IMMUNOGENICITY OF AN INDIGENOUS ISOLATE OF NEWCASTLE DISEASE VIRUS AND ITS USEFULNESS AS A VACCINE STRAIN

By

M. R. MURUGAN

THESIS

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Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Microbiology
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DECLARATION

I hereby declare that this thesis entitled IMMUNOGENICITY OF AN INDIGENOUS ISOLATE OF NEWCASTLE DISEASE VIRUS AND ITS USEFULNESS AS A VACCINE STRAIN is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title of any other University or Society.

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Signature of the candidate : M.R.Murugan

Name of the candidate : M.R.MURUGAN

CERTIFICATE

Certified that this thesis, entitled IMMUNOGENICITY OF AN INDIGENOUS ISOLATE OF NEWCASTLE DISEASE VIRUS AND ITS USEFULNESS AS A VACCINE STRAIN is a record of research work done independently by Sri.M.R.Murugan, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Name of the Guide: Dr.S.Sulochana,
(Chairman, Advisory Board)
Designation : Associate Professor,
Department of Microbiology

Mannuthy,
3--2--1983.

Dedicated

to

My Parents

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INTRODUCTION

INTRODUCTION

Newcastle disease (ND) was recognized as a viral infection after the epidemic in Newcastle-on-Tyne, England (Doyle, 1927) and in Batavia in Java (Kraneveld, 1926). In India, a similar disease was reported in 1928 by Edwards in a farm at Ranikhet in Uttar Pradesh. Cooper (1931) studied the disease in detail and proved that the virus responsible for Indian Ranikhet disease was identical to the Doyle's virus.

Newcastle disease is still the most important fulminating and fatal disease among poultry causing mortality up to 100% in susceptible chicken. Less lethal forms resulting in crippling, impaired growth and poor feed utilization among the surviving birds also lead to heavy economic loss (Berg et al. 1947).

Since 1927, great attention has been paid to evolve suitable methods of prophylaxis including general hygiene, quarantine, compulsory slaughter of ailing and incontact birds and vaccination. The quarantine and slaughter policies have been successful in preventing the establishment of the disease only in certain countries. Methods of spread, the number of birds involved and the prevailing trade and management practices have appreciably interfered

with the eradication of the disease (Lancaster, 1977).

During the period from 1968-73, some countries have adopted strict quarantine and slaughter together with vaccination to reduce the incidence of this disease. Under these conditions the vaccination programme resulted in existance of velogenic form of Newcastle disease in a masked form (Sharman and Lamont, 1974). However, in countries where vaccinated flock could be held under quarantine, the use of live vaccines provided a useful adjunct to slaughter (Lancaster and Alexander 1975) and had given satisfactory results in Scottland, Denmark, Northern Ireland, Southern California and Canada.

The pneumotropic velogenic virus infection had been found to be difficult to eradicate by the conventional quarantine and slaughter or ring vaccination. In such cases intensive vaccination of the whole population is the only safe method (Koapke, 1973). Vaccination could be adopted as a part of the eradication programme as it can reduce the rapidity of spread of an epidemic and in endemic areas systematic vaccination of the total poultry population has been found to be a successful method of control (Allan et al. 1978).

Immunization against Newcastle disease could be effectively adopted due to lack of multiplicity of the antigenic type, the birds below one week of age are vaccinated with a lentogenic strain followed by a booster dose at about 6-8 week with a mesogenic strain. These mesogenic strains include naturally occurring virus of acceptable low level of pathogenicity (Roakin strain) or strains selectively attenuated by serial passage in embryonated hen eggs (Mukteswar R_2B) or in some nongallinaceous hosts (Komarov strain). These vaccines are in use for atleast three decades and have been well studied by Hitchner and Johnson, (1948), Winterfield et al. (1957) and Hanson (1978).

Newcastle disease is endemic in India and continues as a threat to the poultry industry. Vaccination, with a lentogenic strain at the first week of hatch and with a mesogenic virus at the age of 6-8 weeks, is the routine procedure adopted for the control of this disease in our country.

The selection of lentogenic strains for the primary vaccination is not much controversial since they are least virulent and used in chicks, below one week of age, having some amount of maternal antibodies. The mesogenic

strains used for boosting the immunity may be naturally occurring or adapted from virulent virus. The second type of mesogenic strains may have the risk of reverting back to the virulent form and hence the choice of a mesogenic virus for vaccination is of greater importance. In India, Mukteswar R_2B and Komorov strains, developed by serial passages of virulent virus through the chick embryos and ducklings respectively are used as mesogenic strains for secondary vaccination. There are also reports of untoward reactions in the birds vaccinated with these strains.

Mukteswar R_2B is highly pathogenic in young chicks and produced about 6% mortality in chicks of six weeks of age (Haddow and Idrani, 1946). Even in chicks with a base immunity with lentogenic strain, this virus produced post-vaccinal reactions to the extent of 4-21% (Seetharaman and Sinha, 1963; Chakravarty et al. 1981).

Komarov strain is also reported to cause post-vaccinal reactions like the temporary cessation of egg production, nervous derangement and mortality in a very small percentage of birds (Crowther, 1952; Thorne and McLeod, 1960).

During the course of an investigation into the role of free flying birds in the epizootology of ND, Balachana

et al. (1981) isolated a mesogenic strain of ND (MF) from a myna (*Acridotheres tristis tristis*). These authors have also reported that this isolate of ND virus is less virulent than the Komarov strain which is presently being used as the vaccine strain in Kerala and can produce immune response in chicken sufficient enough to protect them against challenge with a virulent virus. Since this is a naturally occurring mesogenic one of local origin, it was felt worthwhile to undertake a detailed study on this isolate and compare its suitability as a vaccine strain with that of Komarov.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Newcastle disease (ND) is possibly the most economically important and fatal disease of domestic poultry, particularly the young ones, throughout the world (Alexander, 1982). Since the time of recognition of this disease, detailed field and laboratory studies have been undertaken to understand the biology, pathogenicity, control and eradication of the disease (Hanson, 1978; Lancaster, 1977, 1981). The economic importance of outbreaks, the world wide distribution and the ability to cause devastating infection in many avian species has kept the Newcastle disease virus in the forefront of serious avian pathogens (Alexander, 1982).

Types of disease

Newcastle disease can occur in a variety of forms according to the tissue tropism of the virus. The pneumotropic form of the virus has a greater affinity to respiratory tract but can be recovered in high titer from many other organs also (Allan, 1975). In this form, the signs manifested by the bird are acute respiratory illness and sudden death from a combination of respiratory

failure and accumulated damage to many cells of the body (Allan, 1975).

For epizootiological studies and management of control programmes, ND is categorized into velogenic mesogenic, lentogenic (Kono, 1971) and asymptomatic form (Lancaster, 1981). The velogenic form may be viscerotropic or neurotropic. The velogenic viscerotropic form (Doyle, 1927) known as the Doyle's form is characterized by greenish blood stained diarrhoea, dehydration, torticollis and paralysis of the leg and wing. The dead birds manifest haemorrhagic lesions in the digestive tract. Mortality is about 90%. In velogenic neurotropic form (Beach, 1942) the lesions are in the respiratory tract and central nervous system and are characterised by respiratory distress, coughing, gasping, lowered egg production and paralysis. Haemorrhages are conspicuously absent from the digestive tract and mortality ranges from 10-50% (Mohanty and Dutta, 1981). Beaudette and Black (1946) described a mesogenic form in which acute respiratory distress and occasional lethal nervous symptoms are noticed in young chicks, but mortality is rare. This form spreads rapidly within a flock manifesting rapid drop in egg production and egg

quality. The lesions are haemorrhagic and inflamatory. Progressive air sacculitis is common (Lancaster, 1981a). Hitchner and Johnson (1948) described a mild respiratory infection of chicken characterized by sudden drop in egg production and reduced feed intake. This was caused by a lentogenic strain of Newcastle disease virus and was described as the lentogenic form of ND. In this form mortality was negligible in adult fowls and complete recovery was effected in 1-2 weeks time. The characteristics in young birds were a mild tracheitis or air sacculitis (Lancaster, 1981). A fourth form of ND described from various countries was diagnosed by chance during routine serological surveillance (Lancaster, 1955; Lancaster and Alexander, 1975). These inapparent outbreaks may be missed if sufficient number of sera are not examined. It was also reported to spread easily to incontact chicks without producing any clinical signs of the disease. Lancaster (1981a) observed that ND virus of this type might occur in the live poultry vaccines including those made from chicken embryo fibroblast cultures. The terms velogenic, mesogenic and lentogenic are also used with respect to the mean death time in eggs (Hanson and Brandly, 1955) and viscerotropic and neurotropic to the

pathology of the disease produced.

Until 1970, the types of ND reported from the western hemisphere were of the Beach's form. In 1970, outbreaks similar to the original Doyle's form were recorded in this region (Hanson et al. 1973). These workers also reported that the virus responsible for the said outbreaks were different from others in that they killed the chick embryos quickly and was lethal for chickens in which it produced haemorrhagic gut lesions when given by the ocular or cloacal route. Spalatin et al. (1973) had reported that marked oedema of eyelid and the tissues of head and neck a feature considered at one time as diagnostic of fowl plague, frequently associated with the velogenic viscerotropic ND infection and such clinical signs were characteristic of only this pathotype.

In Britan, no clear cut division of strains into the four pathotypes have been made and the classification of virus isolates was based mainly on the virulence determined by the intracerebral or intravenous pathogenicity indices (Alexander and Allan, 1973). However, the virus responsible for the U.K. epidemic which began

in 1970 had frequently been reported to produce a disease clinically distinct from earlier isolates in having a high incidence of respiratory distress (Hugh-Jones et al. 1973).

In India, ND was recognised at Ranikhet by Edwards (1928) and Cooper (1931). Kuppuswamy (1935) reported the evidence of fowlpest in the province of Wellesleyand suggested that Ranikhet disease of India, Avian pest in Philippine Islands, Newcastle disease of England and Pseudo-Avian pest of Java were one and the same. Naidu (1938) reported that fowlpest and Ranikhet disease were the two very acute, highly infectious disease of poultry encountered in the state of Mysore causing an unaccountable damage to the poultry industry. An investigation conducted by Nobindro (1945) in the Assam Province revealed that Ranikhet disease was prevalent everywhere in Assam. Aziuddin et al. (1954) detected an outbreak of a mysterious disease in poultry and concluded that it was ND of mild type similar to the one described in United States.

According to the report of the National Commission on Agriculture (NCA) Ministry of Agriculture, Government

of India (1976), this disease was so widespread and devastating in its effects that poultry raising on a commercial scale could not be even thought of till the year 1942 and 1,150 outbreaks occurred annually.

A comparison of the number of annual outbreaks of ND with mortality among the infected birds during 1969-73 and 1964-68 showed that the number of annual outbreaks had remained more or less same while the rate of mortality had gone down considerably. There were on an average 7,513 deaths a year in 1969-73 as against 10,724 in 1964-68. Mortality rate for outbreaks during 1969-73 was worked out to be 6.5 as against 9.5 for 1964-68 and analysis of the pattern of ND outbreaks over last ten years showed that sporadic outbreaks continued to occur throughout the year in various states.

Spread of Newcastle disease and the carrier status

Newcastle disease virus has a very wide range of avian hosts susceptible to natural or experimental exposure (Lancaster, 1963a, 1966; Lancaster and Alexander, 1975). Many of them are believed to act as silent carriers and had been suspected in the spread of this disease (Lancaster, 1963a, 1977; Easterbury and Waddington, 1978). The spread of ND

by wild birds depends upon the species of birds involved and the country of origin (Lancaster, 1974) or the countries involved in the migration of certain birds especially water fowl (Lancaster, 1977).

In U.S.A. (Utterback, 1972; Utterback and Schwartz, 1973) and Canada (Lancaster, 1974), wild birds are not considered as an important factor in the spread of velogenic ND, whereas in other countries such as Japan, Belgium and South America some wild birds caught in the neighbourhood of poultry farms were found to be infected with ND virus (Lancaster and Alexander, 1975).

Schaaf (1974) opined that such birds involved in the spread of ND were local or domestic. In some of the captive and cage birds, virulent ND virus may exist in a symbiotic relationship with the host which cannot be detected either by antibody titration or swab culture (Lancaster, 1977). Cooper (1973) reported that apparently healthy non-psittacine birds can harbour the virus in extremely low titres. In spite of this, cage and captive birds were considered responsible for the introduction of virulent ND to domestic poultry flocks in many countries (Lancaster, 1977).

The rapid spread of ND is a phenomenon of temperate climates in which the temperature is low and humidity is high during autumn and spring (Allan, 1975). Dawson (1973) described transport of live birds, movement of personnel and infected materials between poultry premises as the classic mode of spread of Newcastle disease.

Mode of control of Newcastle disease

Since 1927, much attention had been paid to evolve suitable methods of prophylaxis including general hygiene, quarantine, compulsory slaughter of ailing and incontact birds (Seetharaman, 1951). The frequency with which Newcastle disease occurs in many concentrated poultry producing areas led to the necessity of evolving a suitable vaccine.

The first stage in the development of a prophylactic vaccine was the evolution of a vaccine treated with chemicals such as phenols, carbol-glycerine, ether, chloroform, toluole, and formalin. Dyes like crystal violet and methylene blue were also used. Of these vaccines, the formalinized vaccine (Nakamura et al. 1937) and the aluminium gel adsorbed and formalin treated vaccine (Maddow and Idnani, 1941), were found to be

somewhat satisfactory. The duration of the immunity conferred by any of these vaccines ranged from 15 days to four months (Brandly et al. 1946). Francis (1948) reported that the formalin inactivated vaccine had the capacity to create an immune belt to the spread of the disease.

The observation of Iyer and Hobson (1940) that serial passage of suitable strains of Ranikhet disease virus on the chorioallantoic membrane of developing chick embryos can attenuate the viruses for fowls had made the control of this disease practicable by employing live virus vaccines.

Haddow and Idnani (1946) evolved a live virus vaccine by attenuating an Indian strain of ND virus isolated from natural outbreaks in the field. This vaccine now known as the Mukteswar R_2B was reported to be efficient, cheap and harmless and established resistance in 48 hours through interference phenomenon (Haddow and Idnani, 1946; Beaudette et al. 1949; Laubney and Manay, 1948). Seetharaman (1951) reported that he could obtain immunity with Mukteswar R_2B for a period up to four years when the chicks were vaccinated

at the age of 6-8 weeks. Rao and Agarwal (1960) could observe some untoward effect with vaccines prepared from this strain particularly when it was used in the face of an outbreak. They reported an accumulated tempo of mortality of about 25-30% in day old chickens and only one third of the vaccinates were protected. These authors also demonstrated that F strain of RD which was less virulent (lentogenic) was the vaccine of choice for protection of day old chicks by the intra nasal route when an outbreak of RD was threatened in the brooder house or when the chicks had to be transported to hatcheries over long distances. Since then various vaccine strains were developed for use in young chicks and chicks about 6-8 weeks of age namely the lentogenic strains like B_1 (Hitchner and Johnson, 1948); Roakin (Beaudette et al. 1949); V_{66} (Malik et al. 1969); Ulster (McFerran and Nelsen 1971) and Queensland V_4 strain (Simmons, 1967) and the mesogenic strains like Herts 33 (Iyer and Dobson, 1940); Komarov (Komarov and Goldsmit, 1946); Roakin (Beaudette et al. 1949); NE_{107} (Clancy et al. 1949) and M_{47} (Winterfield and Hitchner, 1961) respectively.

Though several strains of vaccine virus had been used successfully in widely different geographical

regions against different clinical manifestation of the disease (Anon, 1960; Jungherr and Markham, 1962; Mitchell and Walker, 1955; Rao and Agarwal, 1960) failure to protect against indigenous field virus was reported by Blanco (1949) in spain; Flowers et al. (1960) in U.S.A., Correa (1963) and Correa and Rosales (1961) in Guatemala. According to Jansen et al. (1974) if the vaccination was conducted on a regular and systematic basis and the response was determined by laboratory tests, the available vaccines would result in satisfactory control of the disease. Almost all vaccines and the methods by which they were applied would result in the induction of a degree of immunity that will protect chickens from ND for a period of time (Hanson, 1974). This author also stated that depending on the types of challenge virus the vaccinated birds showed an interruption either on weight gain or on egg laying. The degrees of protection provided were also different. Although the vaccinated birds are protected from the clinical disease none of the vaccine or vaccination regime protected the chicken against infection as the virus was shown to become established and multiply in the respiratory tract of some of the chicks and shed

the virus for a period of 1-2 weeks (Kusuchele and Easterday, 1970). Such birds can transmit the disease to unvaccinated birds brought to such flock (Walker et al. 1972). During vaccination almost all chickens in a flock will respond in a predictable fashion to the administration of vaccines, a few will fail to react the way that others do, but has little effect on the success of vaccination programme as measured by economic parameters. However, this may be highly important in understanding the persistance of the disease in nature (Hanson, 1974).

Gomez-Lillo et al. (1974) reported that serological variations and diversity in the antigenic components do occur between different isolates or strains but antigenic difference between vaccine strain and field isolates is insufficient to produce any influence on practical immunology (Allan et al. 1978; Appleton, 1974; Jansen et al. 1974). Hence successful vaccination and field responses might be related to the amount of antigen which enters the tissues or to the difference in antigenic mass produced during infection.

In Singapore velogenic viscerotropic Newcastle disease (VVND) was controlled with a lentogenic vaccine (Strain F) given at the day of hatch followed by

intramuscular injection of R_2B at the 6th week and again at 20th week (Chew and Liow, 1974). Similarly ND was controlled in Mexico by vaccinations at day one with LaSota strain with a titer about 10^5 EID 50/ml and repeating it at frequent intervals before laying and thereafter at every 2-3 months (Lucio, 1974). Repeated vaccination first with a lentogenic strain followed by intramuscular injection of a mesogenic vaccine combined with proper sanitation was found to be effective in controlling VVND in Lebanon (El-Kein, 1974).

Singh (1977) reported that the problems associated with vaccination against Ranikhet disease are improper vaccination, vaccine failure or several other causes. The break down may result from improper storage and handling of vaccine. Improper handling could lead to undesirable change in pathogenicity and immunogenicity of the modified live virus vaccine strain. Break down due to antigenic inadequacy of the vaccine appear to be less important than breaks associated with improper handling of the vaccines. Other factors like age at vaccination, individual immunological capacity, maternal immunity as well as environmental and other factors affecting the

host could mitigate against uniformity of immune response to vaccination. Stress factors like chilling, and other concurrent disease operate on the central nervous system and the release of cortical hormones may minimize inflamatory reaction and limit the violence of body's reaction to the viral infection and production of antibodies (Hungerford, 1969) and impair the cell mediated immunity (Mohn and Klingberg, 1972).

The probable cause of Ranikhet disease outbreaks recorded in unvaccinated poultry flock appears to be the stress factors resulting in the failure of development of the requisite immune response. The presence of residual antibody from vaccinated hens is also important in practical vaccination (Gangopadhyay and Malik, 1970). Hito and Sawai (1973) reported that supression of antibody synthesis due to residual antibody could be considerably reversed by two inoculations of F vaccine. Therefore revaccination in chicks with F strain could perhaps maintain a proper immunogenic level or even induce a strong immunity and would prevent vaccination breakdown (Coleman, 1957; Hirato and Schechtman, 1960).

Beard and Easterday (1967) reported that the method

of exposure of chickens to vaccine contribute significantly in the immune response. Though large number of virions are required to induce an immune response with live virus vaccines the dose appeared to have little significance as it can multiply in the body (Ring, 1972). Differences in the persistence of immunity has also been attributed to the strain of virus (Spalatin and Hanson, 1972). Environmental factors such as temporary deprivation of food and water, social stress inherent in assembling and moving birds, extensive environmental temperature (Sinha et al. 1957) pollution of the air with ammonia dust and carbondioxide (Anderson et al.1964) and amino acid content in the feed (Bhargava et al. 1970) were found to influence the immune response to ND vaccination. Allan (1975) opined that vaccination of commercial chickens was associated with variable results due to the presence of interfering respiratory infections, immunosupresive disease of viral origin, variation in the techniques of vaccination and the programmes of revaccination. There was also an increasing amount of proof to show that local immunity in the respiratory tract was important in the early stage of immune process. Besides vaccination programme slaughter policy was effective in the control

of ND in geographically isolated countries such as Australia. Introduction of exotic ND could be avoided by vigorous quarantine and strict import regulations in countries like U.S.A. (Mohanty and Dutta, 1981).

Vaccine strains

In attempts to control and eradicate ND, both inactivated (Nakamura et al. 1937; Haddow and Idnani, 1941) and live virus vaccines (Haddow and Idnani, 1946; Komarov and Goldsmit, 1947; Van Roekel et al. 1948; Beaudette et al. 1949; Hitchner and Johnson, 1948) were being tried depending upon the local requirement. Inactivated vaccine has the advantage that the virus is killed without destroying its antigenicity and is no longer capable of initiating infection or spreading the disease. Since these vaccines do not generally produce severe reactions they are suitable for use in chickens, young laying stock or birds in poor health. Selection of a strain of the virus for inactivated vaccine is no longer a problem and even fully virulent strains can be safely used to produce such vaccines (Buxton and Fraser, 1977).

Live virus vaccines can be produced from either the

naturally occurring virus of acceptable low level of pathogenicity or a virus which had been selectively attenuated by serial passage through embryonated eggs or some nongallicine host (Asplin, 1952; Allan, 1975). The principle behind using live virus vaccine is to induce mild infection to produce an active immunity (Seetharaman, 1951).

Strains of Newcastle disease virus isolated in different parts of the world vary in their virulence though they are indistinguishable morphologically and serologically (Hanson and Brandly, 1955; Alexander and Allan, 1973). The difference in virulence could be measured in chicks or chick embryos in terms of three parameters viz. the intracerebral pathogenicity index (ICPI), intravenous pathogenicity index (IVPI) and mean death time (MDT) at terminal dilutions (Poultry Biologics, 1963; Waterson et al. 1967). On the basis of these criteria, ND virus could be grouped into three types, the velogenic, mesogenic and lentogenic. The MDT, ICPI and IVPI for velogenic strains are, below 60 hours; $\geq$ 1.5 and $\geq$ 1.5 for mesogenic strains 60-90 hours; 0.8 - 1.5 and 0.05 to 0.31; and that for lentogenic strains over 100 hours; 0.1 - 0.2 and 0.0. The

lentogenic and mesogenic strains usually induce specific antibodies without producing the disease and give protection against infection with virulent strains.

Lentogenic strains

These include naturally occurring strains of low virulence and are less widely distributed than the velogenic strains. They are harmless though some may cause mild respiratory symptoms and other reactions. They are effective for day old chicks and very young birds. The optimum dose is between $10^{6.5}$ - 10^{7} EID 50/bird. These vaccines are generally administered by dust spray, in drinking water, intranasal or intraocular methods.

Strain B_1

This strain is a naturally occurring lentogenic strain identified while screening 105 strains of ND virus in United states by Beaudette. In 1948 Hitchner and Johnson, reported that this strain of low virulence could be used for immunizing fowls against ND. Beaudette's ND strain is known today as the B_1 strain of Hitchner. B_1 strain is used as Newcastle disease vaccine either intranasally or in drinking water (Poultry Biologics, 1963).

This strain is also termed as Blacksburg (Lancaster, 1964). In young chicks clinical effects produced by this B_1 strain depended largely on the route or method of vaccination (Lancaster, 1964). Thus administration by intranasal or conjunctival drop or in drinking water resulted in little or no clinical effect (Mitchner and Johnson, 1948; Winterfield et al. 1957; Baggi and Lee, 1962) but may be more marked in some instance (Doll et al. 1950)

Eidson and Kleven (1976) reported that B_1 strain of ND virus could be administered to one day old chicks subcutaneously, intratracheally, intraocularly or as aerosols and all the methods resulted in increased protection and higher antibody titers.

Ratnaparkhe et al. (1981) reported that B_1 strain by oculo-nasal route in three weeks old chicks gave 100% protection by challenge at the age of 6th, 9th and 11th week and by drinking water the percentage of protection was 95.33 and that for incontact birds 85.7.

In susceptible pullets, vaccination with B_1 virus caused a drop in egg production varying from negligible (Mitchner and Johnson, 1948; Kran et al. 1959) to 20-50%

for two weeks to four weeks (Bell et al. 1950).

The duration of immunity after initial vaccination of day old chicks by intranasal route was four months as determined by 100% survival after challenge (Hitchner and Johnson, 1948; Winterfield et al. 1957).

Initial vaccination at 1 to 7 days of age to 2 weeks and revaccination at the 4th and 20th weeks with B_1 strain gave satisfactory and adequate immunity (Lancaster, 1964).

Strain LaSota

This strain was isolated from the farm of Adam LaSota, in United states in 1946 (Beaudette et al. 1949). LaSota strain is not only used as Newcastle disease vaccine by intranasal and drinking water methods but also as intramuscular vaccine. This lentogenic virus differed from B_1 and F strains in its lower mean death time for chick embryos (Anon, 1959) a greater spreading potential (Marek, 1960) increased postvaccination respiratory symptoms (Winterfield et al. 1957) and a higher serological response by drinking water administration. As a result it was recommended that the LaSota strain could be used at 18 weeks of age to revaccinate chickens which received B_1 or F vaccine at an earlier age (Winterfield et al. 1957).

A clone selected LaSota strain of ND virus reported to be more immunogenic than the B_1 strain while having the same pathogenic index but less immunogenic than the regular LaSota strain (Eidson and Kleven, 1980). The haemagglutination inhibition (HI) titers induced in chickens inoculated with the cloned LaSota strain were higher than those induced in chickens vaccinated with the B_1 strain but were found to be slightly less than the titers recorded in the regular LaSota strain.

The clone-selected LaSota strain had essentially the same spreading potential as the regular LaSota strain as indicated by geometric mean titers and the mortality of challenged uninoculated chickens which were allowed to mingle with the vaccinated chickens. Oral, ocular or aerosol vaccination of maternally immune chickens with the clone-selected LaSota strain gave essentially the same protection as those vaccinated with the regular LaSota strain (Eidson and Kleven, 1980).

According to Borland and Allan (1980) LaSota strain is more heterogenous. Ratnaparkhe et al. (1981) reported that the LaSota strain when given to three weeks old chicks by oculonasal route produced 100% protection as revealed by challenge and the percentage of protection

noted by drinking water method was 100.

CDF_{66} Strain

Malik et al. (1969) isolated a strain of ND virus from the respiratory tract of a pig in Madhya Pradesh and the same was designated as CDF_{66}. These authors reported that the virus strain was completely safe to young chicks and the inoculated chicks showed complete protection against a virulent virus. In a further study this strain showed a satisfactory immunity when the vaccinated chicks were challenged at 60 to 90 days of vaccination (Malik and Dhawedkar, 1970).

Tanwani and Malik, (1978) claimed that the CDF_{66} strain had been used in over 16 lakhs of birds under laboratory and field conditions and was found to be a suitable vaccine against ND. It was well-tolerated by birds of all age groups when given by intranasal and intramuscular routes and not even a single instance of post-vaccinal reaction was recorded. The strain CDF_{66} produced sufficient protection against challenge with Muktesswar virulent virus. The over all protection percentage ranged from 33.88 to 84.70.

Sinha and Malik (1978) reported that birds infected

with CTF_{66} intramuscularly excreted the virus through nasal and tracheal secretions for 20 days and through faeces for 10 days. The authors also observed that the viability of the virus at different temperatures was up to eight months at 4°C, 5 weeks at 25°C, 15 days at 37°C and 12 hours at 45°C.

Samuel et al. (1978) recorded that single vaccination with CTF_{66} strain at hatch could induce only marginal protection to challenge at six weeks of age. Double vaccination with CTF_{66} strain at birth intranasally and intramuscularly at six weeks of age induced very good protection and HI antibody titer correlated with protection.

Tanwani and Malik (1981) were of opinion that the CTF_{66} liquid virus was quite stable at different temperatures. The embryo infectivity was positive at temperatures 4°C, 25°C, 37°C and 43°C for 12 months, 5 weeks, 20 days and 132 hours respectively. Even the haemagglutinins were also quite thermostable. The virus almost showed the same HA titer when the allantoic fluid was held at 37°C for 26 days and at 45°C for 156 hours. Tanwani et al. (1982) further reported the lentogenic strain CTF_{66} of Ranikhet disease as an excellent vaccine as the vaccine was completely devoid of any post vaccinal reactions, afforded life

long immunity, protected birds by interference phenomenon and could be used in the face of an outbreak of the disease. Further they could notice no adverse effect of vaccination on egg production.

Ratnaparkhe et al. (1981) observed that this strain inoculated by oculonasal route could produce 100 per cent protection on challenge but was only 80.66 when administered through drinking water.

Strain F

Asplin (1952) described the suitability of ND virus F strain as a vaccine virus. Anon (1959) was of opinion that strain F closely resembled B_1 in many of its properties and was suitable for the vaccination of chickens of all ages. Single vaccination with F at hatch induced only marginal protection to challenge at six weeks of age. F strain was reported to produce mild respiratory symptoms in young chickens and laying birds (Asplin, 1952; Asplin et al. 1952) but had no effect on egg production (Asplin, 1952; Rao and Agarwal, 1960). However slight nervous and respiratory symptoms in four months old birds were reported by Russeff and Miteff, 1956 and Thorne and Macleod, (1960).

Mesogenic strains

These strains are more virulent than the lentogenic strains, and not recommended for chicks below six to eight weeks of age or for adult birds having no basal immunity. The optimum dose for these vaccines is 10^{5} EID 50 per bird and are given by parentral route (wingweb, feather follicle or intramuscular). Mesogenic strains generally produce long lasting immunity (Buxton and Fraser, 1977).

Strain H.K 107

Attenuated by serial passage in chicken and chicken embryos following isolation (Clancy et al. 1949; Markhams et al. 1949).

Hertfortshire or Herts 33

Isolated from an outbreak of ND in Hertfortshire, England and attenuated by 14-33 passages in chicken embryos (Iyer and Dobson, 1940).

Roakin

A naturally occurring mesogenic strain identified while screening 105 strains of ND virus in United States (Beaudette et al. 1949)

Komarov (K) strain

Isolated in Palestine from an outbreak of ND associate with 80% mortality. Serial intracerebral passages through ducklings (Komaroff and Goldschmidt, 1946 a) modified the virus to the point that adult fowls showed no symptoms after injection and remained healthy when exposed to the virulent virus, 18 days later (Komarov and Goldsmit, 1946 b). The Komarov strain was well tolerated by growing chicken over four weeks of age (Komarov and Goldsmit, 1947).

Based on pathogenicity, the Komarov strain was preferred to the Mukteswar strain (Komarov and Goldsmit, 1947). But a few cases of young chicken in poor condition developed paralysis which lasted for four weeks (Crowther, 1952; Thorne and Macleod, 1960).

Samuel et al. (1979) reported that the mean death time of embryos inoculated with K strain, was 47 hours. These authors also found that the intracerebral pathogenicity index in day old chicks was 1.00 for an eight days observation period.

Serial passage of the Komarov strain in bovine kidney tissue culture had attenuated the neuropathogenicity to the

level of lentogenic strain without loss of antigenicity (Huygelen and Peetermans, 1969).

Ali (1978) reported that the birds vaccinated intranasally with K strain at the age of four weeks resisted challenge with a virulent strain up to one year. Further the HI titers of the birds were also high. There was no significant reaction by intranasal inoculation of the vaccine strain under study for three weeks after vaccination.

Mukteswar R_2B strain

Evolved by Haddow and Idnani (1946) by repeated passage in chicken embryos of a virulent strain isolated from natural outbreaks in India. The strain got attenuated by about 115-126 passages. Mukteswar strain was developed by Banda et al. (1958) and Gupta and Rao (1959) and had been widely used in Asia, (Seetharaman 1951 a, b., Anon, 1960). In young chicks this virus produced a severe reaction and some mortality (Haddow and Idnani, 1946; Gupta and Rao 1959) which may reach up to 30% (Rao and Agarwal, 1960). Though the strain was considered nonpathogenic to six week old chicks (Haddow and Idnani, 1946; Banda et al. 1958), these authors reported occasional mortality ranging

from 1.5 to 6% in this age groups. According to Seetharaman and Sinha (1965) and Chakravarthy et al. (1981) post-vaccinal reactions to the extent of 4-21% was noticed even in chicks with base immunity with a lentogenic strain. The immunity produced with R_2B against virulent strain was for 9-15 months by subcutaneous route (Maddow and Idnani, 1946; Seetharaman, 1951 a; Ockalowa et al. 1955) and 6-8 months by intramuscular route (Banda et al. 1958). Vilakantan et al (1960) opined that there was little difference in the antigenicity of this strain and Komarov strain. As with other mesogenic viruses the Mukteswar strain caused a marked reduction in egg production by about 10-16% which lasted for 1 to 6 weeks. One of the Muktesswar stock of vaccine virus had shown increased pathogenicity to White Leghorn chickens causing 56% mortality compared to a 4% in Rhode Island Reds (Wandi, 1955).

M T strain

This mesogenic strain of ND virus was isolated from the trachea of an ailing mynah (Kulachana et al. 1981) from Trichur, Kerala. These authors also reported that this isolate differed in some properties from that of the Komarov strain and was less virulent. The preliminary

studies carried out by the above authors also revealed that it could induce sufficient immunity in six weeks old chicks to withstand challenge with a virulent virus.

Potency test

The criteria used to assess the potency of the ND vaccines were the reduction in mortality, immunity to systemic infection as evidenced from lack of clinical symptoms and drop in egg production (Lancaster, 1964). The immune status could be studied by haemagglutination inhibition (HI) test and had been documented by Lancaster (1963 b). But, Rao and Agarwal (1969) were of opinion that HI antibody response could not be compared with the immune status as in the case of challenge with virulent virus. Seetharaman and Sinha (1963) opined that the potency of the vaccine could be assessed by the survival of the vaccinated and the death of the control.

Nilakantan et al. (1969) reported that embryo infectivity test combined with the haemagglutination (HA) test could serve as a substitute for the present method of direct challenge in the potency testing of ND vaccines. These authors further added that their test was less costly and less time consuming.

Roepke (1973) reported that the virus content per field dose, measuring the antibody response in vaccinated birds or determining the ability of vaccinated birds to withstand challenge with a virulent virus are the common methods of potency testing of ND vaccines. However, he was of opinion that protective values of a vaccine could not be predicted from the virus content or antibody level because of the difference in properties between and even with n the various strains of ND virus used for vaccine preparation. Beard and Max Brugh (1977) opined that HI test could be effectively used to monitor the immune response of chickens to ND vaccine and it is economic and reliable. By employing this test the need to challenge the birds with virulent virus could also be avoided.

Aygraich et al. (1978) and Borland and Allan (1980) reported that multipoint challenge assay could be effectively used to compare the performance of various ND vaccine strains by measuring the amount of challenge virus withstood by 50% of the vaccinated birds.

Thornton and Hebert (1980) opined that the potency of live ND vaccines could be assessed by multi-point challenge assay in vaccinated chicks. This method was shown to give

reproducible results and the protective capacities of various NP vaccines.

MATERIALS AND METHODS

MATERIALS AND METHODS

Newcastle disease virus

Strain MT

The mesogenic strain of Newcastle disease virus (strain MT) isolated from the trachea of an ailing mynah, at the 10th passage level in chick embryo was received from Dr.S.Sulochana, Department of Microbiology. This isolate on receipt was passaged once in 10 day embryonated eggs by the allantoic route before it was used for experimental purpose.

Komarov (K) strain

This strain of ND virus now being used for vaccine preparation in Kerala was received from the Institute of Veterinary Preventive Medicine, Ranipet. The virus was once passaged in 10 day embryonated egg before use.

F strain

Day old chicks were inoculated with the vaccine prepared with F strain of ND virus. The required dose of the vaccine was received from the Veterinary Biological Institute Palode, Trivandrum District.

Challenge virus (strain V)

Virulent strain of ND virus received from IVRM Ranipet was used for all the challenge experiments after passaging once in ten day embryonated hens eggs. In all cases the challenge dose was 2 x 10^7 ELD 50/bird.

Chicken embryos

White leghorn eggs at the 10th day of incubation received from the University Poultry Farm Mannuthy were used throughout this study.

Chicks

Day old unvaccinated chicks for the first experiment were from the University Poultry Farm Mannuthy and for experiment II and III were from the Regional Poultry Farm Malampusha. All chicks were reared on deep litter system under uniform management practice.

Tryptose phosphate broth (Difco)

The powder was reconstituted as per the manufacturers instructions and then sterilised by autoclaving at 15 lbs pressure for 30 minutes and antibiotics such as penicillin (500 IU/ml) and streptomycin (500 microgram/ml) were added (TPB-A).

Collection of specimens for virus isolations

Throat and cloacal swabs

Throat and cloacal swabs collected from the birds showing clinical symptoms after vaccination were soaked in PBS-A and stored at -20°C until used for inoculation into developing chick embryos.

Tissues

Tissues such as spleen, lung, liver and brain collected under aseptic conditions from dead birds, were preserved at -20°C in PBS-A.

Processing of specimens

Cloacal and throat swabs

Swabs soaked in PBS-A and stored at -20°C were removed from the deep freeze and allowed to thaw at room temperature. Then squeezing of the swabs was done with a sterile pipette for a couple of minutes and centrifuged at 1000 g for 15-20 minutes at 4°C. The clear fluid was collected and the residue was discarded. The supernatant clear fluid was incubated at 37°C for one hour and inoculated into 10 day embryonated eggs by allantoic route.

Tissues

At the time of use the tissues were emulsified in TPB-A with the help of a Tenbroeck tissue grinder to obtain 10-15 per cent (W/V) suspension. In order to remove the coarse particles the tissue homogenate was centrifuged at 1000 g for 15-20 minutes. The clear supernatent fluid was separated, incubated for one hour and then inoculated into the allantoic cavity of 10 day embryonated eggs.

Chick embryo inoculation (Hovenne and Burke, 1973)

After ascertaining the viability of 10 day old chicken embryos the air cell region and the head of embryo was marked. Sterilization of the air cell region was done with tincture of iodine and a hole was made using a dental drill about one centimeter from the margin of the air cell towards its center.

One in 10 dilution of the seed virus (K², K or V) was prepared in TPB-A and 0.2 ml was inoculated into the allantoic cavity with a tuberculin syringe fitted with a 20 guage needle. The hole was then sealed with melted paraffin and incubated at 37°C with the broad end up. Attention was also given to provide 55-60 per cent humidity (Hoskins, 1967) in the incubator. Candling of the eggs

was carried out daily and the embryonated eggs that died after 24 hours of incubation were transferred to the refrigerator for chilling.

Chicken RBC

Blood collected from the wing vein of chicken in Alsever's solution was centrifuged at 2000 rpm for 10 mts and the red blood cells collected. These RBCs were again washed twice in normal saline and resuspended in the required concentration of 5% or 0.5% for Haemagglutination tests.

Harvesting of the Allantoic fluid

The air cell region of the chilled embryonated eggs were disinfected with alcohol. The shell at this region was cut and removed with sterile scissors. The shell membrane and the chorioallantoic membrane were reparated and the allantoic fluid was harvested with sterile pipettes free of erythrocytes. The fluid from each embryonated egg was tested for haemagglutinating activity. The allantoic fluid was then centrifuged at 2000 rpm for 15 minutes and the clear supernatant stored in 3 ml quantities at -20°C until used.

Pathotyping of MT and K strains

Embryo lethal dose 50

Serial ten fold dilutions of either MT or K strain were made in TPB-A from 10^{-7} to 10^{-11}. Each dilution was then inoculated into the allantoic cavity of 10 day embryonated eggs using three eggs per dilution. The eggs were incubated, then candled daily and those that were dead after 24 hours of inoculation were chilled at 4°C and the allantoic fluid was harvested as described above. The allantoic fluid from each egg was tested for haemagglutination activity. Fifty per cent embryo lethal dose was calculated as per the method described by Reed and Muench (1938).

Mean death time (MDT)

Serial ten-fold dilutions of MT or K were prepared from 10^{-1} to 10^{-11} in TPB-A. The last four dilutions were inoculated into 10 day embryonated eggs at the rate of 0.1 ml per embryo by allantoic cavity route using eight eggs for each dilution. All the embryos were incubated as described earlier. The eggs were candled at twelve hours intervals and observations of death and time were recorded. From this, the mean death time was calculated using the formula given in Poultry Biologics (1963).

Intracerebral patnogenicity index (ICPI)
(Hanson, 1975)

Ten, day-old unvaccinated chicks were inoculated with 0.1 ml of a 1 in 10 dilution of the MT or K strain into the cerebral cortex using a 25guage needle. Five, day old unvaccinated chicks which acted as controls were inoculated with 0.1 ml of sterile normal saline intracerebrally. These chicks were kept separately and observed daily for developments of symptoms or deaths till the 8th day. The survival index was based on the time of death and calculated by scoring the factor zero for normal, one for diseased and two for dead. The sum total of all the factors was divided by the number of observations.

Intravenous pathogenicity index (IVPI) in six week old chickens

Eight, six week old unvaccinated chicks were inoculated with 0.1 ml of 1 in 10 dilution of the embryo propogated MT or K strain subcutaneously. Unvaccinated control chicks were inoculated with 0.1 ml of normal saline by the same route. The chicks were housed separately. They were observed for 15 days for the development of clinical symptoms, paralysis, or deaths. The factor for each obser-

vation was zero for normal, one for clinical signs, two for paralysis and three for deaths. Serum samples were collected from birds that did not manifest any of the above clinical signs and were screened for the presence of HI antibodies.

Heat stability of infectivity

Chick embryo propogated virus (allantoic fluid) was distributed in 1 ml volumes into sterile screw capped vials and were submerged in water bath at 56°C and one vial each at various intervals (5, 10, 15, 20 and 30 mts) were transferred to the freezing chamber of the refrigerator. Samples were then assayed for infectivity and haemagglutinin as described below.

Serial ten fold dilutions of each heat treated sample was made in TPB-A and inoculated into the allantoic cavity of 10 day embryonated eggs in 0.2 ml quantities using three eggs per dilution. Postincubation, candling harvesting and infectivity titrations were made as described for titrations of the virus.

Stability of haemagglutinin (Hanson, 1975)

The virus exposed to 56°C at various intervals were

tested for its HA activity and compared to that of untreated sample. Haemagglutination was carried out as described below:

Haemagglutination test (Poultry Biologics, 1963)

Two-fold dilutions of the virus were prepared in normal saline solution in the wells of the perspex plates. An equal quantity of 0.5 per cent washed chicken RBC was added to each dilution and mixed properly. Simultaneous RBC and saline controls were set up side by side. Then the haemagglutination plates were incubated at room temperature for 30-45 minutes. The readings were recorded after the controls had settled.

Detection of HI antibodies

Whole blood was collected by the paper strip method of Nobuto (1967) and evaluated by the method of Beard and Max Brugh (1977) for haemagglutination inhibition antibodies.

Whatman filter paper No.1 was cut into small strips of 20 x 40 mm size which were used for collecting the blood samples.

While collecting the blood the wing vein was punctured

with a hypodermic needle. One tip of the paper strip was passed through the large drop of blood formed on the wing vein surface. When the strip was saturated with blood the strip was placed in penicillin vials and dried at room temperature. The sample area of the blood samples strips was cut into small bits with scissors. The antibody activity in dried strips was eluted in 0.5 ml of normal saline. After the small pieces were soaked and agitated well, the blood samples were kept at 4°C in a refrigerator for complete elution. The next day the paper strips were squeezed with the help of a pasteure pipette and eluted serum samples were used for haemagglutination inhibition tests using 8 HA units of Kumarov strain as the antigen.

Haemagglutination inhibition (HI) test
(Poultry Biologics, 1963)

The Beta procedure of HI test was employed throughout the studies. After ascertaining the HA titer of the virus, the required quantity of 8 HA units of the virus was prepared in normal saline. Serial double fold dilutions of (0.2 ml each) the paper strip elutes were prepared in normal saline and each of these dilutions was well mixed with 0.2 ml of the 8 HA units of the virus and incubated at room

temperature for 30 minutes. After the incubation time 0.4 ml of 0.5% suspension of washed chicken RBC was added to each well and mixed. Simultaneous virus and RBC controls were made. Following incubation for 30 minutes at room temperature the HI antibody titer was taken as the highest dilution of the sera in which there was complete inhibition of HA. The initial dilution of the paper strip eluted samples was taken as 1:20.

Experimental infection and immunization trials

Experiment I (Table 1)

A total of 90 white Leghorn day old unvaccinated chicks were utilized for this experiment. These chicks were grouped into six, each consisting of 15 chicks. The treatments of various groups were as follows:

Group 1

The chicks were inoculated by oculonasal route at the rate of $10^{6.5}$ ELD 50 per bird with MT strain. They were observed for any clinical symptoms or death and were bled at weekly intervals from the second week onwards. Eight of the survivors were given a secondary inoculation with the same virus at the rate of $2 \times 10^{6.5}$ ELD 50 for each

chick. The remaining chicks were challenged with a virulent strain. A rise in antibody titer in these birds were determined by collecting the sera at weekly intervals for six more weeks. At the end of this period the chicks were challenged with the virulent strain and were observed for three more weeks.

Group 2

This group of 15, day old unvaccinated chicks was treated the same way as above except that the primary inoculation was with F strain at the rate of 10^7 EID 50 per chick.

Group 3

Treatments were same as chicks in group 2 but the second inoculation was with Komarov strain at the rate of 2×10^7 EID 50 per bird.

Group 4

Chicks in this group were given 10^7 EID 50 K strain per chick by the oculonasal route. Further treatments, HI antibody titration, challenge, secondary inoculation, detection of rise in antibody titer etc. were the same as birds in group 3.

Group 5

This group of chicks was treated the same way as above except that the second inoculation was with MT strain at the rate of 2 x $10^{6.5}$ ELD 50 per bird.

Group 6

This group of chicks was kept uninfected till the 6th week. At the 6th week five of them were challenged and the remaining chicks were given MT strain and observed for any clinical symptoms or death. Antibody titers were also tested for six weeks and at the end of this period they were challenged to study the protection if any produced by the MT strain.

Experiment II (Table 2)

Forty unvaccinated three week old chicks were divided into four groups of ten each. The experimental studies of different groups were as follows:

Group 1

The chicks were inoculated subcutaneously with MT strain of NIW at the rate of 5 x $10^{6.5}$ ELD 50 per chick.

Group 2

Experimental infection was by oculonasal route with

the same virus as in group 1 but the dose was reduced to $10^{6.5}$ ELD 50 per chick.

Group 3

Route of inoculation was same as in group 1, but the strain of NDV used was Komarov (5×10^{7} ELD 50/chick)

Group 4

Treatment was the same as in group 2 but with Komarov strain.

All the four groups of chicks were kept in separate sheds sufficiently spaced so that there was no chance for cross infection. They were observed closely for development of any clinical symptoms and death. Serum samples were collected at weekly interval for six weeks to detect any increase in HI antibody titer. At the end they were challenged with the virulent virus and observed for three more weeks.

Experiment III (Table 3)

A total of 280, six weeks old chicks vaccinated with F strain at the day of hatch were utilized. They were randomly screened for the presence of HI antibodies and were divided into four groups of 70 each.

Group 1 was given strain NT intramuscularly (I/M) at the rate of 5 x $10^{6.5}$ ELD 50 per bird. While group 2 received the same by subcutaneous route (S/C). Group 3 and 4 received Komarov strain I/M and S/C respectively. The dose per bird was 5 x 10^{7} ELD 50. All the four groups were kept in separate pens and watched closely. Serum samples were collected from 10 randomly selected birds to detect HI antibodies at weekly intervals for a period of six weeks. After this, 10 chicks from each group were challenged with the virulent virus.

Contact transmission

On the day of second inoculation, 8 unvaccinated age matched birds were kept along with each group. They were also bled at weekly intervals for six weeks and at the end challenged with virulent virus.

Cloacal and throat swabs were collected from sick birds and processed for virus isolation. When there was mortality all the dead birds were autopsied and tissues collected for virus isolation.

Controls

Ten age matched unvaccinated chicks were used as controls for each experiment. In experiment 1, five chicks each were challenged at the 6th and 12th week along with their experimental counterparts, while in II and III all the control chicks were challenged at the 9th and 12th week respectively along with the vaccinated birds.

RESULTS

RESULTS

Pathotyping of viruses

MT and K strains

These strains of NDV for the experiments in this study were characterised before they were used. The results of various tests of pathotyping are presented in table 4.

Embryolethal dose 50 (ELD 50)

The ELD 50 of the MT and K strains in developing chick embryos were $10^{9.5}$/0.2 ml and $10^{10.5}$/0.2 ml respectively as determined by Reed and Muench (1938) method. The details are given in table 5 and 6. All the dead embryos had characteristic lesions of NDV infection such as haemorrhages in the occipital region, interdigital space and under aspect of the abdomen.

Mean death time (MDT)

The mean death time of the minimum lethal dose was calculated for MT and K strains as 87 hours and 76.5 hours respectively (Table 7 and 8). The rate of death due to MT strain was as follows. One died at 72 hours, four at 84 hours and three at 96 hours of incubation.

The MDT of A strain was also calculated similarly from the following observations. One embryo died at 60 hours, three at 72 hours and four at 84 hours of incubation. All the dead embryos of both the strains showed specific lesions of ND as described before.

Intracerebral pathogenicity index (ICPI)

Intracerebral pathogenicity index determined by inoculating ten, day old chicks with 0.1 ml each of 1:10 dilution of either MT or A strain are given in tables 9 and 10.

The chicks that received the MT strain were normal for the first two days of infection. On the third day one chick showed clinical symptoms of drowsiness, paralysis of wings, inappetance, diarrhoea and was in sleeping posture. The other chicks were normal. Next day one chick was found dead and another two exhibited symptoms while others were normal. On the 5th day of infection two more chicks were seen dead and another two showed symptoms but the remaining ones were normal. On the 6th day the two sick chicks were found dead and one more chick became sick. On the 7th and 8th day one chick each was found dead. The remaining three chicks were normal.

The ICPI was calculated to be 0.63

Two of the chicks inoculated with K strain showed clinical signs on the first day itself and on the second day of inoculation these two were found dead and another five exhibited clinical signs. On the third day of inoculation five more chicks were seen dead. On the 5th day one more chick showed symptoms while others were normal. The next day one chick was found dead and the remaining two chicks were normal. The ICPI was calculated to be 1.16.

The control chicks inoculated intracerebrally with normal saline was normal throughout the observation period.

Intravenous pathogenicity index

The intravenous pathogenicity index of MI and K strains was studied in six week old chicks. None of the chicks that received either MI or K strain did manifest any clinical symptoms and remained to till the end of the observation period and the IVPI was calculated to be as zero. Sera collected from birds inoculated with MI strain and HI antibody titers ranging from 1:512 to 1:1024 and those received K ranged from 1:4 to 1:256.

Thermostability of infectivity

The strain is considered heat labile if the infectivity titer was decreased by two logarithm (base 10) by heating at 56°C for 10 minutes or less. MT strain was heat labile as two log reduction in the infectivity titer was noticed within 10 minutes. A similar reduction in the titer of A strain was noticed only after heating at 56°C for 20 minutes.

Heat stability of haemagglutinin

The haemagglutinating progerty of MT strain was completely lost within 5 minutes at 56°C. But the haemagglutinins of K strain was comparatively resistant as it took 20 minutes for complete loss of HI activity.

Experimental infection and immunisation trials

Experiment I

Group 1

Out of the 15, day old chicks that received $10^{6.5}$ ELD 90 of MT virus by oculonasal route at the day of hatch three chicks died without specific symptoms of ND and four died after showing clinical symptoms of drowsiness, paralysis

of wings, inappetance and diarrhoea. The virus was reisolated from a few birds. The percentage of mortality was 26.6%.

All the three chicks that were challenged after primary vaccination withstood challenge so also all the chicks six weeks after second vaccination. The percentage of protection was calculated to be 100 during primary as well as secondary inoculation with R2 virus. Following first inoculation the peak titer of 118.9 was reached at the end of 4th week. The geometric means of HI titers after the second inoculation were20.63; 21.33; 142.7; 174.5; 95.13 and 41.25 for the 1st, 2nd, 3rd, 4th, 5th and 6th week. The HI antibody titer reached maximum during 3rd to 5th week with the peak value of 174.5 at the end of 4th week. Details of antibody respon e in this group are presented in table 11 and graph 1.

Group 2

Three chicks from the total of 15 chicks in this group that received F strain died without any specific symptoms of ND. Five birds challenged at the sixth week did not manifest any clinical symptoms or death. There was a rise in the geometric means of the HI titers by the

end of the 2nd week, which declined by the fifth week. After the second inoculation with hT strain, the geometric mean of the HI titer was in the increase reached the peak level at the end of 3rd week and then gradually declined. The geometric mean of the HI titers were 20.75; 82.94; 127.0; 106.8; 89.78 and 45.82 respectively for the 1st, 2nd, 3rd, 4th, 5th and 6th week (Table 12; Graph 2). The percentage of protection was 100 during primary and secondary inoculation.

Group 3

There was no mortality in this group either specific or non-specific. The geometric mean of HI antibody titers was maximum at the end of 4th week and a sudden decrease was noticed by the 5th week. Booster dose with K strain enhanced the antibody level and the maximum level was obtained by the 3rd week and then gradually declined. No post-vaccinal reactions were noticed and all the chicks withstood challange six weeks after the administration of K strain (Table 13; Graph 3). The percentage of protection was 100 in primary and secondary inoculation.

Group 4

Five of the chicks that received K strain manifested

symptoms, suggestive of ND, but recovered after four days. The geometric means of HI antibody titers following primary vaccination were 18.26; 23.35, 4.648 and 4.373 for the 3rd, 4th, 5th and 6th week respectively. Peak level was at the fourth week and then declined. A second dose of K strain did not induce any post-vaccinal reactions. A steady rise in antibody titers from the first week onwards was noticed giving a maximum titer at the 4th week after the second administration. The highest titer obtained at the 4th week was 114.0. On challenge all the birds survived giving a protection percentage of 100 (Table 14; Graph 4).

Group 5

Two of the chicks that received K strain at hatch manifested clinical symptoms of ND. One of them died on the 3rd day and the virus could be isolated from this chick and the other recovered and had sufficient antibodies to withstand challenge after four weeks. Second inoculation with MT strain induced sharp rise in antibody titer which reached its peak at the 2nd week. Again five chicks died without specific symptoms of ND. The HI titer remained more or less uniform from the second to the fourth week and then declined slowly. All the 4 chicks that were challenged six weeks

after secondary inoculation with MT strain survived without any clinical symptoms (Table 15;Graph 5).

Group 6

Chicks in this group did not receive any virus at hatch. They were free from HI antibody. Six chicks died without any specific symptoms of ND. All the five chicks that were challenged at the 6th week succumbed to ND and virus could be isolated from these birds. The remaining chicks which received MT strain did not manifest any clinical symptoms of ND. A rapid and sharp rise in HI antibody titer which reached the peak level at third week and then decline was noticed. None of the four chicks that were challenged died of ND (Table 16; Graph 6).

Experiment II

A comparative pathogenicity and immunogenicity of MT and K strains were also studied in three week old unvaccinated chicks. None of the chicks that received MT strain either by subcutaneous or oculonasal route manifested any clinical symptoms of ND. HI antibodies were demonstrable in both the groups from the 1st week onwards. The geometric mean of HI titers of the chicks that received the virus by subcutaneous

route (group 1) were 41.93; 49.23; 149.3; 80.00; 60.61; and 39.11 respectively for the 1st, 2nd, 3rd, 4th, 5th and 6th week following inoculation. While it was 52.77; 60.61; 211.1; 74.64; 48.15 and 30.31 for the same period with chicks in group 2. In both the cases the peak titers were obtained on the third week and then there was a decline. On challenge after the sixth week none of the chicks succumbed to infection. All of them survived giving a protection percentage of 100 (Table 17 & 18; Graph 7 & 8). Similar observations were also made with chicks in groups 3 and 4 where the inoculation was done with A strain by subcutaneous and oculonasal routes respectively. The geometric mean of HI titers for chicks in group 3 were 28.28; 45.94; 139.3; 74.64; 60.61; 35.89 and that for group 4, 45.94; 64.98; 149.3; 43.20; 34.81 and 26.44 respectively for 1st, 2nd, 3rd, 4th, 5th and 6th week respectively. All the chicks withstood challenge with a virulent strain and the percentage of protection was 100 (Table 19 &20; Graph 9 & 10).

Experiment III

The suitability of MT strain as a vaccine strain was studied by inoculating 70, six week old chicks, either

subcutaneously or intramuscularly. These chicks were vaccinated at hatch with F strain. The immunogenicity and the protective effect of this strain was compared with that of Komarov strain.

All the 70 chicks that received the MT strain of NDV by intramuscular route, did not manifest any postvaccinal reactions and all of them survived. The geometric mean of HI antibody titers of 10 randomly selected chicks from this group were 26.80; 36.50; 394.0; 105.5; 49.23 and 15.19 for the 1st, 2nd, 3rd, 4th, 5th and 6th week respectively. Maximum titer was obtained during the 3rd week which declined thereafter. The percentage of protection was 100 (Table 21; Graph 11).

Antibody response in chicken to this virus by subcutaneous route was also in the same pattern, though the titer was slightly low. The geometric mean of the titers for the six weeks being 34.85; 85.53; 129.9; 112.9; 91.89 and 49.25. None of the ten chicks that were randomly selected and challenged manifested any clinical signs of the disease and all of them survived. The percentage of protection was 100 (Table 22; graph 12).

Out of the 70 chicks that received K strain intra-

muscularly two showed clinical symptoms of ND and died between the third and fifth day. The symptoms manifested by these birds were general depression, gasping and paralysis. On postmortem examination lesions characteristic of ND such as pin point haemorrhages in the proventriculus and caecal tonsils were evident.. Virus could also be isolated from the spleen, liver and lung of these chicks. The percentage of reaction was calculated to be 2.9. The remaining vaccinated chicks were quite normal and survived throughout the experimental period. All the ten chicks that were challenged from this group also withstood challenge. Antibody rise was slightly higher than in group 4. The geometric means of the HI titers were 31.77; 74.63; 105.1; 85.76; 69.85 and 35.85 for the 1st, 2nd, 3rd, 4th, 5th and 6th week after inoculation. All the ten chicks that were challenged did not manifest any clinical symptoms of ND. The percentage of protection was 100 (Table 23; Graph 13).

Chicks in group 4 that received A strain by subcutaneous route did not show any post-vaccinal reaction as in group 3. The geometric means of HI antibody titers for the six weeks following inoculation were 23.02; 45.94; 91.89; 69.65; 42.86 and 13.22 (Table 24; Graph 14). As in the case of group 3 all the chicks withstood challenge and the

percentage of protection was 100.

Contact transmission

The transmissibility of MT and K strain was studied by keeping unvaccinated age matched control chicks along with the inoculated chickens. None of the contact chicks kept along with groups 1 and 2 showed any clinical symptoms. There was no significant rise in antibody titers of these contact chicks. Maximum titer was obtained between 3rd and 4th week giving a geometric mean of 17.5 at the end of 4th week and the titers declined thereafter. On challenge six chicks out of 8, died of typical ND and virus could be isolated from these birds. The percentage of contact transmission in this case was calculated to be 25. One of the unvaccinated contact chicks in group 2 ,died of non-specific causes. The geometric mean of the peak titer obtained in these birds was 21.68 and was seen during the 4th week. On challenge, 2 out of 7 chicks survived while all the remaining five died of ND and virus was isolated from these birds. The percentage of contact spread was calculated to be 28.6.

One of the contact chicks that was kept along with birds in group 3 died on the 16th day of exposure after

manifesting symptoms of ND such as droopiness, off feed, paralysis of the legs and wings, dullness with a tendency to stand in sleeping posture and diarrhoea. Virus could be isolated from the cloacal and throat swabs collected from this bird after death. Virus isolation was also possible from the tissues such as liver, spleen and lung. When remaining chicks were challenged six of them survived. The percentage of contact transmission this group was 75. Though there was no death or clinical manifestation of ND in contact chicks of group 4, 62.5% spread to in contact birds was noticed in this case. In both the groups the antibody titers in contact chicks were lower than that of chicks in groups 1 and 2.

In all the challenge experiments, the control chicks succumbed to ND showing characteristic symptoms in five days time. Postmortem examination of these chicks revealed typical lesions of ND and virus could be isolated from the tissues.

Table 1. Details of treatments of day old chicks in experiment I

Group	Primary inoculation					Secondary inoculation			
	Number of chicks used	Strain	Route	Dose per bird	Number of chicks challenged	Strain	Route	Dose per bird (in ELD_{50}s)	Number of chicks challenged
Group 1	15	MT	ON*	$10^{6.5}$ ELD_{50}	3	MT	SC**	$2x10^{5.5}$	5
Group 2	15	F	,,	10^{7} ELD_{50}	5	MT	,,	$2x10^{6.5}$	7
Group 3	15	F	,,	10^{7} ELD_{50}	5	K	,,	$2x10^{7}$	10
Group 4	15	K	,,	10^{7} ELD_{50}	5	K	,,	$2x10^{7}$	10
Group 5	15	K	,,	10^{7} ELD_{50}	5	MT	,,	$2x10^{6.5}$	4
Group 6	15	..	..	..	5	MT	,,	$2x10^{6.5}$	4

* Oculonasal route

** Subcutaneous route

Table 2. Details of treatments of three week-old unvaccinated chicks in experiment II

Group	Number of chicks used	Strain	Route	Dose per bird (in EID_{50} s)	Number of chicks challenged
Group 1	10	MT	SC*	$5 \times 10^{6.5}$	5
Group 2	10	MT	ON**	$1 \times 10^{6.5}$	5
Group 3	10	K	SC	5×10^{7}	5
Group 4	10	K	ON	1×10^{7}	5

* Subcutaneous route

** Oculonasal route

Table 3. Details of treatments of day old chicks in experiment III

Group	Primary inoculation						Secondary inoculation			
	No. of chicks used	Strain	Route	Dose per bird in EID_{50}s	No. of chicks challenged	No. of chicks survived/percentage	Strain	Route	Dose per bird in EID_{50}s	No. of chicks challenged
Group 1	70	F	ON*	10^{7}	10	3/90	MT	IM**	$5x10^{6.5}$	10
Group 2	70	F	ON*	10^{7}			MT	SC£	$5x10^{6.5}$	10
Group 3	70	F	ON*	10^{7}			K	IM**	$5 x10^{7}$	10
Group 4	70	F	ON*	10^{7}			K	SC£	$5 x10^{7}$	10

* Oculonasal route

** Intramuscular route

£ Subcutaneous route

Table 4. Pathotyping of Newcastle disease virus strains MT and K

Characteristics	Results of MT	Results of K
Embryo lethal dose$_{50}$	$10^{9.5}$/0.2 ml	$10^{10.5}$/0.2 ml
Mean death time	87 hours	76.5 hours
Intracerebral pathogenicity index	0.63	1.16
Intravenous pathogenicity index	0.00	0.00
Heat stability of infectivity	10 minutes	20 minutes
Heat stability of haemagglutinin	$\leq$ 5 minutes	20 minutes

Table 5. Embryo lethal dose$_{50}$ of the MT strain

Dilution	Number of eggs	Number of eggs positive	Number of eggs negative	Cumulative values: Positive	Cumulative values: Negative	Ratio positive	Percentage positive
10^{-7}	3	3	0	9	0	9/9	100
10^{-8}	3	3	0	6	0	6/6	100
10^{-9}	3	3	0	3	0	3/3	100
10^{-10}	3	0	3	0	3	0/3	0*
10^{-11}	3	0	3	0	6	0/6	0

* Fifty per cent infectivity between dilution nine and ten

Proportionate distance $= \frac{100-50}{100-0} = \frac{50}{100} = 0.5$

Embryo lethal dose $_{50} = 10^{9 + 0.5} = 10^{9.5}$ / 0.2 ml

Table 6. Embryo lethal dose $_{50}$ of the K strain

Dilution	Number of eggs	Number of eggs positive	Number of eggs negative	Cumulative values		Ratio positive	Percentage positive
				Positive	Negative		
10^{-7}	3	3	0	12	0	12/12	100
10^{-8}	3	3	0	9	0	9/9	100
10^{-9}	3	3	0	6	0	6/6	100
10^{-10}	3	3	0	3	0	3/3	100 *
10^{-11}	3	0	3	0	3	0/3	0

* Fifty per cent infectivity between dilution ten and eleven

$$\text{Proportionate distance} = \frac{100-50}{100-0} = \frac{50}{100} = 0.5$$

$$\text{Embryo lethal dose}_{50} = 10^{10+0.5} = \frac{10^{10.5}}{0.2 \text{ ml}}$$

Table 7. Mean death time of the minimum lethal dose of MP strain

Dilution	10^{-8}	10^{-9}	10^{-10}	10^{-11}
Death in hours				
12	0	0	0	0
24	0	0	0	0
36	0	0	0	0
48	0	0	0	0
60	3	0	0	0
72	4	1	0	0
84	1	4	0	0
96	0	3	0	0

Mean death time of the minimum lethal dose $= \frac{(72x1) + (84x4) + (96x3)}{8}$

$= \frac{696}{8} = 87$ hours

Table 8. Mean death time of the minimum lethal dose of K strain

Dilution	10^{-8}	10^{-9}	10^{-10}	10^{-11}
Death in hours				
12	0	0	0	0
24	0	0	0	0
36	0	0	0	0
48	1	1	0	0
60	5	4	1	0
72	1	1	3	0
84	1	2	4	0

Mean death time of the minimum lethal dose $= \frac{(60\times1) + (72\times3) + (84\times4)}{8}$

$= \frac{612}{8} = 76.5$ hours

Table 9. Intracerebral pathogenicity index of HP strain

Days / Observations	1	2	3	4	5	6	7	8	Sum	Factor	Sum X Factor
Death	0	0	0	1	3	5	6	7	22	2	44
Signs	0	0	1	2	2	1	1	0	7	1	7
Normal	10	10	9	7	5	4	3	3	51	0	0
Total	-	-	-	-	-	-	-	-	80	-	51

Intracerebral pathogenicity index = $\frac{51}{80}$ = 0.63

Table 10. Intracerebral pathogenicity index of K strain

Days / Observations	1	2	3	4	5	6	7	8	Sum	Factor	Sum X Factor
Death	0	2	7	7	7	8	8	8	31	2	62
Signs	2	5	0	0	1	0	0	0	8	1	8
Normal	8	3	3	3	2	2	2	2	21	0	0
Total	-	-	-	-	-	-	-	-	60	-	70

Intracerebral pathogenicity index = $\frac{70}{60} = 1.16$

Table 11. Results of experimental infection and immunization trials of day old chicks - group 1.

Weeks	Average HI titers	Geometric mean HI titers	No. of chicks inoculated	No. of chicks challenged	Mortality.	Percentage of protection
1	1.5147	32.71	15	..	..	..
2	1.5778	37.83	..	..	..	..
3	1.9783	95.35	..	..	..	..
4	2.0751	118.9	..	..	..	..
5	1.8659	73.43	..	..	..	..
6 (Booster)	1.1518	14.90	5	3	Nil	100
7	1.3144	20.65	..	..	..	..
8	1.3290	21.33	..	..	..	..
9	2.1543	142.7	..	..	..	..
10	2.2417	174.5	..	..	..	..
11	1.9783	95.13	..	..	..	..
12	1.6154	41.25	..	5	nil	100

Graph 1

HI antibody titers of day old chicks at weekly intervals - group 1

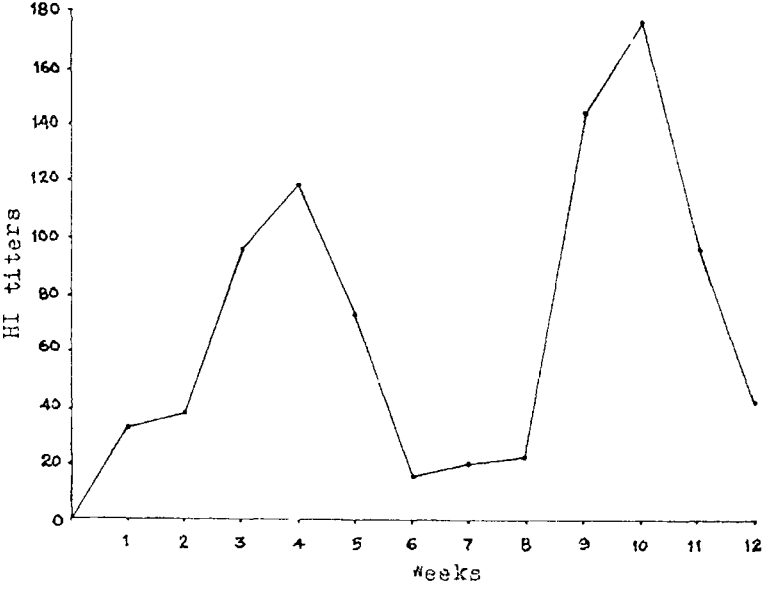


Table 12. Results of experimental infection and immunisation trials of day old chicks - group 2.

Weeks	Average HI titers	Geometric mean HI titers	No. of chicks inoculated	No. of chicks challenged	Mortality	Percentage of protection
1	0.7394	5.488	15	..	..	..
2	0.8088	6.439	..	..	..	..
3	0.5624	3.651	..	..	..	..
4	0.2001	1.585	..	..	..	..
5	Nil	Nil	..	..	..	..
6(Booster)	Nil	Nil	.8	5	Nil	100
7	1.3178	20.75	..	..	..	..
8	1.9188	82.94	..	..	..	..
9	2.1037	127.0	..	..	..	..
10	2.0.285	106.8	..	..	..	..
11	1.9532	89.78	..	..	..	..
12	1.6611	45.82	..	7	Nil	100

Graph 2

HI antibody titers of day old chicks at weekly intervals - group 2

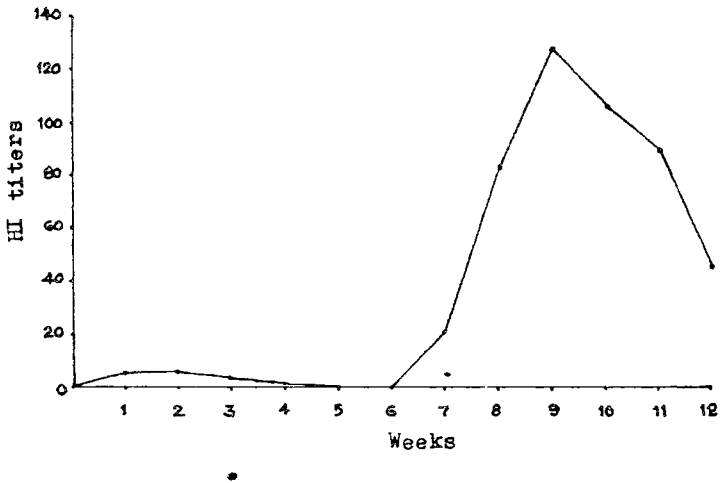


Table 13. Results of experimental infection and immunization trials of day old chicks - group 3.

Weeks	Average HI titers	Geometric mean HI titers	No. of chicks inoculated	No. of chicks challenged	Mortality	Percentage of protection
1	Nil	Nil	15	..	..	..
2	Nil	Nil	..	..	..	..
3	0.1935	1.562	..	..	..	..
4	0.4333	2.172	..	..	..	..
5	Nil	Nil	..	..	..	..
6(Booster)	Nil	Nil	10	5	Nil	100
7	1.2479	17.70	..	..	..	..
8	1.8564	71.65	..	..	..	..
9	1.9367	86.44	..	..	..	..
10	1.8765	75.25	..	..	..	..
11	1.4952	31.27	..	..	..	..
12	1.3884	24.45	..	10	nil	100

Graph 3

HI antibody titers of day old chicks at weekly intervals - group 3

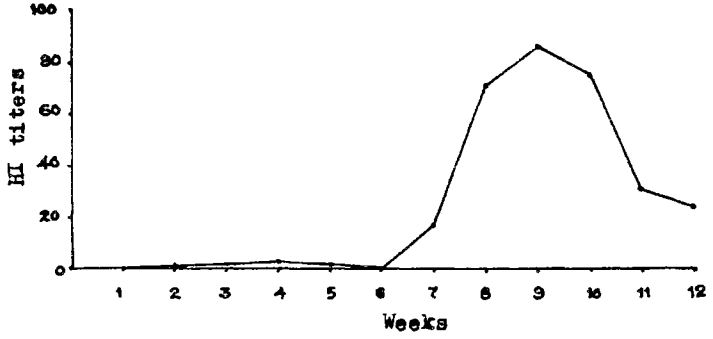


Table 14. Results of experimental infection and immunization trials of day old chicks - group 4.

Weeks	Average HI titers	Geometric mean HI titers	No. of chicks inoculated	No. of chicks challenged	Mortality	Percentage of protection
1	Nil	Nil	15	..	..	..
2	Nil	Nil	..	..	..	..
3	1.2615	18.26	..	..	..	..
4	1.3683	23.35	..	..	..	..
5	0.6673	4.648	..	..	..	..
6(Booster)	0.6408	4.373	10	5	Nil	100
7	1.3418	21.97	..	..	..	..
8	1.4758	29.91	..	..	..	..
9	1.6830	48.19	..	..	..	..
10	2.0571	114.0	..	..	..	..
11	1.8564	68.61	..	..	..	..
12	1.7031	50.47	..	10	Nil	100

Graph 4

HI antibody titers of day old chicks at weekly intervals - group 4

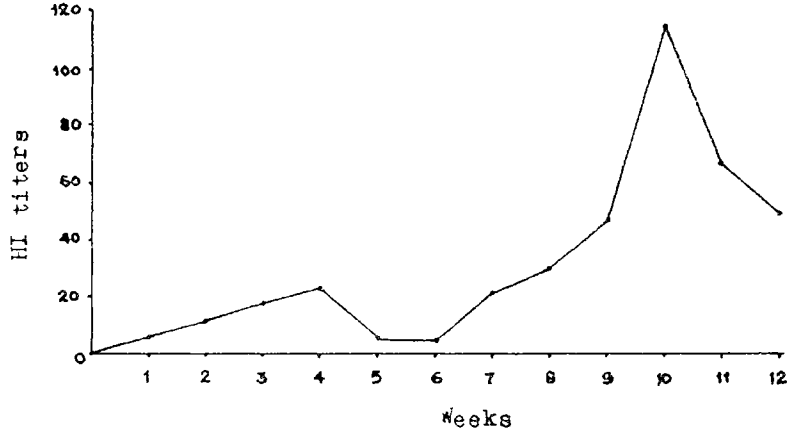


Table 15. Results of experimental infection and immunization trials of day old chicks - group 5.

Weeks	Average HI titers	Geometric mean HI titers	No. of chicks inoculated	No.of chicks challenged	Mortality	Percentage of protection
1	Nil	Nil	15	..	..	..
2	0.7081	5.106	..	..	..	..
3	0.5936	3.922	..	..	..	..
4(Booster)	0.4861	3.063	9	5	Nil	100
5	2.1238	132.9	..	..	..	..
6	2.4649	291.7	..	..	..	..
7	2.1055	126.9	..	..	..	..
8	2.0703	117.6	..	..	..	..
9	1.8696	74.05	..	..	..	..
10	1.5686	37.03	..	4	Nil	100

Graph 5

HI antibody titers of day old chicks at weekly intervals

group 5

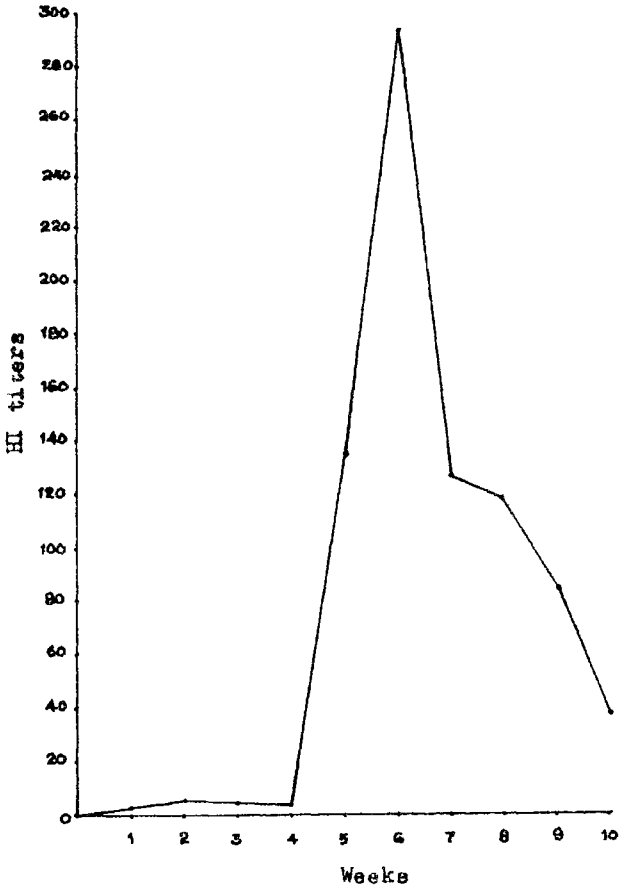


Table 16. Results of experimental infection and immunization trials of day old chicks - group 6.

Weeks	Average HI titers	Geometric mean HI titers	No. of chicks inoculated	No. of chicks challenged	Mortality	Percentage of protection
1	Nil	Nil	15	..	..	..
2	,,	,,	..	..	..	..
3	,,	,,	..	..	..	..
4	,,	,,	..	..	..	..
5	,,	,,	..	..	..	..
6	Vaccinated ,,	,,	5	5	5	0
7	1.7826	60.61	..	..	..	..
8	2.8061	639.8	..	..	..	..
9	2.8663	750.0	..	..	..	..
10	2.0837	121.30	..	..	..	..
11	2.0643	116.0	..	..	..	..
12	2.0536	113.2	..	4	Nil	100

Graph 6

HI antibody titers of day old chicks at weekly intervale

group 6

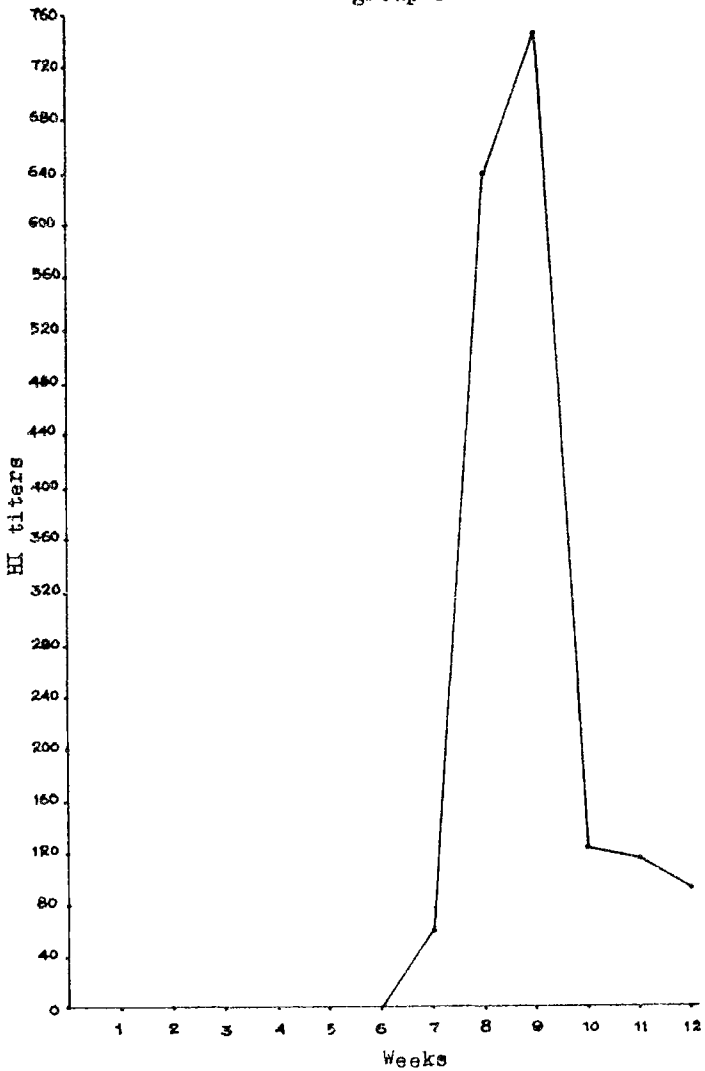


Table 17. Results of experimental infection and immunization trials of three week-old chicks - group 1.

Weeks	Average HI titers	Geometric mean HI titers	No. of chicks inoculated	No. of chicks challenged	Mortality	Percentage of protection
1	NT	NT	..	..	..	..
2	NT	NT	..	..	..	..
3 Inoculated	"	"	10	..	..	..
4	1.6224	41.93	..	..	..	..
5	1.6923	49.25	..	..	..	..
6	2.174	149.3	..	..	..	..
7	1.9051	80.00	..	..	..	..
8	1.7826	60.61	..	..	..	..
9	1.5923	39.11	..	5	Nil	100

NT : Not tested

Graph 7

HI antibody titers of three week old chicks at weekly intervals - Group 1

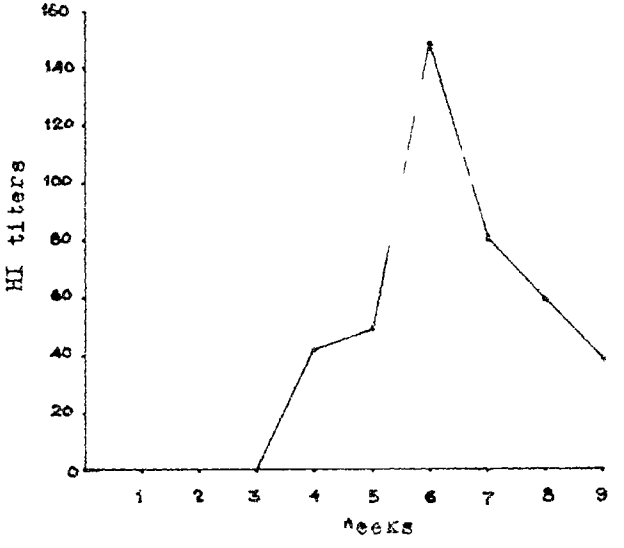


Table 18. Results of experimental infection and immunization trials of three week-old chicks - group 2.

Weeks	Average HI titers	Geometric mean HI titers	No. of chicks inoculated	No. of chicks challenged	Mortality	Percentage of protection
1	NT	NT	..	..	..	..
2	NT	NT	..	..	..	..
3 Inoculated	"	"	10	..	..	..
4	1.7224	52.77	..	..	..	..
5	1.7826	60.61	..	..	..	..
6	2.3245	211.1	..	..	..	..
7	1.8729	74.64	..	..	..	..
8	1.6826	48.15	..	..	..	..
9	1.4816	30.31	..	5	Nil	100

NT : Not tested

Graph 8

HI antibody titers of three week old chicks at weekly intervals - group 2

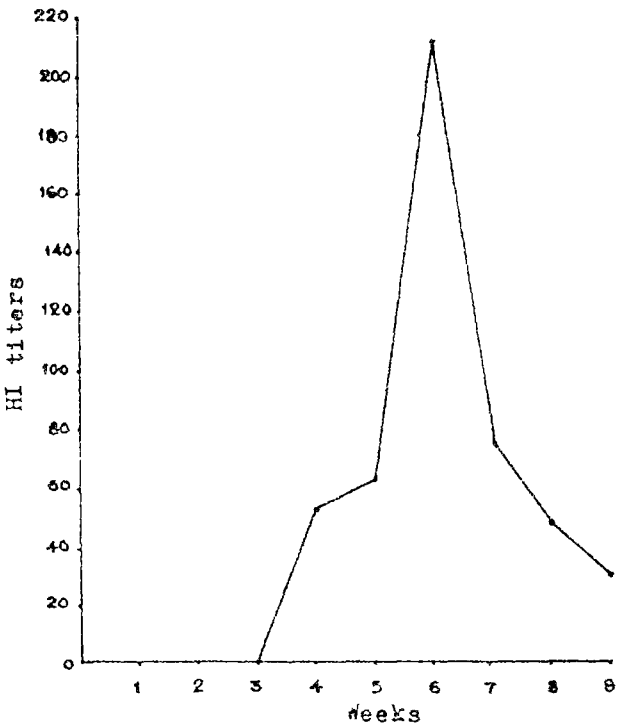


Table 19. Results of experimental infection and immunization trials of three week-old chicks - group 3.

Weeks	Average HI titers	Geometric mean HI titers	No. of chicks inoculated	No. of chicks challenged	Mortality	Percentage of protection
1	NT	NT	..	..	..	..
2	NT	NT	..	..	..	..
3 Inoculated	"	"	10	..	..	..
4	1.4515	28.28	..	..	..	..
5	1.6622	45.94	..	..	..	..
6	2.1439	139.3	..	..	..	..
7	1.8730	74.64	..	..	..	..
8	1.7826	60.61	..	..	..	..
9	1.5525	35.69	..	5	Nil	100

NT : Not tested

Graph 9

HI antibody titers of three week old chicks at weekly intervals - group 3

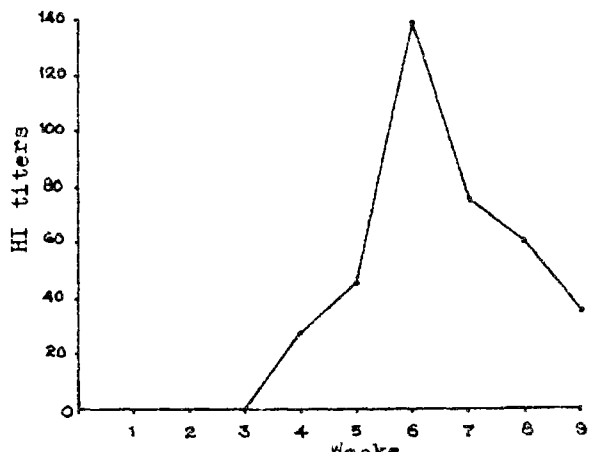


Table 20. Results of experimental infection and immunization trials of three week-old chicks - group 4.

Weeks	Average HI titers	Geometric mean HI titers	No. of chicks inoculated	No. of chicks challenged	Mortality	Percentage of protection
1	NT	NT	..	..	..	..
2	NT	NT	..	..	..	..
3 Inoculated	"	"	10	..	..	..
4	1.6622	45.94	..	..	..	..
5	1.8727	64.98	..	..	..	..
6	2.174	149.3	..	..	..	..
7	1.6355	43.20	..	..	..	..
8	1.5418	34.81	..	..	..	..
9	1.4214	26.44	..	5	Nil	100

NT : Not tested

Graph 10

HI antibody titers of three week old chicks at weekly intervale - group 4

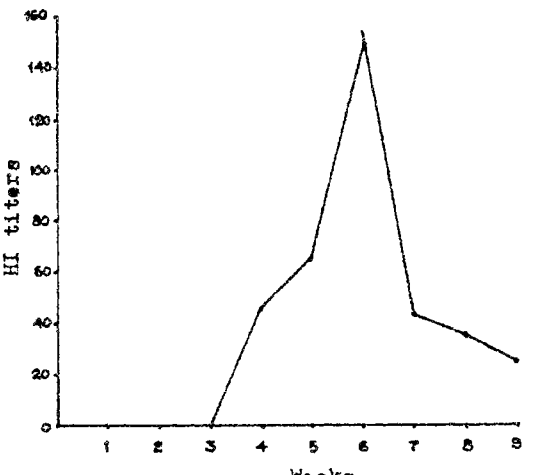


Table 21. Results of experimental infection and immunisation trials of six week-old chicks - group 1.

Weeks	Average HI titers	Geometric mean HI titers	No. of chicks inoculated/ No. of chicks survived in 21 days	No. of chicks challenged	Mortality	Percentage of protection
1 F strain	NT	NT	..	..	..	..
2	"	"	.	..	..	..
3	"	"	..	..	..	..
4	"	"	..	..	..	..
5	"	"	..	..	..	..
6(Booster)	"	"	70/70	..	..	..
7	1.4117	26.80	..	..	..	..
8	1.5622	36.50	..	..	..	..
9	2.5954	394.0	..	..	..	..
10	2.0235	105.5	..	..	..	..
11	1.6923	49.23	..	..	..	..
12	1.1816	15.19	..	10	Nil	100

NT : Not tested

Graph 11

HI antibody titers of six week old chicks at weekly intervals - group 1

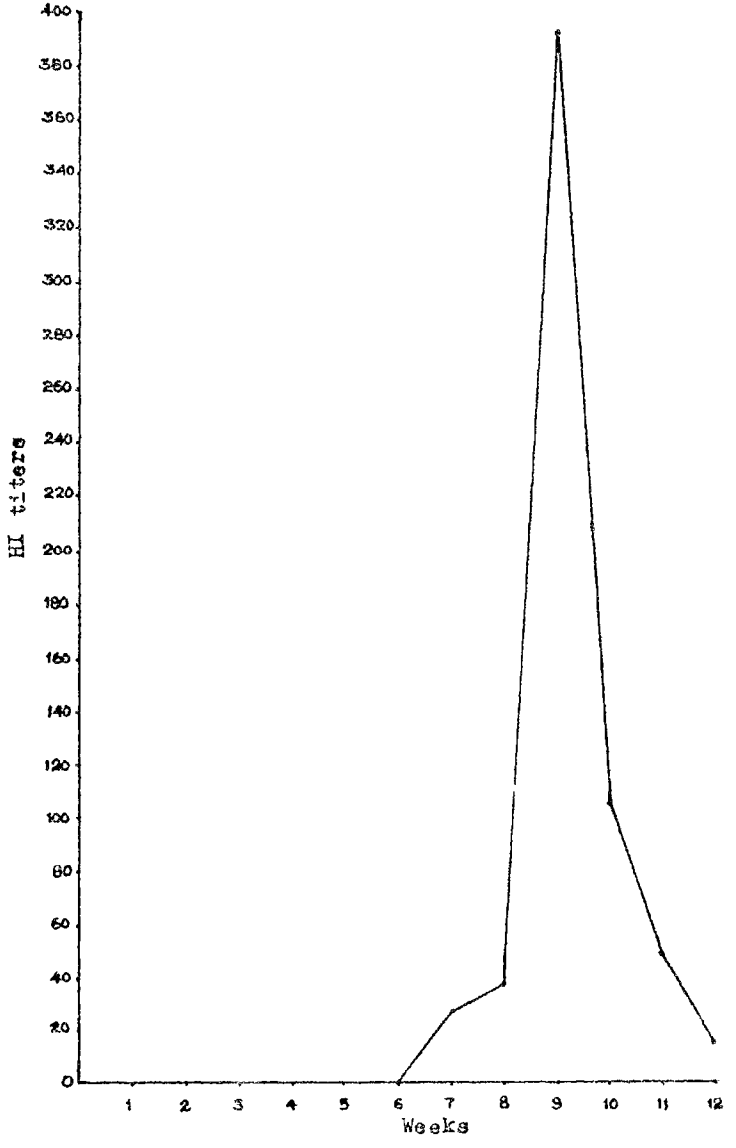


Table 22. Results of experimental infection and immunization trials of six week-old chicks - group 2.

weeks	Average HI titers	Geometric mean HI titers	No. of chicks inoculated/ No. of chicks survived in 21 days	No. of chicks challenged	Mortality	Percentage of protection
1 F strain	NT	NT	..	..	..	..
2	"	"	..	..	..	..
3	"	"	..	..	..	..
4	"	"	..	..	..	..
5	"	"	..	..	..	..
6(Booster)	"	"	70/70	..	..	..
7	1.5418	34.83	..	..	..	..
8	1.9631	85.53	..	..	..	..
9	2.1138	129.9	..	..	..	..
10	2.0536	112.9	..	..	..	..
11	1.9633	91.89	..	..	..	..
12	1.6923	49.23	..	10	Nil	100

NT : Not tested

Graph 12

HI antibody titers of six week old chicks at weekly intervals - group 2

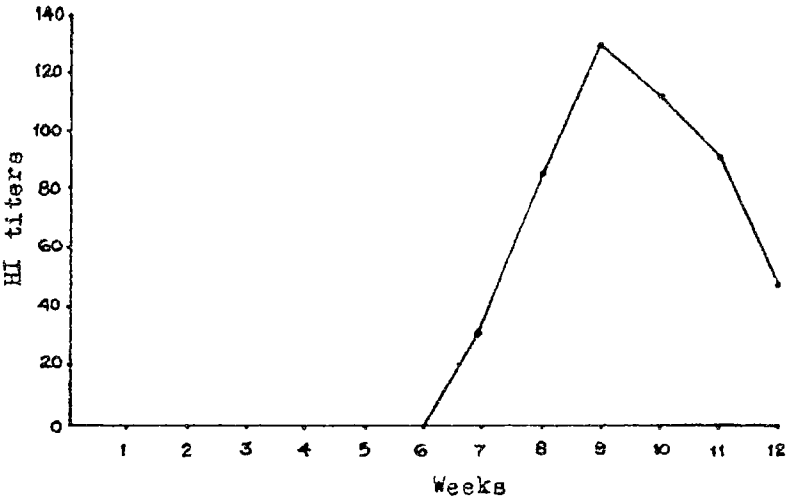


Table 23. Results of experimental infection and immunization trials of six week-old chicks - group 3.

weeks	Average HI titers	Geometric mean HI titers	No. of chicks inoculated/ No. of chicks survived in 21 days	No. of chicks challenged	Mortality	Percentage of protection
1 F strain	NT	NT	..	..	.	..
2	"	"	..	..	..	..
3	"	"	..	..	..	..
4	"	"	..	..	..	..
5	"	"	..	..	..	..
6 (Booster)	"	"	70/68	..	..	..
7	1.5020	>1.77	..	..	..	..
8	1.8729	74.63	..	..	..	.
9	2.0235	105.1	..	..	..	..
10	1.9331	85.76	..	..	..	..
11	1.8429	69.65	..	..	..	..
12	1.5416	35.65	..	10	Nil	100

NT : Not tested

Graph 13

HI antibody titers of six week old chicks at weekly intervals - group 3

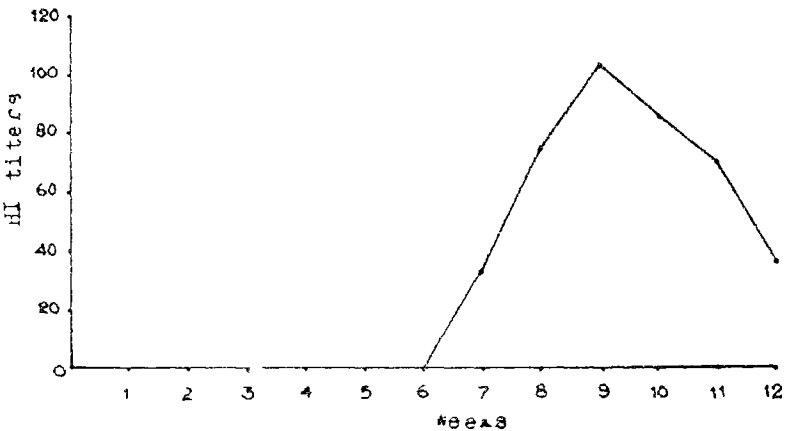


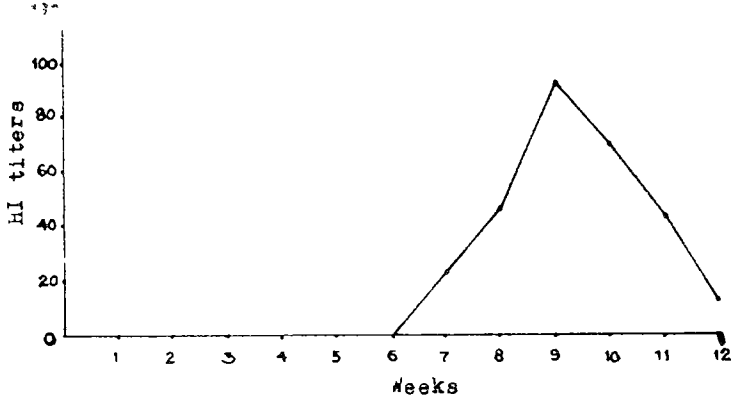
Table 24. Results of experimental infection and immunization trials of six week-old chicks - group 4.

Weeks	Average HI titers	Geometric mean HI titers	No. of chicks inoculated/ No. of chicks survived in 21 days	No. of chicks challenged	Mortality	Percentage of protection
1 F strain	NT	NT	..	..	..	..
2	"	"	..	..	..	..
3	"	"	..	..	..	..
4	"	"	..	..	..	..
5	"	"	..	..	..	..
6 (Booster)	"	"	70/70	..	..	..
7	1.3622	23.02	..	..	..	..
8	1.6622	45.94	..	..	..	..
9	1.9633	91.89	..	..	..	..
10	1.8429	69.65	..	..	..	..
11	1.6321	42.86	..	..	..	..
12	1.1214	13.22	..	10	Nil	100

NT : Not tested

Graph 14

HI antibody titers of six week old chicks at weekly intervals - group 4



DISCUSSION

DISCUSSION

Newcastle disease is virtually world wide in distribution and various methods of prophylaxis such as quarantine, compulsory slaughter of ailing and in contact birds and systematic vaccination had been implemented either singly or combined. Because of the frequency with which ND occurs in many concentrated poultry producing areas, systematic vaccination is accepted by most of the countries for control of this disease and has been adopted voluntarily.

Newcastle disease vaccines are of two kinds, live and inactivated. The inactivated vaccines were the first to be studied (Beaudette, 1943), though the use and preparation of live vaccine were started in the early 1930s (Lopacio, 1954; Iyer and Dobson, 1940).

It has been the general experience that the inactivated vaccines confer only a temporary resistance and injections have to be repeated approximately three months intervals. Even then 'breaks' may occur. Moreover, under some conditions and in some geographical areas live vaccines possessed advantageous and thus resulted in extensive use. Live vaccines are produced from naturally occurring strains of acceptable low level of pathogenicity or from strains that were

selectively attenuated by serial passage either in embryonated hen eggs or some non gallicine hosts (Asplin, 1952; Allan, 1975).

Pathotyping of viruses

On the basis of MDT at terminal dilution, ICPI and IVPI, these strains may be either lentogenic or mesogenic, the former being the less pathogenic used in day old chicks while the latter in chicks above six weeks of age. The mesogenic strains are used usually in chicks with a base immunity with some lentogenic strains.

All the mesogenic strains of ND virus are lethal to chicken embryos irrespective of route of inoculation, temperature of incubation and age of the embryo. However, presence of antibodies may interfere with multiplication if given into the allentoic cavity at the 16th day of incubation.

Newcastle disease virus, usually produces a high titer in chick embryos giving an ELD_{50} of 10^{9}/ml (Poultry Biologics, 1963). The MT strain of ND virus which is a new isolate also multiplied at the same extent giving a titer of $10^{9.5}$/ 0.2 ml. Similar result was also obtained with the known mesogenic strain K.

Mean death time at terminal dilutions is one of the criteria used for pathotying an isolate of ND virus. The time taken by lentogenic, mesogenic and velogenic strains are over 100 hours; 60-90 hours and below 50 hours respectively (Poultry Biologics, 1963). In this study the new isolate MT had a MDT of 87 hours while that of F strain was 76.5 hours. The mean death time reported for other mesogenic strain was 62 hours for Beaudette C (Hanson et al. 1973; Spalatin et al. 1973); 68.8 hours for R_2B (Chandra et al. 1972); 64 hours for Roakin and 65 hours for Mk 107 respectively (Poultry Biologics, 1963). In contrast to the observation made in this study and that of Sulochana et al. (1981), Samuel et al. (1979) obtained a low MDT of 47 hours for the F strain. This MDT of 47 hours is nearing the value of a velogenic strain and this discrepancy cannot be explained.

Newcastle disease virus strains also differ in their neuropathogenicity to day old chicks. Lentogenic strains rarely kill the chicks and the virus cannot be transmitted serially in day old chicks by the brain material. The mesogenic strain on the other hand kills all the chicks within a period of eight days. Velogenic strains also kill all the chicks but at a faster rate. The values for

lentogenic, mesogenic and velogenic strains were 0.1 to 0.2; 0.8-1.5 and $\angle$ 1.5 respectively (Poultry Biologics, 1963). The ICPI for day old chicks in the case of MP and K strain was 0.63 and 1.16 respectively. Other known mesogenic strains such as Beaudette C; M2B; Roakin and MK 107 had the above value as 1.46 (Hanson et al. 1975; Spaletin et al. 1973) 1.06 (Chandra et al. 1972); 0.8 and 1.5 (Poultry Biologics, 1963). Samuel et al. (1979) obtained a value of 1.00 as ICPI for K strain. According to Marczewski et al. (1969) the intracerebral pathogenicity index of K strain varied between 0.37 and 1.05.

Intravenous pathogenicity index is the criterian used to find out the difference in lethality of the virus for chicken by extra neural route. The lentogenic and mesogenic strains usually produce specific antibodies without producing the disease associated with NDV infection while the velogenic strains kill an appreciable number of chickens into which a minute quantity of virus is introduced (Poultry Biologics, 1963).

The IVPI reported for the lentogenic, mesogenic and velogenic strains are 0.0; 0.05 to 0.31 and $\angle$ 1.5 in the order. The strains of the virus used in this study did not kill or produce any clinical manifestation of the virus

infection. Hence IVPI for both the strains was 0.0. On the other hand other mesogenic strains such as Beaudette C and R2B had higher values being 1.23 (Hanson, et al. 1973 and Spalatin et al. 1973) and 0.19 (Chandra et al. 1972) respectively. However Alexander and Allan (1974) were of opinion that a better differentiation between the isolates could be obtained by mean death time of chicks following infection by cloacal swab.

The results obtained with the above criteria used for pathotyping of NDV isolates, have clearly shown that the MT strain, is a mesogenic strain. Moreover the values also show that this strain is less virulent than other mesogenic strains referred, particularly to the K strain which is presently being used as the vaccine strain in Kerala.

The haemagglutinins of MT and K strains were inactivated in less than five minutes and in 20 minutes respectively at 56°C. Only a marginal reduction in the HA titer was reported by Samuel et al. (1979) with K strain at 56°C in 60 minutes. While complete loss of HA activity in 30 minutes was reported for R2B by Chandra et al. (1972)

The thermostability of HA is a stable character and

is retained essentially unchanged through many serial passages (Chu, 1948; DiGioia et al. 1970). Hanson, et al. (1949) reported that the virulence of a strain of ND virus was appeared to be related to the thermostability of the haemagglutinin, as the haemagglutinin of a group of virulent strains resisted heating at 56°C for 30-120 minutes while that of avirulent strains were destroyed in five minutes or less. According to Hanson and Spalatin (1978) no such relationship existed between these two factors. Westerbury and Waddington (1978) opined that the thermostability of HA and infectivity need not be considered as criteria for typing a strain as velogenic, mesogenic or lentogenic as they could observe the presence of both stable and labile population in the same strain.

The biological characteristics described above for MT and K strain show that these two are distinct strains. Though both of them are classified as mesogenic the former is less virulent than the latter. Mesogenic strain R2B which is also used in some parts of India as a vaccine strain is also different from the MT isolate as evinced from its characteristic cited above.

Experimental infection and immunization trials

Experiment 1.

Infection of day old chicks by oculonasal route at the rate of $10^{6.5}$ ELD 50 per bird showed that it was pathogenic to this age group with a mortality rate of 26.6%. The infection was confirmed by virus isolation from the dead birds. Sulochana et al. (1981) reported a higher percentage (46%) of mortality. This discrepency could probably be due to the lesser number of passages the isolate MY had gone through in developing chick embryos. In the present study virus at the 10th passage level was used while Sulochana et al. (1981) used the virus when it was at the 6th passage. The virulence of the virus might have reduced by serial passage in chick embryos. Some strains of ND virus had shown to reduce its virulence by serial passage in chick embryos, without effecting its antigenicity. Mesogenic vaccine strains such as R_2B (Haddow and Idnani, 1946); Beaudette 72 (Reeve et al. 1974); Herts 33 (Iyer and Robson, 1940) were evolved in this way. The possibility of the infected day old chicks possessing a reasonable titer of HI antibody cannot be ruled out as they were not screened for maternal antibodies.

The chicks that survived had GMTs ranging from 32.71, 37.83, 95.35, 118.9, 73.43 and 14.9 for the 1st, 2nd, 3rd, 4th, 5th and 6th week respectively. So the peak titer was obtained between 3rd and 5th week. None of the survived chicks that were challenged six weeks after the primary inoculation succumbed to the disease. On second inoculation with the same virus the chicks did not manifest any clinical symptoms. A sharp rise in antibody titer was noticed and the titers were higher than in primary inoculation and all of them withstood challenge after six weeks. These observations suggest that NF strain of RNV is a good immunogen.

Birds that received primary vaccination with F strain at the day of hatch MF produced sufficient HI titers when given as a booster after six weeks. The HI titers were compared with that produced by K strain and it was seen that the F strain produced slightly higher titers to a though statistically not significant. Though pathotyping revealed that MF is less virulent than K, the latter was found to be less pathogenic to day old chicks. Though 23.5% of them had clinical symptoms only 5.3% died and remaining chicks recovered within four days. This discrepancy in pathogenicity of MF and K strain to day

old chicks in contrast to pathotyping could probably be due to the presence of maternal antibody in chicks infected with K strain as this batch of chicks was a different one from that used for MT strain and interference by congenital antibodies in NDV is a well established factor (Lancaster, 1966). Markham et al. (1951) reported that the immune response to primary vaccination and revaccination could be suppressed in birds possessing residual antibody and this could be attributed to the low HI titers obtained in this group. According to Gibson and Cleven (1976) birds with maternal antibody were more effectively protected against challenge when they were vaccinated by aerosol or ocular method. None of the six week old unvaccinated chicks manifested any clinical symptoms or death following infection with either MT or K strain.

The results obtained in experiment 1 indicated that MT strain is pathogenic to day old chicks. Its immunogenicity is comparable with that of K strain not only in the HI antibody titers in the serum but also in the capacity of vaccinated birds to withstand challenge with a virulent strain. The comparison between different combination of

strains revealed that, though there is no significant difference in the HI values MT has a higher mean value than K, the mean being 2.048 and 1.9012 respectively.

Experiment II

Experimental infection of three week old unvaccinated chicks by subcutaneous or oculonasal route with MT or K strain revealed that both were well tolerated by the chicks without manifesting any clinical symptoms. The immune response of these chicks was also similar and was comparable to that obtained in experiment I. The G.M's of HI antibodies in chicks receiving MT by S/C route, were 41.93, 49.23, 149.3, 80.00, 60.61 and 39.11 for the 1st, 2nd, 3rd, 4th, 5th and 6th week respectively. The peak titer of 149.3 was obtained at the end of 3rd week. A slightly higher values were obtained for birds in group II that received the virus by oculonasal route. Similar results were also obtained with K strain. However the titers obtained with MT strain were always higher, though statistically not significant. Chicks in all the four groups withstood challenge giving 100% protection.

Analysis of variance of HI titers in different weeks showed no significant difference between the four groups.

However, Mann Whitney U test has shown that MT had higher mean than K being 1.8491 and 1.7564 respectively.

Sulochana et al. (1982) observed a mortality of 24% and 20% by S/C and oculonasal route respectively with MT strain in three week old chicks. This could be due to the reasons given above for experiment I.

Experiment III

The usefulness of MT as a vaccine strain was studied by inoculating 70 chicks each by I/M or S/C route. Primary immunization of these chicks were done with strain F. The effect of strain MT was compared with strain K. The evaluation of immunogenicity and protective effect of these vaccines were done by titrating the HI antibodies at weekly intervals and their capacity to resist challenge with a virulent virus.

The maximum GMTs of HI antibodies in chicks that received MT strain by I/M and S/C routes were 394.0 and 129.9 respectively. In both cases the peak titer was observed at the end of the third week. None of the 70 chicks in either group developed clinical symptoms or died due to ND. Ten chicks from each group were challenged

at the end of six weeks and all of them survived for 21 days without manifesting any untoward reactions.

Post-vaccinal reactions evinced by clinical manifestation of ND leading to death was noticed in 2.9% of chicks that were inoculated with strain K by I/M route. No such reactions were observed in the S/C group. The HI antibody titers in chicks of these groups were comparatively less. However all the 10 chicks from each group survived on challenge.

Analysis of variance of the GMTs of HI antibody showed no significant difference between the four groups in this experiment. However, MWU test revealed that MT strain had a significantly higher effect than the K strain. The mean values for MT and K were 1.8491 and 1.7564 respectively.

Post-vaccinal reactions in the form of paralysis lasting for a period of four weeks was reported with K strain by Crowther (1952); Thorne and MacLeod (1960). Others two mesogenic strains such as Roakin and MK 107 were found to cause a severe mortality in birds under six weeks of age (Cole and Hutt, 1961; Anon, 1962) and in week old chicks it was 50% (Clancy et al. 1949)

Mortality was negligible in chicks vaccinated with Roakin strain at five weeks of age (Beaudette et al. 1949; Cordier-Boullangier et al. 1955). A mortality rate of 1.3% (Veneraso and Mendozoa, 1950); 6% (Haddow and and Idnani, 1946) and 16% (Van Waveren and Muidam, 1953) was reported in chicks vaccinated with R_2B. This strain was also reported to produce nervous symptoms in vaccinated chicks (Jaksic and Stefanovic, 1957). Similar post-vaccinal reactions were also reported with Hertfordshire-33 (Iyer and Dobson, 1940; Schneider, 1954). From these reports it is clear that strain MT is quite safe when compared to other mesogenic strains.

Most of the mesogenic strains cause a marked reduction in egg production lasting for about 1-3 weeks (Lancaster, 1964). But, the effect of MT strain in this respect has not been worked out.

Contact transmission

Westbury and Waddington (1978) reported that transmissibility is an important characteristic of a vaccine strain. Strain that spreads quickly within a flock could ensure that most birds would be immunized. In this

regard high transmissibility is advantageous. But it could be undesirable if the vaccine virus spread naturally from the vaccinated to unvaccinated farms.

The ability of MF strain to spread to incontact unvaccinated chicken was also compared with that of R. This was studied by detecting a rise in antibody titer in the incontact chicks or their capacity to withstand challenge. The transmissibility of MF strain was poor as it was only 25.0% and 28.6% by I/M and S/C routes respectively. A higher percentage of transmissibility over 60% was noticed with R strain by either route.

Though the actual effect of transmissibility under field conditions is speculative, it could be an important factor in selecting a vaccine strain under certain field conditions.

The pathotyping of the newly isolated strain of ND virus indicates that it is a mesogenic strain. It was a pathogenic to day old chicks producing 26.6% mortality. Chicks of three weeks of age and above were well tolerated without producing any untoward effects. In inoculated chicks the strain produced satisfactory level of antibody which was comparable to that of

K strain. It should also be noted that there was no post vaccinal reactions unlike K strain in which 2.9% of the vaccinated birds died of ND. Though the present study indicates that MF strain possesses the characteristics of a good vaccine strain more of field trials and its effects on egg production need to be worked out before it is recommended as a vaccine strain.

SUMMARY

SUMMARY

A strain of Newcastle disease virus (MT) isolate from an ailing mynah at the 10th passage level was characterised and its pathogenicity and immunogenicity were studied and compared with that of Komarov strain, the presently used vaccine strain in Kerala.

Embryo lethal dose 50 of MT and K strain in developing chick embryos was $10^{9.5}$/0.2 ml and $10^{10.5}$/0.2 ml respectively. The mean death time of the minimum lethal dose was calculated for MT and K strains as 87 hours and 76.5 hours respectively. The intracerebral pathogenicity index determined by inoculating day old chicks with the virus into the cerebral cortex was found to be 0.63 for MT and 1.16 for K strain. The intravenous pathogenicity index was zero for both MT and K strains. The sera collected from the birds inoculated with MT strain had HI antibody titers ranging from 1:512 to 1:1024 and those received K ranged from 1:4 to 1:256 indicating that the birds had picked up infection.

Regarding thermostability of infectivity at 56°C the MT strain was heat labile as two log reduction in the infectivity titer was noticed within 10 minutes but the

same reduction in the titer of K was found only in 20 minutes time. The haemagglutinating property of MT strain was completely lost within five minutes at 56°C while that of K strain was comparatively resistant as it took 20 minutes for complete loss of HI activity.

Preliminary studies on pathogenicity and immunogenicity were conducted in day old and three week old chicks in comparison with K strain. MT strain was found to be pathogenic for day old chicks giving 26.6% mortality. Satisfactory HI antibody titers were obtained in all the survived chicks and all of them withstood challenge with a virulent virus. A second dose of the same virus at the rate of $5 \times 10^{6.5}$ EID 50 per bird did not produce any untoward effect but produced high HI titers and all the birds were protected against challenge. In chicks that received primary immunization with F strain, MT at the 6th week as a booster dose, induced good antibody response without any clinical manifestations and gave 100% protection against challenge after six weeks.

Similar treatments with K strain had shown that this strain produced 3.3% mortality on the 3rd day, and 23.3% of the chicks reacted severely. The recovered chicks

had HI antibody titers sufficient enough to protect them against challenge. Booster dose at the 6th week in birds with a base immunity with F strain also protected the birds. In all these cases HI titers were always higher in birds that received MT strain though statistically not significant.

In three weeks old chicks both the strains did not produce any clinical illness or death either by S/C or oculonasal route of inoculation. The strains were highly immunogenic producing HI antibody titers and protected 100% of the chicks against challenge with a virulent strain. In both the cases the peak titers were obtained at the end of the 3rd week following inoculation. Though not statistically significant MT strain had high HI titers.

The suitability of MT strain as a vaccine strain was investigated by inoculating 70, six week old chicks immunized by F strain at the day of hatch either intramuscularly or subcutaneously. The immunogenicity and protective effect was compared with that of A strain. The chicks that received MT strain by intramuscular route or subcutaneous route did not manifest any post vaccinal reactions and all of them survived. The maximum geometric

mean of HI titers was obtained at the end of the third week in both cases. The geometric means of HI titers were 26.80, 36.50, 394.0, 105.5, 49.23 and 15.9 and 34.85, 85.53, 129.9, 112.9, 91.89 and 49.23 in 1st, 2nd, 3rd, 4th, 5th and 6th week respectively for I/M and S/C routes.

Out of the 70 chicks that received K strain intramuscularly, two showed clinical symptoms of ND and died between the third and fourth day. The percentage of reaction and mortality was calculated to be 2.9%. GMTs of HI antibody titers in this group of chicks were 31.77, 74.63, 103.1, 85.76, 69.65 and 35.65 for the 1st, 2nd, 3rd, 4th, 5th and 6th week after inoculation. All the 10 chicks that were exposed to virulent ND virus at the end of six weeks survived giving 100% protection.

The chicks that received K strain by subcutaneous route did not show any post-vaccinal reactions. The geometric means of HI antibody titers were 23.02, 45.94, 91.89, 69.65, 42.86 and 13.22 for the first, second, third, fourth fifth and sixth week respectively and were slightly lower than for the chicks in the previous group. However the difference was not statistically significant,

hundred per cent protection was also obtained in this group.

The transmissibility of MT and K strain was compared by keeping unvaccinated age matched control chicks with the inoculated chickens. The percentage of contact spread was 25 and 28.6 respectively for the chicks that were kept along with I/M and S/C group of MT strain. The percentage of contact transmission was higher from K infected chicks being 75 and 62.5 respectively for the I/M and S/C group.

Though the newly isolated MT strain of ND virus is lentogenic and possesses comparable immunogenicity with that of K strain detailed field trials and its effects on egg production need to be undertaken before it is recommended as a vaccine strain.

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IMMUNOGENICITY OF AN INDIGENOUS ISOLATE OF NEWCASTLE DISEASE VIRUS AND ITS USEFULNESS AS A VACCINE STRAIN

By

M. R. MURUGAN

ABSTRACT OF A THESIS

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Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Microbiology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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ABSTRACT

The newly isolated mesogenic strain of Newcastle disease virus (MT) from an ailing mynah was studied in detail with particular reference to its biological characteristics, pathogenicity and immunogenicity. The results of various studies were compared with that of Komarov strain, a known mesogenic strain.

The titer attained in developing chick embryos, mean death time of inoculated chick embryos at terminal dilutions, neuropathogenicity index in day old chicks and intravenous pathogenicity index were $10^{9.5}$/0.2 ml, 87 hours 0.63 and 0.00 respectively for the MT strain. The above values in order were $10^{10.5}$/0.2 ml, 76.5 hours, 1.16 and 0.000 for the Komarov strain. The infectivity of MT strain was labile at 56°C for 10 minutes and the haemagglutinin was completely lost within five minutes. On the other hand the infectivity and haemagglutinin of A strain were comparatively resistant.

Strain MT was pathogenic to day old chicks in which 26.6% mortality was noticed. In recovered chicks sufficient HI antibodies were seen and all of them withstood challenge. Although comparable results were obtained for Komarov strain, it was less pathogenic to day old chicks.

Though 23.3% of chicks manifested clinical symptoms only 3.3% died and the remaining birds recovered.

In three weeks old chicks MT and K strain were found to be nonpathogenic either by S/C or oculonasal route. The inoculated chicks were immune when challenged six weeks later.

Even in six weeks old chicks having no base immunity no post-inoculation reactions could be detected. All the chicks showed a rise in antibody titer reaching the peak level by the end of the third week and were resistant to challenge after six weeks.

Thechicks aged six weeks having a base immunity with F strain were also free from any post infection reaction either by I/M or S/C route or innoculation. Chicks in both the groups produced HI antibodies and was always higher in those received infection by I/M route. The peak titers were obtained at the end of the third week and then declined. Though the titers were low by the end of the 6th week all the chicks were resistant to ND when exposed to a virulent virus. 2.9% of the chicks that received K strain by I/M route showed post inoculation reaction and died of ND. The remaining chicks and those in the S/C group

behaved the same way as those received MT strain.

Though the antibody response of chicken to MT and K were not statistically significant in all the three experiments, MWU test revealed that MT has a significantly higher immunogenic effect than K as the former always had a higher means than the latter.

The ability to infect in contact chicks was also investigated. Strain MT was less efficient in this property giving only 25.0% to 28.6% transmission. On the other hand K strain revealed significantly higher transmissibility as it could spread to 62.5 to 75% of the inoculated incontact chicks.

The mesogenic strain MT is quite safe in chicks of three weeks of age and above. It is also a good immunogen producing HI antibodies which protected the chicks from challenge even after six weeks. However the strain can be recommended as a vaccine strain only after further field trials and its effects on egg production are worked out.