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THE EFFICACY OF INACTIVATED OIL EMULSION NEWCASTLE DISEASE VA

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The efficacy of inactivated oil emulsion Newcastle Disease Va

by

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This thesis attached hereto, entitled "The Efficacy of Inactivated Oil Emulsion Newcastle Disease Vaccine" prepared and submitted by Rahaju Ernawati in partial fulfilment of the requirements for the degree of Master of Science, is hereby accepted.

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ABSTRACT

THE EFFICACY OF INACTIVATED OIL EMULSION

NEWCASTLE DISEASE VACCINE

The preparation of an experimental oil emulsion Newcastle disease vaccine is described and its efficacy was evaluated in broiler chickens.

A plaque purified clone of Mukteswar strain of Newcastle disease virus designated UPM-AC/2 was used for the preparation of the vaccine. The virus had a titre of 10^{11} fifty percent egg infective dose. The vaccine virus was inactivated with betapropiolactone at a final concentration of 0.1 percent. The vaccine was prepared by mixing the antigen with variable concentration of Arlacel A and Tween 80. Two types of vaccines were prepared, a single oil emulsion and a double oil emulsion vaccine. Both vaccines were evaluated for their stability, viscosity emulsion type, safety and antibody response in chicken. The double emulsion vaccine containing 6% Arlacel A and 1.5% Tween 80 had low viscosity and was stable for at least 6 months at room temperature. The vaccine induced marked antibody response in chickens which were previously vaccinated with lentogenic live Newcastle disease vaccine.

The vaccine was also evaluated for its efficacy in broiler chickens which had been previously vaccinated with live Newcastle disease vaccine. Broiler chickens which had been vaccinated when day old with the live Newcastle disease vaccine and revaccinated when 3 weeks and 8 weeks old with the emulsion vaccine were protected when challenged with a viscerotropic velogenic Newcastle

disease virus. Between 90 to 100 per cent of the vaccinated chickens were resistant to the challenge compared to 100% mortality in the non vaccinated control chickens.

I. INTRODUCTION

Newcastle disease (ND) is a very important disease of poultry in many parts of the world including Malaysia.

The disease was first reported on the island of Java in Indonesia by Kraneveld in 1926. "A disease with similar characteristics was reported the same year in the seaport town of Newcastle-on-Tyne, England, by Doyle (1927) from which its common name was derived. During the next six months, eleven other outbreaks occured in nearby English counties, with mortality of 98 to 100 per cent. Newcastle disease was also recognized in Korea in 1926 by Konno et al (1929) who described it as an acute and usually fatal respiratory and nervous disease. The infection spread rapidly and numerous chickens were lost by many farmers during the following two years. In India, the first recorded outbreak was in 1927, in Ranikhet" (Brandly, 1964). In immediately succeeding years, the disease was seen throughout Malaysia, Singapore, Japan, China and Australia (Wong, 1981). In the United States, Newcastle disease was first reported by Beach and Stover (1942), as a respiratory nervous disease of young and mature chickens. It has average mortality rates ranging from about 15 per cent to 100 per cent (Brandly, 1964).

Newcastle disease virus strains can be differentiated into three pathotypes according to virulence for chickens: lentogenic, mesogenic and velogenic. The three techniques commonly used to determine whether a virus is a lentogenic, mesogenic or velogenic strain are as follows (Hanson and Brandly, 1955):

1. The Mean Death Time (MDT) of infected 10 day-old embryonated chicken egg; the lentogenic strains take 96-168 hours to kill embryos following allantoic cavity inoculation, whereas the mesogenic strains take 44-70 hours to kill, and the velogenic strains take 40-70 hours.

2. Intracerebral Pathogenecity Index (ICPI) in day-old chicks. The ICPI for the lentogenic strains is less than 0.4, for the mesogenic strains is between 0.4-1.9, and for the velogenic strains is between 2.0-3.0.

3. Intravenous Pathogenecity Index (IVPI) in 6 weeks old chicks. The lentogenic and mesogenic strains are not lethal to 6 week-old chickens when the 50% embryo lethal dose (EID_{50}) is inoculated, while the velogenic strains are lethal.

Four forms of Newcastle disease have been described (Hanson, 1963). These forms are clinically and pathologically distinctive:

1. Doyle's form of the disease, first recognized in 1927 by Doyle, is an acute lethal infection of all ages of chickens. Haemorrhagic lesions of the digestive tract are a prominent pathologic feature. This form is caused by certain velogenic strains, Asiatic Newcastle disease strain and more recently velogenic viscerotropic Newcastle disease (VVND) strain.

2. Beach's form of the disease, described 15 years later by Beach, is an acute and frequently lethal infection of chickens of all ages. This form is characterized by lesions in the respiratory tract and nervous system. Haemorrhages are conspicuously absent from the digestive tract. It was initially called nervous respiratory disease or pneumoencephalitis and is caused by

neurotropic velogenic strains.

3. Beaudette's form of the disease, recognized by Beaudette a few years later, is an acute respiratory and sometimes lethal nervous infection of young chickens. In older birds mortality is rare. Some of the mesogenic strains which produce this form have been used as vaccines.

4. Hitchner's form of the disease, described by Hitchner, is a mild or inapparent respiratory infection of chickens caused by lentogenic strains. Several lentogenic strains are also used as vaccines.

Birds of all ages and breeds are susceptible to Newcastle disease virus. The factors involved in the spread of the disease within an area are the movement of live birds and the mechanical transport of infection by normal trade practices such as carcasses, meat products, vaccines and feedstuffs. Natural spread of Newcastle disease has been chiefly through the media of exudates, excreta and offals of infected birds. The digestive and respiratory routes obviously constitute the major channels of natural infection, although entry of Newcastle disease by the occular and cloacal routes may be quite common. Spontaneous infections have been reported among pheasants, gammets and other free living species and it is probable that wild birds acting as mechanical carriers were the source of these infections (Hanson, 1963 and Dawson, 1973). The subject of windborne spread has been reviewed (Dawson, 1973).

The world spread of the disease has been fully reviewed by Lancaster (1966). He has traced the spread of the fully virulent disease from Asia, the mild disease from the New World and the spread through Europe and Africa that occured prior to and during the Second World

War. By 1966, a fairly stable situation seemed to have developed in which fully virulent virus has become endemic in the tropics, milder disease in North America and Western Europe and an intermediate form in Iran and the Arab countries to the West. In 1968 an upsurge of the disease was reported in Iraq. In 1971 the United States reported cases of fully virulent Newcastle disease occuring along its southern and western borders and designated such strains as velogenic viscero-tropic Newcastle disease.

Newcastle disease continues to be a major threat to the poultry industry in many parts of the world especially in Asia. In many countries the disease is controlled through a vaccination programme. However, the need for ND vaccination may not be the same for all countries. In countries where the disease is endemic live lentogenic vaccines (F, La Sota and B_1) are used and the vaccines have to be given at frequent intervals in order to maintain satisfactory haemagglutinating antibody titre. Unfortunately repeated administration of the vaccine is usually not very convenient for a large farm and at the same time the chickens are subjected to stress. In many countries in South East Asia the problem is avoided by using the mesogenic live ND vaccine (Mukteswar) which is highly immunogenic and gives long lasting immunity (Table I). This vaccine is used as a booster vaccine. However, the use of such vaccine in partially immune chickens can cause adverse reactions leading to respiratory distress, loss of weight, or loss of egg production. Moreover, the potency of live vaccines often deteriorates in hot tropical climates. This problem may be avoided by the use of killed Newcastle disease virus (NDV) vaccine.

During recent years, there has been an increased interest in the use of inactivated oil emulsion ND vaccine in Europe and

America (Stone et al., 1978; Box and Furminger, 1975 and Eidson et al., 1980). However the use of such vaccines in Malaysia has not been reported. This is because the production of an effective inactivated oil emulsion ND vaccine is often difficult and not always achieved in all laboratories. One of the main factors that has to be taken into consideration when preparing an inactivated oil emulsion ND vaccine is the quality the vaccine virus. They must be able to grow to a very high titre so that virus concentration is not required. In many of the work on inactivated OE ND vaccine the virus had to be concentrated (Stone et al., 1978; Brugh Jr., 1978). The vaccine must be stable and of low viscosity to ensure injectibility and ease of handling. The vaccine must be stable to stimulate satisfactory haemagglutinating inhibition response in vaccinated chickens.

The objectives of this project are:

1) To develop an effective experimental oil emulsion Newcastle disease vaccine.

2) To study the serologic response of chickens vaccinated with the oil emulsion Newcastle disease vaccine containing different doses of antigen and emulsion composition.

3) To study the response of vaccinated chickens to challenge with viscerotropic velogenic Newcastle disease virus.

II. LITERATURE REVIEW

STUDIES OF INACTIVATED NEWCASTLE DISEASE VACCINE

The control of Newcastle disease represent a continued and unresolved problem. In general they are based on vaccination, quarantine measures and slaughter or both (Robertson. 1964). In many countries the disease is controlled through a vaccination programme. A variety of vaccines and vaccination programmes have been introduced and it is important for poultry farmers to use the most efficient vaccine and vaccination programme. Different countries have developed various types of living and inactivated vaccines, depending upon local requirements.

The live lentogenic strains most commonly used for production of vaccines are: the B_1, La Sota and F strains. The B_1 strain was first described by Hitchner et al., (1948) who used it a It has been widely used in birds of all ages. The immune response in individual chickens has depended largely on the method of administration of the vaccine. The La Sota strain was introduced as a commercial vaccine in 1952 (Hitchner, 1964). It often causes post vaccination respiratory symptom (Allan, et al., 1978) and is of greatest use in flocks which are mycoplasma free. The F strain of lentogenic Newcastle disease virus was first reported by Asplin (1952). This strain was isolated from a mild outbreak in England. Strain F has now been used as an intranasal vaccine both for broilers and layers. Respiratory symptoms have appeared in a variable proportion of inoculated chickens but no nervous or intestinal symptoms have been seen (Aspl
F strain has the lowest virulence of the common lentogenic vaccine strains (Allan et al., 1978).

The mesogenic strains of Newcastle disease virus which had been used for production of vaccines are: the Mukteswar strain (Haddow and Idnani, 1946), the Hertfordshire (or Herts) strain (Iyer and Dobson, 1940), the Komarov (or Haifa) strain (Komarov and Goldsmit, 1946) and the Roakin strain (Beaudette et al., 1949). The Mukteswar is the most virulent of the mesogenic strains and therefore provides the greatest and the most durable immunity (Asplin, 1952; Allan et al., 1978). The Hertfordshire strain is recommended for a booster vaccination of chickens over 8 weeks of age which have been immunized at an earlier age with lentogenic vaccine. The Komarov strain has also provided adequate immunity. The Roakin strain has been used mostly in the United States (Allan et al., 1978).

Control of Newcastle disease through the use of inactivated vaccine was reported by Beach (1944) in California. The results showed that inactivated virus vaccines gave a significant degree of protection against paralysis, mortality, and drop in egg production caused by virulent strains, but did not protect the respiratory system. Inactivated vaccines have the advantages that the virus is inactivated without destroying its antigenecity, and it is no longer capable of initiating infection or spreading the disease. Inactivated vaccines are of value in flocks with an intercurrent respiratory infection which could increase the risk of an excessive reaction to a live vaccine. Inactivated oil emulsion vaccines can play a valuable role in healthy blocks by preventing drops in egg production.

A number of inactivating agents, such as crystal violet, formalin, betapropiolactone, gamma radiation, low and high temperature and urethane have been used for inactivation of viruses.

Doyle and Wright in 1950 reported that a crystal violet-ethylene glycol inactivated Newcastle disease virus vaccine was capable of stimulating immunity and that the immunity persisted for at least 12 months. Legenhausen and Sinkiewicz (1959) found that a crystal violet inactivated vaccine and a commercial ultra violet inactivated Newcastle disease virus vaccine were ineffective in inducing resistance to challenge following single dose vaccination at 10 days of age. However, when a booster dose was given 32 or 288 days after the first injection, there was an increase in resistance to challenge.

The effect of urethane as an inactivating agent for Newcastle disease virus has been studied by Bower and Einstark (1954). In preliminary experiments urethane inactivated Newcastle disease virus stimulated a high antibody level in chickens, suggesting a high level of immunity.

Formalin has been the most widely used inactivating agent in the production of Newcastle disease virus vaccines. Many investigators have reported generally good results when formalin inactivated preparations of Newcastle disease virus were used (Dardiri *et al.*, 1957). Others (Hofstad, 1953) have reported poor or partial immunity following its use. Hanson *et al.* (1951) compared the immunogenecity of five strains of Newcastle disease virus as formalinized vaccines. They found two strains to be superior to the other three, but could not ascribe their superiority to heat stability or virulence. The virulent strain did not produce better inactivated vaccine than the other strain. Doll *et al.* (1951) observed that all chickens vaccinated with a formalin inactivated virus were susceptible to respiratory infection when challenge by the respiratory route; however, all the birds were refractory to fatal infections. Mitchell and Walker (1952) reported

that birds vaccinated with a formalin inactivated vaccine were resistance to challenge up to 13 months. Levine and Fabricant (1952) found that chickens vaccinated with formalin inactivated vaccine were susceptible when challenged at 4 and 8 weeks after vaccination. None had a positive hemagglutination inhibition titre. Waller and Gardiner (1953) reported 96 per cent and 84 per cent resistance to challenge at 8 and 12 weeks of age, respectively, following use of formalin inactivated Newcastle disease virus vaccine in 10 day-old chicks. The results were 72 per cent and 56 per cent for the same challenge when chicks were vaccinated at one day of age.

Recently, betapropiolactone has come into use as an inactivating agent for a number of vaccines. Betapropiolactone is a highly alkylating and acylating agent. It has been especially useful for the production of vaccines, as it causes loss of infectivity while preserving antigenecity. Newcastle disease virus inactivated by betapropiolactone was found to lose RNA-dependent RNA polymerase activity. Betapropiolactone was shown to react with both virus proteins and RNA (Garlick and Avery, 1976). Betapropiolactone has the advantages of acting very rapidly, and although the chemical itself is regarded as having a carcinogenic hazard, it is degraded to propionic acid within a few minutes of coming in contact with organic material and so becomes nontoxic (Allan, et al., 1978). Another benefit from the choice of betapropiolactone is that it is able to inactivate the leucosis agent and hence allow inactivated vaccines to be made from non-leucosis tested eggs, an aspect that might not be completely safe if formalin is used as the inactivating agent (Allan, et al., 1978; Levy and Zakay, 1973).

Hofstad in 1963 carried out an experiment to compare the immunogenecity of Newcastle disease virus inactivated by ionizing gamma radiation, formalin or betapropiolactone. The immunogenic properties of these preparations were determined by intramuscular inoculation of young chickens and challenging them about 6 weeks later. He found that the immunogenecity of irradiated preparations in general was similar to that found for formalin inactivated preparations. Newcastle disease virus preparations inactivated with betapropiolactone induced a better immunity in chickens than similar preparations inactivated with gamma radiation or formalin.

The effectiveness of many vaccines can be greatly increased by incorporating the antigen in, or mixing it with an adjuvant. An adjuvant, can be defined as any substance which enhances the immune response. Adjuvants as an immunity stimulating substance, act (i) on a hapten or an antigen by enhancing its antigenic properties, or (ii) on the cells involved in the immune response (Jolles and Paraf, 1973). Two main types of adjuvant have been used with veterinary vaccines, a gel of aluminium hydroxide or aluminium phosphate, and a water-in-mineral oil emulsion. Both adjuvant types act in varying degrees by creating a depot from which the antigen is slowly and continuously released over a period of days or weeks. The alum adjuvants and probably the oil emulsion adjuvants also help to make the antigen more particulate, which again enhance the antibody response (Herbert, 1970).

Mixtures of mineral oil and aqueous solutions or suspensions of antigens were introduced many years ago by Freund, who further increased the adjuvant effect by the addition of killed tubercle

bacilli being known as Freund's complete adjuvant and that without the tubercle bacilli as Freund's incomplete adjuvant (Freund et al., 1948). Freund's incomplete adjuvant for the strengthening of inactivated vaccines originally assayed by Salk in 1952 for human influenza vaccine - has been used widely since 1965 in the preparation of poultry vaccines, particularly Newcastle disease vaccine (Cessi and Nardelli, 1974b; Levy and Zakay-Rones, 1973).

An emulsion can be viewed as a heterogenous system, consisting of at least one immiscible liquid intimately dispersed in another liquid in the form of droplets. The suspended droplets are referred to as the dispersed or internal phase; the medium in which they are suspended is the external or continuous phase. The addition of a third component, acting at the interface to retard phase separation is called emulsifier or emulsifying agent (Autian, 1966; Petrowski, 1976). From a practical definition, emulsions are normally formed when two immiscible liquids are mechanically agitated, both phases initially tend to form droplets. When the agitation is stopped, the droplets quickly coalesce, and the two liquids separate. The lifetime of the droplets is materially increased if an emulsifier is added to the two immiscible liquids. Usually only one phase persists in droplet form for a prolonged period of time (Rieger, 1976). When the oil droplets are dispersed in a continuous aqueous phase, the emulsion is termed oil-in-water; when the oil is the continuous phase, the emulsion is of the water-in-oil type (fig. 1).

Recently, a water-in-mineral oil emulsion adjuvant has come into use. In this adjuvant the antigen which has been dissolved or suspended in water is dispersed in the oil as very tiny droplets so as to form a water-in-oil emulsion. The oil selected for use is a

pharmaceutical grade white mineral oil of the light liquid paraffin. Water-in-oil emulsions cannot readily be prepared from vegetable oils except by the addition of large quantities of aluminium monostearate as a stabilizer, e.g. in Hilleman's adjuvant 65 (Hilleman et al., 1973).

To achieve a stable mixture of water and mineral oil in the form of emulsion, it is necessary to add an emulsifier which is capable of promoting good emulsion. Arlacel A[1] is commonly used as emulsifier of the type promoting water in oil emulsion while Tween 80[2] is commonly used as emulsifier of the type promoting oil in water emulsion (Herbert, 1978).

Emulsion of the water in oil type are difficult to prepare. According to Herbert (1978) various forms of emulsion may result from attempts to prepare a water in oil emulsion. It is essential that before use the water in oil emulsion be tested to determine the emulsion type under the oil immersion objective of a microscope.

A modified type of emulsion called a multiple emulsion can be prepared by reemulsifying a simple water in oil emulsion in an outer aqueous phase containing Tween 80. A stable multiple emulsion is formed which contains the antigen in the secondary dispersed phase within the oil droplets of the primary dispersed phase (fig. 1). This type of emulsion of low viscosity and produces a superior and more rapid immune response than a simple water in oil emulsion.

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1 = Mannide monooleate, Sigma Chemical Company Saint Louis, Missouri 63178 U.S.A.

2 = Polyoxyethylenesorbitan monooleate, Sigma Chemical Company Saint Louis, Missouri 63178 U.S.A.

The serological response induced by inactivated Newcastle disease vaccine is dependent on factors such as inactivating agents, adjuvants, dose of antigen and age at which the chickens were vaccinated.

One of the major problems in controlling Newcastle disease virus is the lack of immune response of young chicks up to 21 days old. Although this age group is very sensitive to infection, invariability in the level of maternal antibodies and presumed immunologic immaturity make it difficult to induce an adequate immune response. To investigate this problem, Zakay and Levy (1973) carried out a series of experiments in which groups of chicks 3-5 days old with maternal antibodies and another groups of chicks of the same age but lacking maternal antibodies were immunized intramuscularly with a $10^{8.3}$ EID_{50} dose of inactivated oil adjuvant vaccine. Chicks in the group lacking maternal antibodies, responded to this vaccine with high titres of haemagglutination inhibition (HI) antibodies. The antibody titres remained at a high level for as long as 3 months. In the presence of maternal antibodies, a very low titre of HI antibodies could be detected after immunization with the same dose of antigen. The results indicate that chicks can respond immunologically to intramuscular administration of antigen in the absence of maternal antibodies. In another experiment, chicks with a high titre of maternal antibodies were immunized intranasally at one day old with inactivated antigen followed by a second dose on the 15^{th} day with an equivalent of $10^{9.5}$-10^{10} EID_{50} of inactivated virus. The chicks developed local antibodies in the respiratory tract, and 75% survived when exposed to a virulent strain of Newcastle disease virus by contact 14 days after the second dose. These results indicate that maternally derived antibodies do not interfere with local vaccination.

According to Cessi and Nardelli (1974b) vaccination using injectable inactivated oil emulsion Newcastle disease vaccine at 21 days of age stimulated high and persistent HI antibody levels. High levels of maternal antibodies had a negative influence on immunity when chickens were vaccinated during the first 3 weeks of age, but not when performed later. The results of the experiment showed that the field dose of 0.5 ml containing inactivated oil emulsion Newcastle disease vaccine produced high levels of antibodies and persisted for at least 60 weeks in chickens without maternal antibodies. The influence of maternal antibodies in chickens up to 3 weeks old showed the existence of an interference which was inversely proportional to the age of chickens and practically absent at and after the third week of life.

Extension use of the oil emulsion vaccine during the epizootic in the United Kingdom in 1970-1971 has confirmed their value, first by serological demonstration of the extremely high level of antibodies they produced, and later by the clinical demonstration of freedom of vaccinated chickens from both overt disease and any drop in egg production as reported by Phillips (1973). He found that live vaccine administered during rearing gives adequate protection against mortality, but if revaccination is not carried out during the laying period, this protection is reduced, and protection against egg loss is not good. A combination of live vaccine administered during rearing and one injection of inactivated oil emulsion vaccine at or near point of lay followed by live vaccine during the laying period gives satisfactory protection against egg drop.