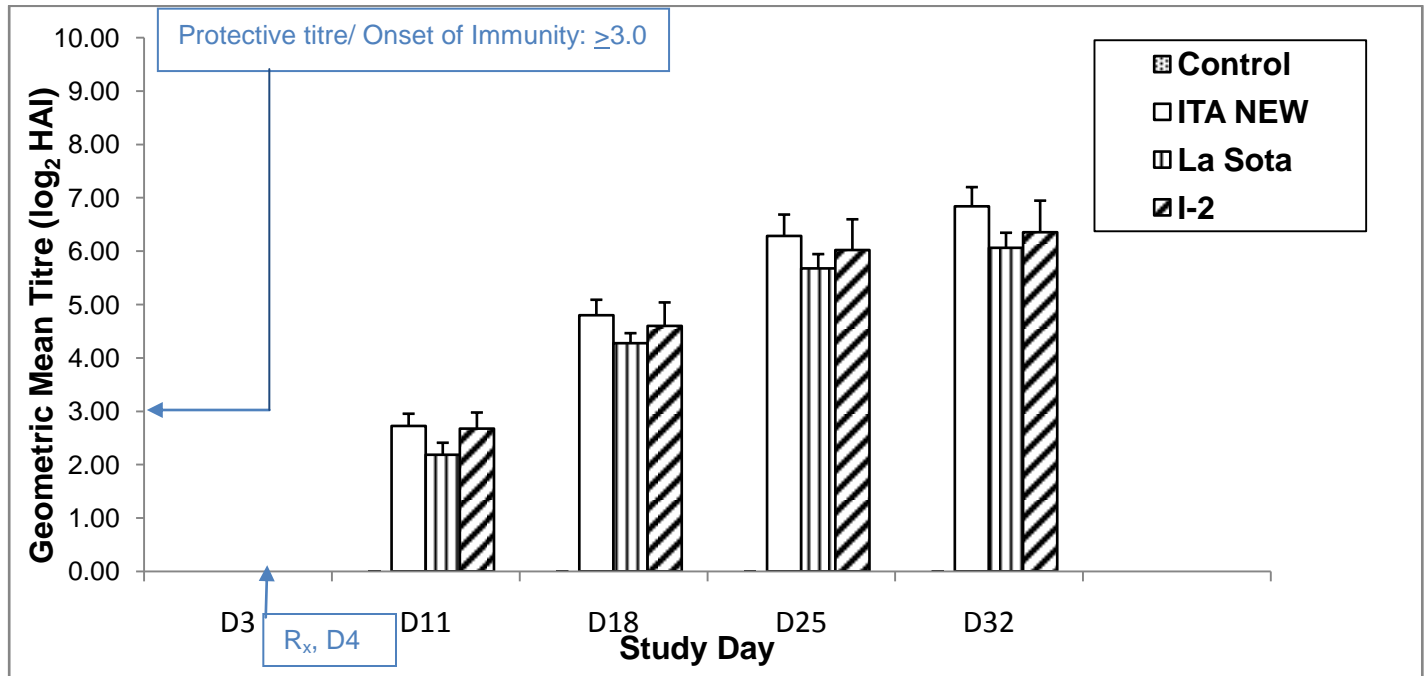
In study 1, Geometric Mean Titres (GMTs; log<sub>2</sub>), were produced from the raw HAI data and analysed using one-way ANOVA. Differences among treatment means were compared using Fisher's Protected Least Significant Difference test, PLSD, at a 5% probability (StatView®). In study 2, due to the study design, GMTs for the treatment groups and for individual bird titres were compared for differences using Tukey's pair-wise comparison test. Differences in mean values were considered statistically significant at the 5% level.

## RESULTS AND DISCUSSION

In both studies all pre-study and baseline sera samples were negative for NDV antibodies. There were no mortalities or local or systemic reactions following vaccination in any vaccinated groups indicating that all vaccines were safe when used under these conditions.

Herd immunity, HI, (Diekmann and Heesterbeek 2000) is achieved if a fraction of birds with a high level of protection is equal to or greater than a certain critical

**Figure 2A.** Study 1 HAI GMTs Induced by the Three ND Vaccines in 8 day old chicks ( $\pm$  standard error; D, Study Day; R<sub>x</sub>, treatment).



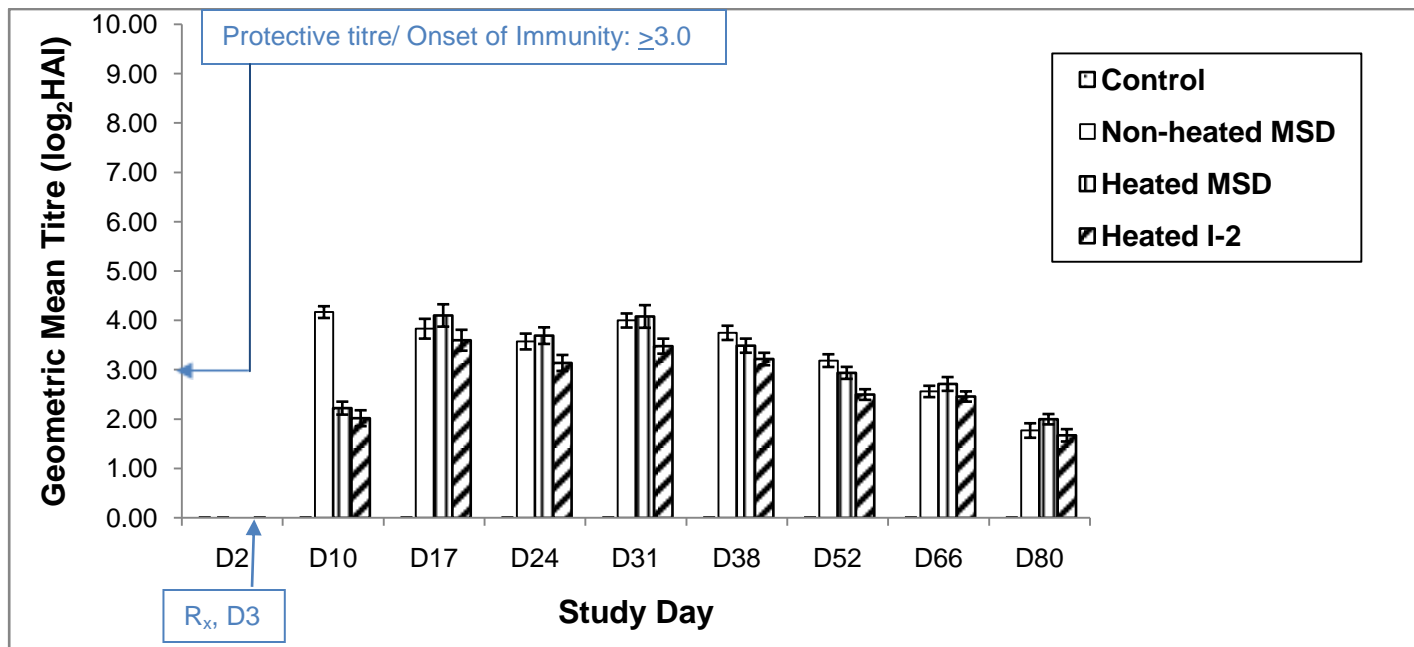
fraction of potential host birds. For ND, a high level of protection in the HAI test is defined as a  $\log_2$  titre  $\geq 2^3$  or a GMT  $\geq 3.0$  (ACIAR, 2002, Allan and Gough, 1974, van Boven et al., 2008). This interpretation was used in our studies, i.e. HI would be achieved if  $\geq 85\%$  of target birds, the critical fraction, had a GMT  $\geq 3.0$  (van Boven et al., 2008).

The first study was carried out as although several ND vaccines brands are available in east Africa, there was a seeming lack of GCP standard data on the safety and efficacy of the main candidates likely to be used in very young village chicks. Here, birds were not challenged with live virus for ethical reasons. Instead the serological response was used as a marker of immunogenicity. Mean antibody titres for the three vaccinated groups are shown in Figure 2A. After vaccination NDV antibody levels increased significantly in all three groups by Study Day 11, D11 (i.e. 7 days post-vaccination) and by D18 they had exceeded the protective level of  $2^3$ . Peak mean GMTs were obtained for all vaccines at D32. There were no significant differences in response between the three vaccine groups, but all vaccinated groups showed significant differences ( $P < 0.001$ ) from the negative control group. The duration of immunity, DOI (the time during which HAI titres  $\geq 2^3$  are sustained) in this short study for all vaccines was from before D18 to study end. The IO route of administration was used for the

attenuated LaSota and I-2 (and MSD in study 2) as this is the standard approach with live attenuated ND vaccines. It seems that IO is equivalent to other routes due to the close proximity to the Harderian gland, which has a role in immunity at the mucosal or secretory level (Alders et al. 2009; Degefa et al., 2004; Illango et al. 2008). The ITA-NEW vaccine being an inactivated, adjuvanted vaccine was administered by IM injection. The results of this GCP standard study which used very young backyard chicks with undetermined genetics, compared well to results published for LaSota (Awa et al. 2009; Degefa et al. 2004; Illango et al., 2008), for I-2 (ACIAR 2002, Alders et al. 2009; Illango et al., 2008; Wambura et al. 2006) and for ITA-NEW (Bennejean et al. 1978; Dossa et al. 2005; Magand, 2009). Further work here could include a challenge phase.

Study 2 evaluated thermotolerant properties i.e. the resultant immunogenicity after heat stress of the MSD and KEVEVAPI-produced I-2 vaccines. I-2 is considered a thermotolerant strain (ACIAR 2002) but for vaccine registration purposes, there were few controlled data on its protection against challenge after being heat stressed. Titration revealed that pre-heating before reconstitution for 24h at  $32.3^\circ\text{C}$  in the dark (conditions representative of field conditions), had caused drops in titre of 3 log and 2 log for MSD and I-2 respectively to  $10^6$  EID<sub>50</sub> per vial. Titres for all vaccinated groups reached  $\geq 2^3$  by D17

Figure 2B. Study 2 HAI GMTs Induced by the Two ND vaccines in 35 day old pullets ( $\pm$  standard error; D, Study Day; R<sub>x</sub>, treatment).



(Figure 2B) but in the non-pre-heated MSD group (V2.1) this was achieved surprisingly early by Study Day 10, D10 (i.e. by 7 days post-vaccination) which was also when the group's peak GMT was reached. The peak GMTs for heated MSD and heated I-2 were detected at D17 and lasted to D31. The DOI for heated MSD was from before D17 to just before D52, c.f. that for heated I-2 which was from before D17 to just after D38. Generally titres began to decline for the heated vaccine groups after D38 and with minor differences in titre between them. The non-pre-heated MSD appeared to induce a slower decline after peak concentrations had been reached and its DOI was from before D10 to just after D52.

The term "thermotolerant" and not "thermostable" or "heat-resistant" is preferred by the authors in recognition that any vaccine has its heat tolerance limits. These relate to the local conditions of transport and use (ACIAR, 2002; GALVmed, PANVAC and IIAM Conference, 2009). It was hypothesised that if the MSD vaccine was used, it would retain sufficient potency to be protective, even after the type of heat stress seen in the field, and that use at this dose could still be cost effective. The data for I-2 closely matched previous in-house results (KEVEVAPI) and the data upon the effects of temperature upon infectivity and immunogenicity from non-GCP standard studies (ACIAR 2002; Wambura et al. 2006). The data for the MSD vaccine are novel as there are no published GCP standard results for a 10x concentrated Clone 30 and heat-stressing as implemented here (D. Goovaerts,

MSD, The Netherlands, individual communication). Using a 10x field dose to offset a heat-induced drop in titre was effective as the heated vaccine still elicited satisfactory serological responses. The results were also consistent with other Clone 30 data (Rahman et al., 2002; van Eck and Goren, 1991; D. Goovaerts, MSD, The Netherlands, individual communication). Since the antigen used to determine the HAI titres was derived from the V4 virus, which is a virus closely related to the I-2 strain, HAI titres in the test could possibly be slightly artificially higher in favour of the I-2 vaccinated chickens. Nevertheless titres found in the MSD vaccinated groups were slightly higher compared to the I-2 vaccinated groups, indicating the good immunogenicity of this vaccine including in the heat treatment group. It was clear that heat treatment had a negative effect but the vaccines still showed good evidence of potency. Serological titres in all groups had declined to about 2 log<sub>2</sub> by D80 (77 days post-vaccination suggesting that the putative Duration of Immunity, DOI, induced by these vaccines is relatively short in practical terms, which has implications for control programmes. However, the DOI is based not only on titre values but on protection in challenge experiments, and is likely to be longer due to mucosally-based protection even in the apparent absence of titres (D. Goovaerts, MSD, The Netherlands, individual communication). Clearly the safety and efficacy of the MSD vaccine may need further study especially if very young local ecotype chicks were to be considered.



However, Clone 30 is acknowledged to be safe (Rahman et al., 2002; van Eck and Goren, 1991) even at titres far exceeding the normal  $10^6$  EID<sub>50</sub> dose (D.Goovaerts, MSD, The Netherlands, individual communication). If the 10x field dose approach to counteract thermolability in the field can be confirmed in larger GCP-standard trials incorporating a challenge phase, it may represent a simple, cheap, and pragmatic approach to the increased availability of quality vaccines made and produced to Good Manufacturing Practice standards in hot climates.

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