# Development and Manufacture of an Inactive Oil Emulsion Newcastle Disease Vaccine in Iran

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**Summary**

*Inactivated oil emulsion Newcastle disease (ND) vaccine was prepared and evaluated at laboratory and put to field tests with success. Oil adjuvant, ISA 70, gave a very satisfactory result causing desirable immunological responses. The oil emulsion vaccine engendered immunological responses both in live-vaccine primed birds and unprimed birds. However, immunological responses were noticeably higher in the birds that were first primed with a live vaccine. Birds which were primed with B1 vaccine alone demonstrated almost similar reactions as those that were first primed with B1 and later with La Sota live vaccine. The vaccinated birds when challenged, by intramuscular injection of* $10^{5.5}$ $ELD_{50}$ *of a VV NDV strain, showed a very strong resistance.*

## Introduction

Newcastle disease (ND) is highly contagious and attempts to control it by slaughter, sanitary measures and quarantine are often unsuccessful. When it becomes endemic, vaccination of flock at risk is a highly effective method of control (Cross, 1988).

Vaccines, either live or inactive, are produced for control of the disease. Each of these vaccines has advantages and disadvantages. Studies in the 1930s on the attenuation of virulent Newcastle disease virus (NDV) strains by Iyer and Dobson (1940) and Haddow and Idnani (1946) led to the development of mesogenic vaccine strains. This was followed by introduction of lentogenic strains (B1 and La sota) for vaccine production.

There are several disadvantages with live vaccines including: a)the vaccine may cause disease, depending upon environmental conditions and the presence of complicating infections. b) Although the abilty of vaccine virus to spread may be an advantage within the flock, spread to susceptible flocks, especially on multiage sites, can cause severe disease problems.
Early studies demonstrated that inactivated infective material conferred protection on inoculated chickens, but problems in production and standardization discouraged its use on a large scale. Inactivated vaccines are usually produced from infective allantoic fluid treated with betapropiolactone or formalin to kill the virus and mixed with a carrier adjuvant. Early inactivated vaccines used aluminium hydroxide adjuvants but the development of oil-emulsion-based vaccines proved a major advancement. Different oil-emulsion vaccines vary in their formulation of emulsifiers, antigen, and water-to-oil ratios; most now use mineral oil (Cross, 1988). The value of inactivated oil emulsion vaccine for avian virus infections has been demonstrated by the successsful use of oil emulsion Newcasle diseae vaccines for several years. The use of this kind of vacccine (Zanella, 1969, Allan, 1972; Dawson and Allan, 1973; Beard, 1975) and its preparation and potency evaluation under experimental conditions have been reviewed (Zanella, 1966; Papparella, 1973; Cesssi and Nardelli, 1974; Gough et al. 1974; Levi and Zakay-Rones, 1974; Box and Frminger1975). Potency of this vaccine under experimental conditions has been confirmed by high levels of protection in the field for long periods of time (Philips, 1973). It has an advantage over live vaccines because the duration of immunity it produces is quite long and it is enough, under normal conditions, for one egg production season and, hence, revaccination may not be required. The disadvantage with inactivated oil emulsion vaccines is their higher cost of production that can be offset by producing multivalent vaccines. In the present paper we report on successful production of ND vaccine in Iran.

## Materials and methods

**Eggs**: Specific pathogen free (SPF) eggs were purchased from Lohmann (Cuxhaven, Germany).

**Chickens used for laboratory experiments**: These originated from the above mentioned SPF eggs. These were used at different ages that are mentioned when appropriate.

**Chickens used in the field**: 20 days old replacer chickens (Shaver breed) and broiler chickens were used.

**Adjuvant**: Oil adjuvant ISA-70 (SEPPIC, Cosmotics/Pharmacy Division, Paris, France) was used. Ratio of adjuvant to antigen was 80/20 (V/V).

**Antigen**: The V4 NDV was grown on 9-day old embryonated SPF eggs. Allantoic and amniotic fluids were collected, embryo infective dose 50 ($EID_{50}$) was calculated. The antigen was diluted in order to adjust the $EID_{50}$ to $Log_{10}^{8.5}$/0.1 ml. The inactivation was carried out, using 0.1% formalin, for 16 h while liquid being continuously shaken. All necessary measures were undertaken to garantee that no virus was left active. This was realised by inoculation of inactivated material into embryonated eggs to make sure no viable virus had remained.

**Vaccine production**: A water in oil emulsion was produced mechanically by using a homogeniser (Rannie, Model Mini-Lab Type 8.30H). The antigen, prepared as mentioned above, was emulsified in the adjuvanted oil. Each dose of the vaccine contained 0.4 ml oil, 0.1ml antigen and 0.05mg Thiomersal. The whole procedure of vaccine production was carried out in controlled air conditions under a Lamin Air flow unit. The vaccine was stored at +4°C.

**Control tests**: These comprised sterilty, stability, safety and potency tests.

*Sterility test*: Antigen was tested for bacterial and fungal contaminants. The final product underwent the same checks.

*Stability test*: The vaccine was tested for stability for one week at 37°C and for different periods of time at +4°C.

*Safety test*: A group of 10 SPF chickens were inoculated with the vaccine and were observed for a period of 10 days for any possible untoward manifestations.

*Potency test*: A group of 20 chickens (SPF) were vaccinated, one dose/bird, and were bled regularly for detection of changes in the serum antibodies, measured by haemagglutination inhibition test. Twenty days from the vaccination day these birds were challenged by a highly virulent field strain of NDV.

**Vaccination**: Vaccine was inoculated IM into pectoral muscles. At the laboratory, SPF birds were vaccinated with 0.5 ml (one dose) of the vaccine. In the field, the oil vaccine was inoculated simultaneously with one dose of a live vaccine (La Sota strain) in birds which had previously been primed with B1 Strain live vaccine.

**Blood samples**: The birds at the laboratory were bled weekly by cardiac puncture. Blood samples from field trials were prepared by wing vein puncture.

**Haemagglutination test (HA)**: This was carried out according to the method described by Allan and Gough (1976) using microtitre plates. Briefly, twofold dilutions of 0.025ml amounts of allantoic fluid (antigen) were made in phosphate buffered saline (PBS) pH 7.0-7.4. To each dilution 0.025ml of PBS and 0.025ml of 1% v/v chicken red blood cells were added. forty-five

minutes later tests were read, the dilution in the well showing 100% agglutination was taken as the titre.

**Haemagglutination inhibition test.** Standard method was employed, using 4 HA units and 1% v/v chicken red blood cells. The HI titres were the highest dilutions of sera causing complete inhibition of 4 HA units.

**Challenge test** : Thirty-five days after vaccination, vaccinated birds along with unvaccinated (control) chickens were challenged, by intramuscular injection, with $10^{5.5}$ $ELD_{50}$ of a VV NDV strain.

## Results

The virus yield was very high and titres as high as $10^{10.5}$ $EID_{50}$ were achieved. The viscosity test showed that the proportion at which oil and antigen was mixed was appropriate and resulted in a viscosity of 19 cp. Vaccine stability showed that it was stable at +4°C for a period of one year (the time thus far the vaccine is tested) and a month at 37°C. At the latter temperature a liquid phase not more than 1 mm high appeared after one week storage but easily disappeared after shaking the mixture which thereafter maintained its normal texture at 4°C. Vaccine was safe for chickens and none of the inoculated birds showed noticeable untoward sign. Feed intake and water drinking of the vaccinated birds did not differ form those of the the control groups. No macropathological changes were observed at the site of injections. The results of serological reaction of SPF chicken to the vaccine are shown in Fig.1. The rise in HI titres were already detectable 10 days after vaccination. These titres steadily continued to rise and reached a plateau 40 days after vaccination. The vaccine significantly stimulated the immune system on its own without a need to priming, these birds were kept under controlled conditions, in adjacent to control birds, to exclude chances of aberrant natural contamination. Fig.3 shows the results of vaccination of birds which had been first primed with a live B1 vaccine 15 days prior to revaccination by the oil emulsion vaccine. The results of the field trials carried out in flocks that had been primed first, when one week old, with live B1 vaccine (eye drop) and receiving La Sota vaccine (in drinking water), simultaneoulsy, at the time of vaccination by oil-emulsion vaccines are depicted in Fig. 2. These flocks were divided in two groups, each receiving either RazPassol 101 or a commercial imported vaccine referred to in this paper as the imported vaccine. Results indicate that RazPassol showed higher HI titres. Field observations later proved that the resistance of birds vaccinated with RazPassol was extremely higher than birds vaccinated with the imported vaccine. The results of challenge test are shown in Table 1. The challenge strain caused 100% mortality in control birds, with typical ND pathological lesions being demonstrable after

necropsy, whereas vaccinated birds did not deviate from the normal behaviour.

| DPV[1] | Treatment | HI titres ($L0g_2$) | | Mortality | |
|---|---|---|---|---|---|
| | | V[3] | C[2] | C[2] | V[3] |
| -10 | | 2 | 2 | | |
| 0 ← | RazPassol 101 vaccine | 2 | 2 | | |
| 10 | | 7.25 | 2 | | |
| 20 | | 10 | 2 | | |
| 35 ← | Challenge | 9 | 2 | | |
| 36 | | | | | |
| 37 | | | | | |
| 38 | | | | 3/6[4] | 0/10 |
| 39 | | | | 2/3 | 0/10 |
| 41 | | | | 1/1 | 0/10 |
| 42 | | | | | 0/10 |
| 41 | | | | | 0/10 |
| 42 | | | | | 0/10 |

1) Days post vaccination
2) Control birds
3) Vaccinated birds
4) Dead/ total

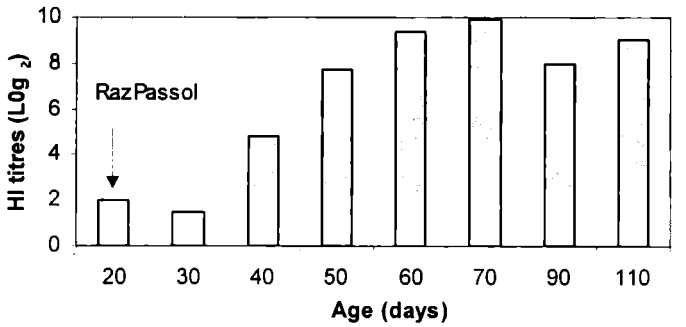


**Fig. 1 Mean HI titres of 20-day old SPF chickens to RazF .ssol inactive oil-adjuvanted ND vaccine**

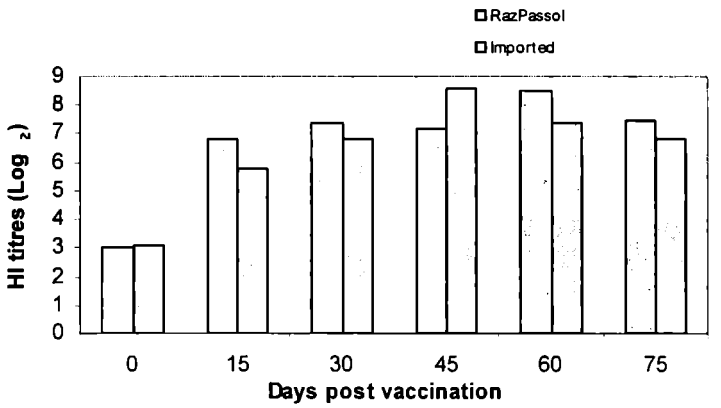


**Fig. 2 Mean HI titres of 20-day old layer chickens to 2 inactive oil-adjuvanted ND vaccines, RazPassol and an imported one**

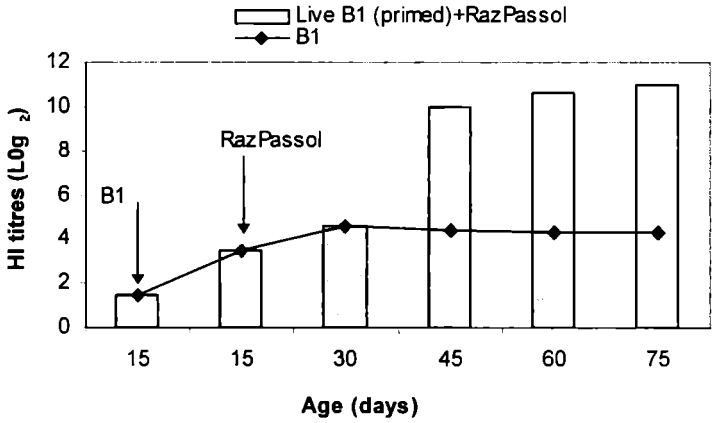


**Fig. 3-Serological (HI) reaction of SPF birds received B1 and later Razpassol**

## Discussion

The best method to measure antibodies capable of protecting the host is virus neutralization (VN) tests. However, since the VN response appears to parall the HI response, the latter test is frequently used to assess protective response, especially after vaccination (Allan et al., 1978). In the present work antibody response was measured by this method and was found to be compatible with ELISA test (results are not shown). Level of HI antibodies

was also compatible with those of challenge test, therefore, the test was deemed efficient for the control tests of the vaccine.

Various seed viruses used in the production of oil-emulsion vaccines include Ulstster 2C, B1, La Sota, Roakin, and several virulent viruses. The selection criterion should be the amount of antigen produced when it is grown in embryonated eggs. Apathoghenic viruses grow to the highest titres (Gough *et al.*, 1977), therefore, it would seem an unnecessary risk to use a virus virulent for chickens.The strain V4 used in production of the present vaccine was found to be highly antigenic and engendered very high immune

responses, besides, it propagated efficiently in embryonated eggs, giving $EID_{50}$ titres of not less than $10^{10}$

Different adjuvants have been added to NDV for production of vaccine. These adjuvants are aluminium hydroxide, vegetable oil, Freund's complete and incomplete adjuvants and mineral oil including ISA 70. Pagnini *et al* (1969) and Zanella (1970) reported that an oil emulsion was superior to aluminium hydroxide as adjuvant when used in ND vaccines. This had been the results obtained by Gough *et al.* (1977) who concluded 3 to 6 fold higher potency with oil emulsion than with aluminium hydroxide. Stone *et al.* (1978) pointed out that one major constraint in developing oil-emulsion vaccines is the difficulty of preparing stable water in oil emulsions with low viscosity which is essential for assuring injectablity and easy handling. It is well established that Freund's adjuvant is one of the most effective adjuvants (Freund, 1956; Woodhour *et al.*, 1964). However, the use of this adjuvant has been prohibited in commercial vaccine products because of high viscosity and the severe local reactions it produces. On the other hand, the oil adjuvant ISA-70 gives a less viscous water in oil type emulsion (41 45 cp) than Freund's adjuvants (350 506) and its enhancing effect on NDV

antigens, measured by serology, is roughly the same as Freund's adjuvant (Yamanaka *et al.*, 1993). We did not perform histopathological studies on chickens vaccinated with the vaccine adjuvanted with ISA 70, but macroscopically no tissue reaction could be noticed. However, histopathological reaction of vaccine produced with ISA 70 has been described by Ymanaka *et al.* (1993) who has maintaned that the elevated and longterm persistent NDV HI antibodies by ISA 70 adjuvant is correlated with the extent and persistence of such histological changes as marked plasma cell and lymphocyte infiltration at the injection site.

Inactivated vaccines are far easier to store than viable vaccines. They are not as adveresly affected by maternal immunity as live vaccine and can be used in day old chickens (Box et al., 1976). We are in agreement with these authors as chickens hatched, under laboratory conditions, with high maternal antibody titres showed satisfactory immunological responses.

The major advantages of inactivated vaccines are the very low level of adverse reactions in vaccinated birds. The ability to use them in situations unsuited for live vaccines, especially, if complicating pathogens are present and the extremely high levels of protective antibodies of long duration is desired.

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