www.bti.org.in
ISSN 0974-1453
Research Article

CHARACTERISTICS OF NOVEL CLONE (Super LaSota™) DEVELOPED FROM LaSota STRAIN OF RANIKHET DISEASE VIRUS AND ITS USE AS VACCINE

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ABSTRACT

A clone derived from LaSota strain of Ranikhet Disease (RD) virus was characterized for pathogenicity and antigenicity/immunogenicity. The pathogenic parameters were mean death time (MDT) 111.5 hours in 10 days old embroyonated eggs; Intra-cerebral Pathogenicity Index (ICPI) 0.0125 in day-old chicks ; Intra-venous Pathogenicity Index (IVPI) 0.0 in 6 weeks-old sero negative chicks. It was non-invasive to respiratory system after administration by oral route. The virus did not spread to intact healthy chicks from vaccinated chicks indicating that it is not released by respiratory, secretory or excretory route. The clone was found to be highly antigenic when administered orally @ 10^6 EID_{50} in 0.2 ml to chicks leading to early onset of immunity inducing base line protective HI-antibody titer of 1:16 in a week time and a peak titer of ≥1:80 by day 21 post-vaccination that declined gradually to base line protective HI-antibody titer of 1:16 by 7 weeks. It conferred 100% protection to chicks against virulent challenge on day 22 post-vaccination.

Key words: Ranikhet Disease, LaSota Strain, Pathogenicity

INTRODUCTION

Poultry Industry in India is one of the largest egg and poultry meat producers in the world. Age old Ranikhet Disease (RD) continues to be serious threat to the poultry industry despite availability of effective vaccines. Hence, this disease continues to attract the attention of vaccinologists to develop least reactogenic but potent vaccines.

Ranikhet Disease was first recorded in Newcastle (UK) by Doyle (1927) and simultaneously in Ranikhet town at Kumaon Hills in India by Edwards. Hence, the disease is known as Newcastle Disease (ND) and Ranikhet Disease (RD). The disease is caused by a RNA virus classified as Paramyxovirus Group-1 (PMV-1), under Paramyxoviridae. The disease is most commonly seen in domestic Poultry including guinea fowl.

The disease is of relatively milder form in Western countries, whereas, in India and other Asian countries it is highly virulent and causes acute form of pathology in the gastro-intestinal system and reproductive system. The disease affects all the four vital systems viz. Respiratory, Neural, Visceral and Reproductive system (Alexander and Allan, 1973).ND virus strains are classified as "Lentogenic", "Mesogenic" and "Velogenic" based on their pathogenic parameters, Mean Death Time (MDT) in embryonated chicken eggs, Intra-cerebral Pathogenicity Index (ICPI) in day-old chicks and Intra-Venous Pathogenicity Index (IVPI) in 6 weeks old chicks (FAO, 1978).

The disease control measures are centered around standard hygienic and sanitary measures and by use of effective vaccine in planned manner (Garg & Pankaj 1993). Two kinds of vaccines, viz live vaccine and inactivated vaccine are used. Live vaccines are of two categories: Lentogenic strains (F, B1 Hitchner & LaSota) and Mesogenic strains (Komarov, R_2B, and RB). Lentogenic strains are used from day 1 to 35 days of age; whereas, `Mesogenic strains' are used from 35 days to 14 weeks of age of chicks. Inactivated adjuvanted vaccines are generally recommended after 16-18 weeks of age to prevent the shedding of live virus in the environment to avoid affecting birds at lay as well as younger chicks in the same farm and to maintain the required antibody titer over longer period to prevent infection and subsequent loss in egg production and mortality.

Ideally, a live vaccine is expected to be mild enough not to precipitate `Respiratory', `Digestive' or `Neural' reactogenicity and confer long lasting immunity. One of the important steps in this direction was taken up by Bio-Med (P) Ltd in 1975 when it introduced its Ranikhet Disease `RB' strain vaccine as an alternative to R_2B strain vaccine that causes serious post vaccination stress.

At present LaSota strain is being used in India as vaccine of choice to vaccinate young chicks at 1-7 days age and also as booster vaccine in drinking water several times even during laying periods. LaSota strain is the most pathogenic of all the three lentogenic strains viz F, B1 and LaSota. LaSota is more prone to give rise to respiratory complications specially in mycoplasma infected flock as it leads to development of a serious disease– C.R.D. LaSota strain is shed by respiratory, secretory and excretory routes and effective spread occurs.

Further, the immunity conferred by LaSota strain is of weak type and lasts for only 4 weeks (Kreimar, 1969). Considering the above scenario, scientists of Bio-Med's R & D centre undertook this work to develop a mutant which could be mild enough but would induce long lasting immunity in day 1 to 7 weeks age groups of chicks.

MATERIALS AND METHODS

SPF Chicken Eggs (Venkey's, Pune), Chicks-day old & 42 days old (Susceptible to ND), NDV (LaSota) (From seed Bank, Bio-Med), NDV (Super La Sota) (Evolved at R&D Lab Bio-Med), NDV (Virulent Challenge Virus) from Q.C. Lab, Bio-Med were used.

Pathogenic Characterization

Mean Death Time (MDT) in 10 day-old SPF-Eggs, Intracerebral Pathogenicity Index (ICPI) in day-old chicks, Intravenous

Pathogenicity Index (IVPI) in 6 weeks old chicks were assessed for Super LaSota as per standard procedures (FAO Manual, 1978).

Assessment of Pathogenicity to Lungs

Five chicks (3 days old) primed with Super LaSota @ 10^6 EID_{50} in 0.2 ml by oral drops, were sacrificed after 48 hours, lungs were collected, triturated and processed for testing the presence of virus by inoculation into 10 day-old SPF chicken eggs. The eggs were incubated for 4 days, then chilled and the allantoic fluid was tested for the presence of the virus.

Assessment of antigenicity

A total of 60 chicks (3 days old) susceptible to RD were grouped and conducted the study as follows:

Group A (40 chicks)

Administered primary dose of Super LaSota @ 10^6 EID_{50} in 0.2 ml by oral drops. On day 36 post-primary vaccination 20 chicks were given booster dose of Super La Sota @ 10^6 EID_{50} in 0.2 ml by oral drops. The balance 20 chicks were maintained as such.

Group B (10 chicks)

Administered primary dose of Standard La Sota @ 10^6 EID_{50} in 0.2 ml by oral drops.

Group C (10 chicks)

This group was maintained as un-vaccinated in-contact control along with Super LaSota group. Sero-conversion was assessed by HI test in micro-titer plates using 4HA units of super LaSota following the standard procedure (FAO Manual, 1978).

Virulent challenge study in chicks

In a separate experiment twenty four chicks (4 days old) were primed with Super LaSota @ 10^6 EID_{50} in 0.2 ml by oral drops. On 22^{nd} day post-vaccination the chicks were challenged along with 10 un-vaccinated control chicks by intranasal and oral administration of RD virulent virus @ 10^5 LD_{50} in 0.2 ml. Before virulent challenge, sero-conversion (HI-antibody titer) was assessed at weekly intervals.

RESULTS

Pathogenic Characterization

The results of present study on Super LaSota^{TM} and parameters of LaSota, B1 and F strains as per FAO manual (1978) are presented in Table 1 for comparison.

Table 1. Parameters of Super LaSota, LaSota, B1 and F Strain

Sl. No.	Parameters	Super La Sota	La Sota	F-Strain	B1
1.	MDT	111.5 hours.	103 hours	119 hours	117
2.	ICPI	0.0125	0.15	0.25	0.25
3.	IVPI	0.0	0.0	0.0	0.0

The results indicated that Super LaSota is the least pathogenic among the lentogenic strains (F, La Sota, B1) used as vaccine. In the ICPI study of Super LaSota^{TM}, only one chick became sick on the last day of observation (day 8 post-injection) and did not die even after the observation period. All other chicks

were normal throughout the period of experiment.

Pathogenicity to lungs

No virus could be isolated from the lungs of chicks vaccinated with Super LaSota^{TM} by inoculation of lungs-homogenate in 10 days-old SPF chicken eggs which clearly points to the non-invasive property of "Super LaSota" to lung tissue. Whereas invasiveness is a common feature of standard LaSota.

Sero-conversion studies in chicks

The results of sero-conversion in chicks to primary vaccination with Super LaSota^{TM} and Standard LaSota are presented in Table 2 and Figure1.The booster effect of Super LaSota in chicks primed with Super LaSota has been depicted in Figure 1.

Table 2. Sero-conversion in chicks vaccinated with Super La Sota/Standard La Sota

Weeks post-vaccination/ Age in weeks	HI-antibody titer (Reciprocal dilution) to primary vaccination with		
	Super La Sota	Standard La Sota	Un-vaccinated control
1	16.0	12.0	2.0
2	44.8	16.0	2.0
3	80.0	32.0	<2
4	80.0	16.0	<2
5	44.8	8.0	<2
6	25.6	6.0	<2
7	16.0	4.0	<2
8	13.4	4.0	<2
9	12.8	4.0	<2
10	9.6	4.0	<2
11	9.0	4.0	<2
12	8.0	3.8	<2

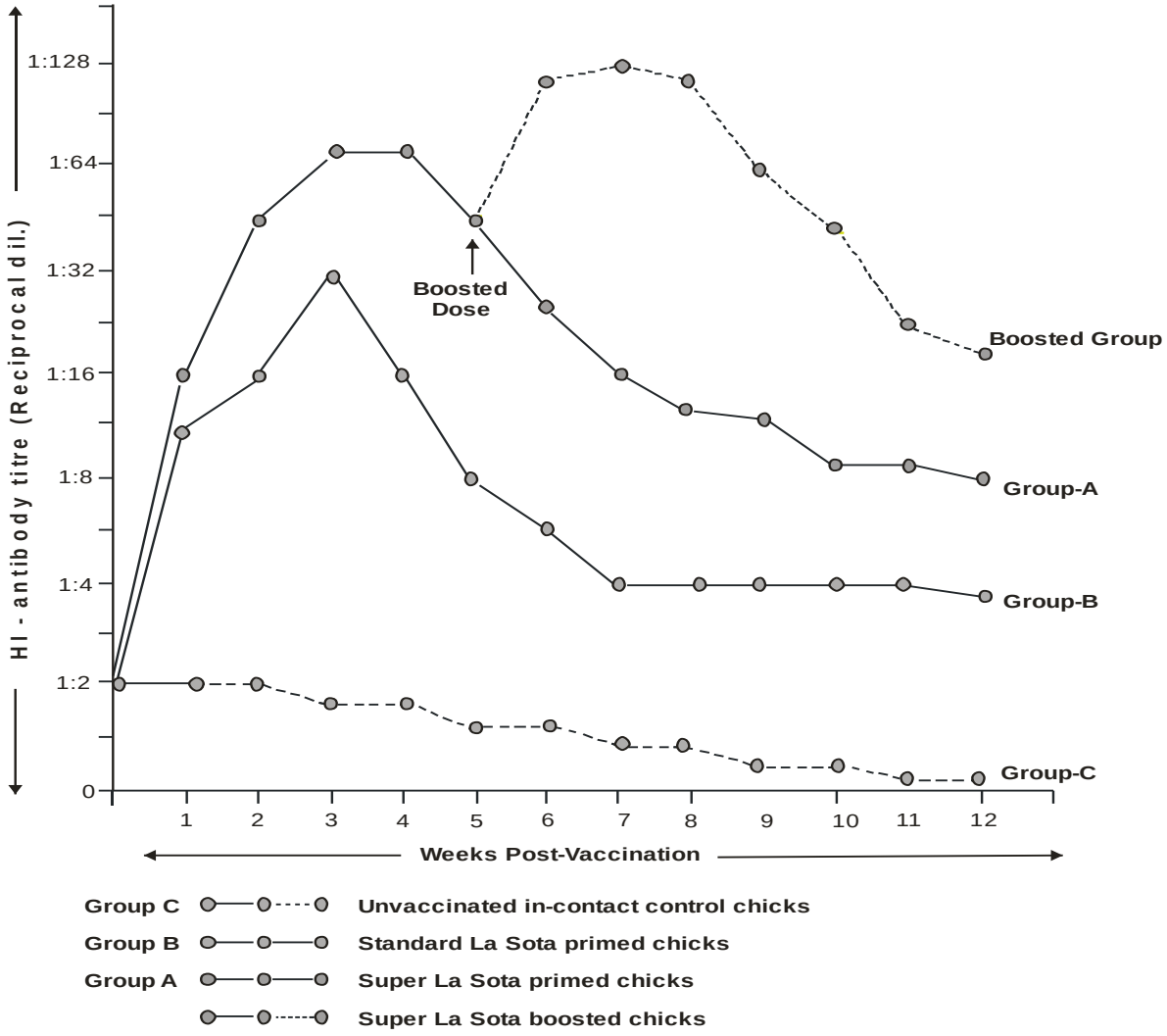
It is inferred from the results that primary vaccination with Super LaSota induced protective level of HI antibodies (>1:16) by day 7 and peak titer of 1:80 by day 21,

maintained a plateau for one week and gradually declined to the base line protective titer of 1:16 in 7 weeks. Whereas, standard LaSota (Group B) vaccine induced HI antibody titer of 1:16 by day 14 and peak titer of 1:32 by day 21 which declined rapidly to unprotective level by 5th week of vaccination. This observation clearly proves superiority of Super LaSota^{TM} over LaSota strain in terms of higher levels of immune response and for longer period.

A booster dose with Super LaSota^{TM} to 20 out of 40 chicks on day 36 post-primary vaccination induced anamnestic HI antibody response ≥1:120 (Fig.1) within 6 days and the titer attained a peak 1:128 by day 14. The titer declined gradually to just above the baseline protective titer of >1:16 after 12 weeks. The control group of chicks (Group C) had insignificant level of HI antibodies with declining trend viz. ≤1:2 throughout the period of experiment.

Protective effect of super LaSota

FIG.1 HI antibody response in chicks vaccinated with RDV - Super LaSota^{TM} / LaSota



DISCUSSION

A new clone has been developed from standard LaSota strain of Ranikhet disease virus identified as "Super LaSota^{TM}" at Bio-Med (P) Ltd Laboratories is unique. It is the mildest of all strains/clones in the lentogenic category reported so far (FAO, 1978). Super LaSota is completely non-pathogenic to lung tissue and not excreted by the vaccinated chicks. These qualities of Super LaSota^{TM}, differentiated it from Standard LaSota strain which is known to grow in lungs leading to distress of respiratory system resulting in precipitation of CRD in some cases and is excreted for about 10-15 days in the atmosphere.

"Super LaSota^{TM}" induced very strong serological response and the protective titer lasts upto 7 weeks when compared to Standard LaSota whose serological response lasts upto 4 weeks only.

No other cloned vaccine e.g. ND clone 30, ND Clone VH Tanuvas D-58 strain has been claimed to be free of pneumotropic character, secretion/excretion free and confer mild (HI titer~ 1:32) immunity lasting for 4 weeks only.

Thus "Super LaSota" vaccine could prove very beneficial to broiler farmers who can use it to vaccinate broiler chicks at 2-4 days of age. In layer chicks two times vaccination, the first at 2-4 days of age and the second (booster) at 6-7 weeks of age will protect them upto 14 weeks of age, thus resulting in huge saving in terms of vaccine and labour cost with minimal handling of chicks. Super LaSota^{TM} can also be used for boosting immunity in laying flocks in the face of outbreaks of RD in the area without any post vaccination stress and danger of spread into atmosphere by vaccinated birds.

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