REVIEW
OF
LITERATURE
Newcastle disease: Is an acute viral disease affecting many domestic and wild avian species with respiratory, gastrointestinal, and central nervous system involvement. Also known as avian pneumoencephalitis, avian pseudoplague, and is classified as a list A disease by the World Animal Health Organization, Office International des Epizooties, (OIE) because it is highly contagious and responsible for severe disease and high mortality in susceptible birds (Alexander, 2004).

2. 1- History:

Several reviews have dealt with the history of ND (Lancaster, 1962, 1966; Beard and Hanson, 1984; Spradbrow, 1987; Kaleta and Baldauf, 1988; Alexander, 2003 and 2004 and Alexander et al., 2004). It is generally considered that the first outbreaks to be recognized and termed (ND) occurred in poultry in 1926, in Java, Indonesia and in Newcastle-upon-Tyne, England. However, there are earlier reports of similar disease outbreaks in central Europe before this date (Halasz, 1912). The name “Newcastle disease” was coined by Doyle as a temporary measure because he wished to avoid a descriptive name that might be confused with other diseases (Doyle, 1927). The name has, however, continued to be used although when referring to the ND virus, the synonym ‘avian paramyxovirus type 1’ (APMV-1) is now often employed.

2. 2- Etiology:

ND, is caused by avian paramyxovirus serotype 1 (APMV-1) In 2002 the International Committee on the Taxonomy of Viruses re-arranged the order of the Paramyxovirus genus and separate the avian paramyxoviruses include NDV from other rubulaviruses and then placed them in a new genus called Avulavirus genus among the paramyxovirinae of the family
paramyxoviridae. The virus families paramyxoviridae, filoviridae, and rhabdoviridae form the virus order Mononegavirales (i.e., the single-stranded, nonsegmented, negative-sense RNA viruses showing helical capsid symmetry) (Fauquet et al., 2005). Eight other serotypes of avian paramyxoviruses are recognized, namely: APMV-2 to APMV-9. Most of these serotypes appear to be present in natural reservoirs of specific feral avian species, although other host species are usually susceptible. Only APMV-2 and APMV-3 viruses have made a significant disease and economic impact on poultry production (Alexander, 2003).

The definition of ND adopted at the 67th General Session of the Office International des Epizooties (OIE) held in Paris in May 1999 was: ND is defined as an infection of birds caused by a virus of avian paramyxovirus serotype 1 (APMV-1) that meets one of the following criteria for virulence: a) The virus has an intracerebral pathogenicity index (ICPI) in day-old chicks (Gallus Gallus) of 0.7 or greater. Or, b) Multiple basic amino acids have been demonstrated in the virus (either directly or by deduction) at the C-terminus of the F2 protein and phenylalanine at residue 117, which is the N-terminus of the F1 protein. The term multiple basic amino acids refer to at least three arginine or lysine residues between residues 113 to 116. Failure to demonstrate the characteristic pattern of amino acid residues as described above would require characterization of the isolated virus by an ICPI test' (Alexander, 2001a; Alexander 2004 and Fauquet et al., 2005)

2. 2. 1- Morphology and structure:

Negative contrast electron microscopy of purified NDV reveals very pleomorphic virus particles. Generally, they are roughly spherical with 100-500 nm in diameter, although filamentous forms of about 100 nm across and of variable length are often seen. Virion core contains a
nucleocapsid about 18 nm across and showing helical symmetry “herring bone” that comprised nucleic acid of a negative-sense, single-stranded 15 kd RNA genome containing genes, 3_-NP-P-M-F-HN-L-_5, those encode six major structural proteins. The majority nucleocapsid protein (NP) the less abundant phosphoprotein (P) and a few molecules of the large protein (L) are the three core proteins and the three envelope proteins include a large glycoprotein with both hemagglutinin and neuraminidase activities (HN) a smaller glycoprotein with cell fusing activity (F) and a non-glycosylated protein (M) localized at the inner surface of the envelope (matrix protein). The virion is enveloped with a lipid bilayer membrane derived from the host cell membrane. The two glycoproteins, F and HN are different glycoprotein, which appear as tiny spikes projecting from the external surface of the membrane about 8 nm in length when observed under an electron microscope, they are important for virus infectivity and virulence, and either of these proteins can induce protective immunity (De Leeuw and Peeters, 1999; Aldous and Collins, 2001; Mayo, 2002; Alexander 2003 and Mohan et al., 2006).

2. 2. 2- Strain Classification:

Although NDV has essentially only one serotype, there is a considerably difference in the pathogenicity of different strains, ranging from those that cause virtually no signs to those that kill within few days. All NDV isolates are categorized as three pathotypes based on the disease severity they induce: velogenic, mesogenic or lentogenic, (Panda et al., 2004 and Alexander et al., 2004). For NDV isolates, this has inevitably meant that the distinction between viruses of high and low virulence for chickens or perhaps more pertinently between enzootic and epizootic viruses. Protein patterns of different NDV isolates are usually similar but a number of
previous studies have demonstrated a minor difference in molecular weight and protein patterns of NDV isolates (Kumanan and Mustay-Ahamed, 1994).

Alexander, (2000a) stated that strains of NDV have been distinguished on the basis of the clinical signs produced in infected chickens. Beard and Hanson, (1984) defined the following five groups or pathotypes: 1) Viscerotropic Velogenic: viruses responsible for disease characterised by acute lethal infections, usually with haemorrhagic lesions in the intestines of dead birds. 2) Neurotropic Velogenic: viruses causing disease characterised by high mortality, which follows respiratory and neurological disease, but in which gut lesions are usually absent. 3) Mesogenic: viruses causing clinical signs consisting of respiratory and neurological signs, with low mortality. 4) Lentogenic: viruses causing mild infections of the respiratory tract. 5) Asymptomatic enteric: viruses causing a virulent infection in which replication appear to occur primarily in the gut.

Alexander, (2003) discussed that certain unrelated biologic properties of viruses vary with different strains and isolates, these properties are: antigenicity, pathogenicity tests, plaque formation, elution, thermostability, structural polypeptides, oligonucleotide fingerprinting, lectin binding and genetic characterization. These properties have been used to characterize and group isolates.

2. 2. 3- Laboratory hosts:

Animals: NDV can infect and multiply in a range of nonavian as well as avian species following laboratory infection. The chicken, however, remains the most readily available and frequently used laboratory animal,
as well as the most important natural host of the disease (Lancaster, 1966 and Kaleta and Baldauf, 1988)

**Chicken Embryos:** All avian paramyxoviruses replicate in embryonated chicken eggs. Because of their availability (especially from specific pathogen-free sources) their sensitivity for virus growth and the high titres to which viruses grow in them, they are generally used for virus isolation and propagation. ND virus strains and isolates vary in their capacity and time taken to kill chick embryos. Virus titres are also influenced by strain, with the highest titres obtainable by those causing slow or no death (Gough et al., 1974). With some strains, embryo death and virus growth is affected by the presence of maternal antibodies in the yolk (French et al., 1967). The route of inoculation is also important (Beard and Hanson, 1984). Inoculation of NDV via the yolk sac, as compared with the allantoic cavity, produced more rapid embryo deaths and caused deaths by strains that do not consistently kill by the latter route (Estupinan et al., 1968).

2. 2. 4- Natural and Experimental Hosts:

**Birds:** ND viruses have been reported to infect animals other than birds, ranging from reptiles to humans (Lancaster, 1966). Kaleta and Baldauf, (1988) concluded that NDV infections have been established in at least 241 species of birds representing 27 of the 50 orders of the class. All birds are probably susceptible to infection, but, the disease observed with any given virus may vary enormously from one species to another (Alexander, 2000 b).

ITO et al., (1999) compared the receptor specificity of ND viruses from a variety of avian species, including chickens and wild waterfowls, using hemagglutination tests with erythrocytes from different animal species. All
isolates from wild waterfowl agglutinated horse erythrocytes, while the chicken isolates did not. They suggested that the receptor specificity of ND viruses is different, depending on the avian species from which the viruses are.

Swayne and King, (2003) maintained that humans are among the many species that can be infected with NDV. Many of the documented cases involved infections in laboratory workers who accidentally splashed high-titre NDV-infected egg fluids into their eyes, veterinary laboratory diagnosticians who performed postmortem examinations on infected birds or handled infectious tissues, workers in poultry processing plants, and poultry vaccination crews. The absence of many recent reports suggests that such infections may be commonplace and that when they occur, they are mild and self-limiting.

The distribution of ND is dependent on the attempts at eradication and control made in different countries. The success of such measures is in turn dependent on the nature of the poultry industry (i.e., countries with mostly village chicken flocks have far greater problems than those with mostly large commercial flocks) (Alexander, 2003).

There is little evidence for the spread of ND virus through the egg (true vertical transmission) though transmission of virus on the shell of infected eggs is well recognized. Embryos inoculated at about 9 days of incubation with avirulent viruses (that do not kill embryos by this route) have been allowed to hatch. However the same viruses given by yolk sac route at 4 days of incubation kill the embryos (Kim and Spradbrow, 1978). Recently, virulent NDV was detected in uninfected cell cultures prepared from embryonated chicken eggs. However, chicks hatched from eggs collected from NDV infected flocks were virus negative even though dead
embryos and infertile eggs in the same incubator contained NDV (Seal et al., 2000) but other researchers (Chen and Wang, 2002) confirmed that a few chicken embryos infected in ovo with a low titre of NDV can hatch and contain NDV after hatching, which results in NDV spreading through eggs.

2. 3- Economic Significance:

The global economic impact of ND is enormous. It probably represents the bigger drain on the world’s economy than any other animal virus, although the current epizootics of H5N1 avian influenza in Southeast Asia are challenging (if not surpassing) this status (Van Boven et al., 2008). Every outbreak of ND in the industrialized part of the world has great economical impact on the producers of poultry products because of trade restrictions (Alexander, 2001b). In developed countries with established poultry industries or where ND may be considered to be controlled, the heavy economic losses caused by ND necessitated the introduction of legislation to control the spread of the disease. This legislation varies from country to country and represents in compulsory vaccination for all birds in most countries while in other countries for example, Sweden, Finland, Norway, Denmark and on the island of Ireland, which does not permit vaccination (except of racing pigeons) whose flocks do not contain vaccinated birds and where the breeding flocks are monitored once a year for ND, there is a complete reliance on a stamping out policy with a ban on the use of vaccines (Box et al., 1988; Kouwenhoven, 1993 and Moynagh, 1994). In most developing countries, ND is the most important infectious disease affecting village chickens (Aini, 1990 and Spradbrow, 1999). It causes a great loss in domestic village chickens which often provide the only or one of the main
sources for marginal diets of animal protein to poor villagers especially for women and children living in remote areas throughout the developing countries (Martin, 1992 and Spradbrow, 1994) so that the economic impact of NDV should not only be measured in direct commercial losses, but in some countries in the effect on human health and loss of potential socioeconomic gain, if such food was plentiful (Bouzari et al., 2004).

2. 4- Clinical signs:

Clinical signs alone do not present a reliable basis for diagnosis of ND. Immune birds infected with virulent strains of NDV may show greatly diminished clinical signs (Allan et al., 1978 and Parede and Young, 1990). Gastrointestinal, respiratory and/or neurological signs have been described, but the clinical presentation of infection with NDV is very variable. Factors such as the virulence and tissue tropism of the virus, the species and age of bird, pre-existing immunity, environmental stress and concurrent diseases influence the outcome of infection with NDV (Alexander, 2000a). In some studies, a reduction in egg production was the main clinical sign reported in infected layers (Murray, 2002). The mortality rate in susceptible flocks varies between negligible (lentogenic ND) and 100% (velogenic ND) (Alexander, 2003).

2. 5 - Pathology:

The gross lesions of ND reflect the clinical presentation. Congestion and haemorrhage in various organs and tissues with necrohemorrhagic plugs in the lymph aggregates and intestine are the main findings (Brown et al., 1999 and Reece et al., 2000). Congestion and sometimes haemorrhage on tracheal mucosa, petechiae and small ecchymoses on the mucosa of the proventriculus, concentrated around the orifices of the mucous glands are observed (Kommers et al., 2002). No pathognomonic lesions are
associated with any form of the disease; gross lesions may also be absent (Alexander, 2003). Lesions present predominantly in lymphoid, intestinal, and central nervous tissues (Piacenti et al., 2006).

2.6- Diagnosis:

Immunohistologic techniques, Immunofluorescence, impression smears or immunoperoxidase technique for thin sections offer a rapid methods for the specific demonstration of the presence of NDV or viral antigens in organs or tissues (Lockaby et al., 1993).

Virus isolation and virulence testing involves the inoculation of tissue cultures or embryonating chicken eggs (Rodriguez et al., 1967; Hightower and Bratt 1974, and Ahamed et al., 2004). Once the presence of NDV infection is confirmed, virulence can be assessed using the intracerebral pathogenicity index in one day-old chicks, the mean death time in chicken embryos, the intravenous pathogenicity in six-week-old chicks or nucleotide sequencing (Alexander, 2004).

There have been several reports of the use of an RT-nested PCR ELISA for detection of NDV (Kho et al., 2000) RT-PCR techniques to detect ND virus in clinical specimens (Gohm et al., 2000) have the advantage of extremely rapid demonstration of the presence of virus and even its virulence if primers covering the part of the genome coding for the F0 cleavage site are used (Barbezange and Jestin, 2002; Creelan et al., 2002; Wise et al., 2004 and Kong et al., 2007).

Monoclonal antibodies (MAbs) directed against strains of ND virus have been used in HI tests to allow rapid identification of ND virus without the possible cross-reactions with other APMV serotypes that may occur with polyclonal sera. This has proven to be a valuable method for grouping
and differentiating isolates of ND virus, and has been particularly valuable to the understanding of the epidemiology of outbreaks (Alexander, 1997a and Alexander et al., 1997).

2.7- Control:

2.7.1- Non specific control:

Mazija, (1990) summarized the principles that prevent the spread of ND and almost all other poultry diseases. These principles are: all in - all out, one age on a farm, one production purpose, one genetic basis and a long-enough pause between cycles so a radical break in transmitting biological contamination from the processing cycle of previous poultry generations possible.

Alexander, (1997b) emphasized that there are no circumstances in which vaccination can be regarded as an alternative to good management practice, biosecurity or good hygiene in rearing domestic poultry.

Gallili and Ben-Nathan, (1998) pointed the measures available for preventing the spread of ND virulent viruses as follows: (i) Vaccination of susceptible poultry with live or killed ND vaccines; (ii) stamping out of infected flocks; (iii) environmentally controlled isolated growth premises; (iv) strict sanitary measures in poultry houses and processing plants; (v) periodic checks for adequate serum antibodies titres followed by revaccination; (vi) prohibition of the importation of susceptible poultry and poultry meat from ND endemic countries; and (vii) control of backyard poultry health.
Review of literature

2. 7. 2- Control by Vaccines:

2. 7. 2. 1- Vaccines:

Quaglio et al., (1977) stated that chicks with no specific antibodies were initially vaccinated against ND at 21 days old with the live 'LaSota' strain or beta-propiolactone-killed virus emulsified in mineral oils. After a second vaccination 15 or 21 days later with the live 'LaSota' strain, the interference produced by the first vaccination on the antibody production induced by the second vaccination was investigated. It was found that the live virus vaccine used for the second immunization was affected by the antibodies induced by the first vaccination,

Allan et al., (1978) have published comprehensive reports on the preparation and use of ND vaccines. They summarized the points that should be considered in a vaccination program in the following: A) The pathogenicity of the vaccine virus. B) The immune competence of the chicken. C) The levels of humoral maternally derived antibody. D) The levels of residual antibody from a previous vaccination. E) The effect of the vaccination on the general health of the birds. F) Vaccination programs for other disease. G) The general level of immunity required.

Partadiredja et al., (1979) demonstrated that antibody response was rapid and high in broiler breeder chickens receiving 1 or 2 vaccinations with oil-emulsion vaccine against ND at 23 or at 23 and 26 weeks old but it was higher in the later. The antibody titres remained high during the 41-week (experimental period). The immune response to live-virus vaccine given at 2, 9, 20, 30, 42, or 54 weeks of age via the drinking water was high, but uniformity was lacking in the antibody response in the breeders and maternal antibody response in the progeny. Maternal antibody levels in one-day-old chicks were related to the titres of antibody in the dams.
Maternal antibody titres of chicks originated from breeder flocks that were vaccinated with the oil-emulsion vaccine remained high for all hatches.

**Stone et al., (1980)** evaluated several European oil-emulsion (OE) vaccines under controlled laboratory conditions and compare them with USA-licensed inactivated AI (OH) and live NDV vaccines, includes HI response and response to challenge with neurotropic and VVND viruses. They found that OE vaccines induced higher and generally more sustained HI antibody titres than AI (HO) or live vaccines. They concluded that vaccination did not prevent infection with the challenge viruses; however the infection rates were lowest in LaSota vaccinates and highest in AI (OH) vaccinates.

**Eidson et al., (1982a)** compared between live and inactivated vaccines in broiler breeders. They found high and more consistent HI antibody titres in case of live ND vaccine and subsequently vaccination with inactivated oil emulsion vaccine. Also they observed that, between 8-32 weeks after onset of egg production, geometric mean maternal antibody titres in 1-day old chicks derived from breeders vaccinated with live ND vaccine ranged from 7 to 24, whereas, chicks from breeders vaccinated with the inactivated oil emulsion vaccine had maternal antibody titres that ranged from 64 to 89.

**Mazija, (1990)** underlined that the current vaccination program against ND using the exclusive application of live viral vaccines differ from country to country and very often between regions, or even between production organizations. There are no legislative norms by which the application of these vaccines could be defined, which is perfectly understandable because the analyses of the disease are still incomplete and the possibilities of its control are still insufficiently investigated.
Alexander, (1995a) explained that inactivated oil emulsion vaccines can be efficacious if used as primary vaccine, but their additional cost, both direct and in administration, means that live vaccines are usually preferred. Vaccination program for laying birds are usually based on the use of several doses of live lentogenic vaccine followed by inactivated vaccine at point of lay. Mesogenic live vaccines tend to be used only in countries where virulent NDV is widespread and it is important to maintain high antibody titres to prevent serious disease. The presence of enzootic disease is usually linked to severe economic restrictions, which rule out the use of oil emulsion vaccine. The most popular live vaccines have evolved from field isolates of low virulence. Most are based on the "lentogenic" viruses Hitchner B1 or LaSota or similar viruses, although some "asymptomatic enteric" viruses have also been used as live vaccines. LaSota, for example, gives better protective antibody titres than Hitchner B1 but is more likely to cause reactions.

Erganifl et al., (1997) showed that the antibody titres to NDV in a flock vaccinated with live vaccines decreased more rapidly than those of flocks vaccinated by live and inactivated strains.

Folitse et al., (1998 b) confirmed that the concurrent administration of oil emulsion and live NDV vaccines induced the best antibody response, but there was no significant difference in protection with those vaccinated either with live or killed vaccine alone among the vaccinated groups.

Spradbrow, (1999) stated that the more invasive mesogenic viruses produce a better secondary response, as well as a better primary response, and they are widely used as booster vaccines. He classified the common vaccinal strains as strain F and B1 are examples of lentogenic vaccines and
Komarove and Mukteswar or (standerd) are mesogenic vaccines. Strain V4 is an example of an avirulent virus that has been used as a conventional vaccine in commercial and village chickens.

An attempt was made by Roy et al., (1999) to potentiate the immune response of a live mesogenic vaccine (RDVK) by incorporating two different adjuvants, a mineral oil (liquid paraffin) and a vegetable oil (groundnut oil) in the live mesogenic vaccine (RDVK). They found a significant difference in immune response between the adjuvanted and non-adjuvanted groups.

Alexander, (2000a) mentioned that choice of vaccine is governed by three main considerations, namely: The immunogenicity of the vaccine, the type of vaccine (inactivated or live) the virulence of live vaccine strains, not only for the recipient host species, but also for other species, which may become infected due to lateral spread of the vaccine.

Seal et al., (2000) established that the mildly virulent B1 and LaSota strains of NDV are currently the most widely used efficacious live-virus vaccines for prevention of ND that are marketed worldwide. These live-virus vaccines induce high levels of IgA, IgY and IgM antibodies in sera of newly hatched chicks. They also induce local antibody response such as IgA production in the Harderian gland along with lachrymal IgM following intraocular inoculation with NDV.

Alexander, (2003) maintained that in the poultry industry, vaccination against ND with live vaccines is common practice and even obligatory in many countries. The mildly virulent B1 and LaSota strains of NDV are currently the most widely used as live-virus vaccines for prevention of ND. The objective is to establish a mild infection in the flock, preferably in each bird at the time of application.
Ken Opengart, (2003) Summarized, the important components that have been needed and can be developed in the breeder vaccination program for optimal protection with minimal reaction as following:

A. Timing of the various vaccines given is critical and has been developed based on:
   1) When field exposure to certain diseases may occur.
   2) Spacing of vaccines for the same disease in order to maximize the antibody response that is generated.
   3) Spacing of vaccines for different diseases in order to minimize interference between vaccine responses.

B. Method of administration
   1. Closely mimics the natural route of exposure of the bird to the disease.
   2. Maximizes the bird’s response to the vaccine

C. Every bird receives a full dose of vaccine.

Erganis and Ucan, (2003) evaluated the effect of alterations made to the ND vaccination regime in 2,230,000 chickens in control Anatolia Turkey. The alteration was based on the use of an inactivated NDV + IBDV and the application of aerosol vaccines with different timing compared to traditional programs. Satisfactory humoral protection (HI titre 7 log2) was achieved in birds up to 9-11 weeks of age by the early administration of inactivated NDV+ IBDV vaccine.

Alexander, (2004) classified the strains of NDV use in vaccines production according to their virulence into three categories. 1- The lentogenic strains are very mild and naturally inhabit healthy flocks. They can be used as live vaccines even for young chicks. Killed vaccines can be produced from the same viruses following inactivation. 2- Mesogenic ND viruses, which cause mild or inapparent respiratory infections, have recently been banned in many countries even for killed vaccine production.
due to fears of disease emergence. 3- Velogenic strains are the causative agents of the disease and can be used for the purpose of vaccine challenge test. Isolates of low virulence, Hitchner B1 and LaSota, became the most used veterinary vaccines throughout the world.

**Senne et al., (2004)** stressed that the types of vaccines and vaccination schedules used vary depending on the potential threat, virulence of the field challenge virus, type of production, and production schedules.

**Alexander et al., (2004)** numerated and explained three types of vaccines which are used for ND: live lentogenic, live mesogenic and inactivated vaccines. Live lentogenic vaccines are usually derived from field viruses that have been shown to have low pathogenicity for poultry but produce an adequate immune response. Typical vaccine strains are Hitchner B1, LaSota and F strain and some viruses from the asymptomatic enteric pathotype, which are usually based on the V4 or Ulster 2C viruses. However, these viruses have been frequently subjected to selection pressures by manufacturers in order to improve their immunogenicity or to enable their use by a particular method of application.

**Kapczynski and King, (2005)** reported that all unvaccinated control chickens challenged with exotic ND (END) died within 6 days post-challenge (pc). They found that protection from disease was correlated with the presence of antibody titres (determined by ELISA or HI) at day of challenge.

**Oladele et al., (2006)** observed that chickens which received ND Komarov vaccine had higher daily mean values of HI antibody titre than their counterparts which received NDV LaSota vaccine.
Azzam and Ahmed, (2006) recommended that the LaSota vaccines for the protection of chickens at different ages due to efficacy and safety. The antibody levels reached protected the vaccinated chicks against NDV infections. This protection depends on recurrent infection and factors affecting the immune systems of the susceptible birds.

There has recently been growing interest in using subunit vaccines against poultry diseases including ND. Several recombinant vaccines have been developed that provide protection against ND. Poxvirus-type vectors expressing the F or HN proteins are protective when using vaccinia (Meulemans et al., 1988 and Nishino et al., 1991) fowlpox (Boursnell et al., 1990 a and b) as the recombinant vector. Herpes virus of turkeys expressing the F and HN of NDV is protective against Marek's and ND simultaneously whether inoculated parenterally (Morgan et al., 1992).

A number of studies in last years (Krishnamurthy et al., 2000; Huang et al., 2001; Nakaya et al., 2001) highlighted the potential of ND virus to be used as a vaccine vector for avian diseases.

At least two vaccines that basically allow serological differentiation of infected poultry from vaccinated poultry have been developed. One recombinant chimeric vaccine was generated by using a reverse genetics system (Peeters et al., 2001). The other vaccine consists of a recombinant vaccine virus with a deletion of an immunodominant epitope in the nucleoprotein gene. This epitope could also be replaced by a foreign epitope (Mebatsion et al., 2002).

Kapczynski and Tumpey, (2003) had produced a nonreplicating virosome vaccine by solubilization of ND virus with Triton X-100 followed by detergent removal with SM2 Bio-Beads. They reported that the NDV virosomes had similar characteristics as the parent virus and
Review of literature

contained both the fusion and HN proteins using biochemical analysis. Their results demonstrate the potential of virosomes as an effective tool for ND vaccination.

Huang et al., (2003) summarize the developments and methodologies employed and success achieved in using NDV as a vaccine vector and the potential of this approach in designing next generation vaccines to prevent diseases of poultry.

By using the reverse genetics approach, Huang et al., (2004) developed a bivalent recombinant NDV/IBDV vaccine for protection against both of these economically important diseases. They devised a recombinant ND virus vector from a commonly used vaccine strain LaSota to express the host protective immunogen VP2 of a variant IBDV strain GLS-5, and they concluded that the recombinant NDV can be used as a vaccine vector for other avian pathogens.

Bukreyev et al., (2005) evaluated the lentogenic vaccine strain LaSota (NDV-LS) and the mesogenic strain Beaudette C (NDV-BC) as vectors to express the HN protein of human parainfluenza virus type 3 (HPIV3) as a test antigen. After one dose, an efficient HPIV3-specific serum antibody response was detected; after the second dose, it became equal to or greater than that induced by HPIV3 infection.

Park et al., (2006) and Veits et al., (2006) have developed and evaluated a bivalent vaccine against both AIV and NDV for poultry that is based on the fusogenic r NDV vector expressing a chimeric HA protein. A single immunization of chickens with this improved vaccine prototype virus induced 90% protection against an H7N7 and highly pathogenic avian influenza virus, and complete immunity against a highly virulent NDV.
Qin et al., (2007) first reported the recombination in the F gene of a mutative velogenic ND virus strain. Complete genome sequences analysis indicated that the N-terminal of F gene originated from a genotype II NDV strain, designated as SRZ03, whereas the C-terminal of F gene and the rest of the genes originated from a prevalent velogenic genotype VII NDV strain. They provided valuable information for understanding the recombination of nonsegmented negative-sense RNA viruses.

2. 7. 2. 2- Vaccination:

With regard to the method of vaccine administration, Allan, (1971) and Allan et al., (1973) reported that under field condition the method of vaccination will very much influence the level of immunity in a flock. The beak or head dipping, intraocular, intranasal, and injection methods are for individual immunization which results, when properly applied in the most uniform dosage and immune response in a flock. Their chief disadvantage is the penning and handling of the birds, which are stressing to poultry and expensive in terms of labor.

Devos et al., (1975) stated that the HI titres were strongly dependent both of the vaccine strain used and on the vaccination method. The highest post-challenge titres were seen after vaccinations with the lentogenic and avirulent strains and the lowest after-challenge titres were seen when the mesogenic Beaudette strain was used. Birds with high HI titres showed a marked decrease of these HI titres two weeks after challenge, while there was an increase of HI titres in birds with low post-vaccinal titres. It may be concluded that clinical resistance to ND infection is not directly correlated with high-vaccinal HI titres.

Quaglio et al., (1977) studied the effect of the length of time between two vaccinations on the immune response of the chicken to vaccination.
against ND. Their results were clearly showed how the choice of vaccine type and the time between vaccinations were correlated and of considerable importance. They utilized 1-day-old chicks with no specific antibodies were initially vaccinated against ND at 21 days old with the live 'LaSota' strain or beta-propiolactone-killed virus emulsified in mineral oils. They found that after a second vaccination 15 or 21 days later with the live "LaSota' strain, was an interference produced by the first vaccination on the antibody production induced by the second vaccination. They found that the live virus vaccine used for the second immunization was affected by the antibodies induced by the first vaccination. They concluded that the choice of vaccine type and the time between vaccination were correlated and of considerable importance.

Satyanarayan et al., (1977) reported that maternal antibody was insufficient to give protection against ND beyond 48 hours of hatching of chicks. Immunization of chicks at the age of 3, 5, and 7 days by the nasal route gave protection even at the end of the 1st week without showing significant rise in HI titres, probably due to the interference phenomenon by F strain virus rather than to specific antibodies. Chicks of all three groups survived challenged at the end of 8th week, despite low titres of HI antibody.

Allan et al., (1978) reported that, if the time interval between the primary and secondary vaccination is less than 21 days, the antibody produced by the first dose of vaccine is more likely interfere with the multiplication of the second dose of the virus. Therefore, there is little to be gained by reducing the interval between vaccinations. As the response to the first vaccination declines with time, delaying the second dose can be expected to elicit a better response. They demonstrated that the response to revaccination at 9 weeks with Hitchner B₁ or F strains by the drinking
water route, did not lead to an increase in the immune level over the levels obtained following primary vaccination at 3 weeks by the same vaccines. In contrast, the secondary response obtained by revaccination with LaSota vaccine in the drinking water was similar to vaccination by individual application using the intranasal or ocular route.

**Partadiredja et al., (1979)** compared the immune responses of broiler chickens to different methods of vaccination, aerosol, intratracheal, drinking water and unvaccinated against ND. They found that aerosol vaccination induced higher serologic responses than water and intratracheal vaccination, as well as giving the highest levels of protection against challenge. Results of challenge tests were in agreement with results of HI tests, further; they demonstrated that intratracheal vaccination at one day of age does not elicit a satisfactory immune response in birds with a maternal antibody titre of 15 or higher.

**Ewert et al., (1979)** immunized adult chickens by two methods: (i) combined intratracheal- intranasal vaccination followed by intratracheal revaccination or (ii) intramuscular vaccination followed by intratracheal revaccination. They found that the first method produced the highest antibody levels in both serum and saliva and, in addition, prevented detectable virus multiplication in the respiratory tracts upon revaccination 4 weeks later.

**Gordon and Jordan, (1982)** numerated and discussed the factors affecting the immune response. They stressed that programs must be so timed that successful immunization is not jeopardized either by interference between sequentially administered live viruses of similar tropism or by antigenic competition, if birds are given two or more vaccines at the same time.
Giambrone, (1985) examined the efficacy of various commercial vaccination programs for the prevention of ND, in broilers. In all, chicks were from breeders vaccinated against ND via drinking water at 75-day intervals. The chicks which were vaccinated at 1 day by coarse spray or by tracheal instillation and vaccinated at 7 days via drinking water, failed to produce measurable antibody to NDV, all methods resulted in protection against NDV challenge at 35 and 49 days.

Giambrone and Closser, (1990) carried out two experiments to determine the effect of breeder vaccination program and maternal antibody on the efficacy of ND immunization of 1 day old chicks. Experimental protocol was the same for both. Broilers were from breeders aged either 32 or 50 weeks old. Breeders received three live ND viruses, vaccines and either a killed vaccine at 18 weeks old or continual live boosting at 60 – 70 – day intervals through lay. In the first experiment, maternal antibody was higher in chicks from the younger breeders given the inactivated vaccine, and in the second experiment maternal antibody was higher in chicks from older breeders given continual live vaccines.

Van Eck, (1990) stated that during the egg production period mean log2 HI ND titre of the parents slowly decreased from 11.6 at 26 weeks of age to 10.0 at 53 weeks of age. The titres of day-old progeny paralleled those of their parents. Mean protection of broilers against mortality following challenge with Herts 33 virus at the ages of 1, 2, 3 and 4 weeks was 86%, 73%, 54% and 44%, respectively It is concluded that passive ND protection of broilers by hyperimmunization of the dams is realizable from an economic point of view and is suitable for low-risk area significantly increase antibody titres in parent flocks and the level and duration of passive protection in broilers.
Alexander, (1995b) confirmed that the method of application is important. Individual application methods such as the use of eye drops and beak dipping are costly; mass application of live vaccine in generated aerosols or sprays or in drinking water is cheaper and more convenient. However, sprays and aerosols in particular may result in severe respiratory reaction and, especially with LaSota virus, even high mortality.

Takada and Kida, (1996) showed that vaccination with inactivated virus via the mucosal surface induced local antibody responses and protected birds from lethal challenge with virulent virus. The local antibodies, especially IgA inhibit primary replication of the virus at the site of entry. They concluded that NDV infection would be controlled by the development of a vaccination strategy designed to stimulate not only systemic but also mucosal immune responses by administration of noninvasive antigens which have the advantage of safety and convenience.

Stone et al., (1997) described the serologic response, seroconversion rates, hatchability and challenge exposure responses of chickens vaccinated as 18-day old embryos with ND and AI inactivated oil-emulsion vaccines. They concluded that the hatchability, seroconversion rates, and protective immunity can be attained with in ovo inoculation of ND or IA OE vaccines if the vaccines are prepared with sufficient antigen and administered properly.

Gallili and Ben-Nathan, (1998) demonstrated a several factors determine the degree and duration of immunity imparted by a vaccine. These factors include the age of vaccinated fowls, virus content of the vaccine, vaccine storage regime, vaccine potency and the vaccination route. High temperatures severely affect ND vaccines; improper transportation conditions cause rapid deterioration. Vaccines held at 37-45°C for 4-5 days
may lose all their live virus content. They pointed out the measures available for preventing the spread of ND virulent viruses as follows: (i) Vaccination of susceptible poultry with live or killed ND vaccines; (ii) stamping out of infected flocks; (iii) environmentally controlled isolated growth premises; (iv) strict sanitary measures in poultry houses and processing plants; (v) periodic checks for adequate serum antibodies titres followed by revaccination; (vi) prohibition of the importation of susceptible poultry and poultry meat from ND endemic countries; and (vii) control of backyard poultry health.

Spradbrow, (1999) notified that in most developing countries, many chickens are reared as scavenging village chickens, which frequently act as reservoirs for NDV. Satisfactory vaccination of such birds represents a considerable challenge. Recently the use of thermo-stable symptomatic or lentogenic viruses which can be presented by coating food have been evaluated and have had a good success under field condition.

Permin et al., (2001) demonstrated that the protection against ND infection is not only based antibodies, but also on the cellular immune response.

Kapczynski and King, (2005) studied the protection efficacy of existed commercial live and inactivated ND virus, vaccines against the 2002–2003 END viruses, and the protection potency of the current commercial NDV-vaccination programs for broiler-breeders (BB) and broilers (Br) against END-challenge. Results indicated that both the live and inactivated vaccines protected against morbidity and mortality and significantly reduced the incidence and viral titres shed from chickens in comparison with simulation controls, but did not prevent infection and virus shedding. In addition, both doses of live vaccine protected birds and significantly
decreased the number of birds shedding virus. They reported that commercial BB was protected from disease and exhibited low incidence and titre of challenge virus shed. In contrast, commercial Br exhibited 66% mortality and shed significantly more virus than the BB birds. They concluded that the need to develop new NDV vaccines and vaccine strategies for use during outbreak situations to protect birds from both disease and infection to reduce virus shedding.

Mast et al., (2006) envisaged the production of strains with mutated F and HN-glycoproteins, so-called double escape mutants, to further reduce virulence and reduce risks of reversal. For the obtained escape mutants, they investigated the relation between the introduced mutations and their biological activity and to examine whether protective immune responses against NDV could be induced by vaccination of 18-day-old embryos. They found a significant effect of the vaccine dose was observed in chicks having maternal antibodies and vaccinated in ovo with the F +HN mutant and they concluded that immuno selection using antibodies against the HN- and F glycoproteins can be used as alternative technique for the production of attenuated NDV strains, which are candidate in ovo vaccines.

Zhang et al., (2007) concluded that the method of orally mucosal immunization is simple, convenient and cause little stress in chicken; it is applicable in big hennery because the oral immunization with attenuated virus vaccine can promote lymphocytes to produce IgA and many cytokines in intestinal mucosa. The reasons of oral immunization have been under focus in vaccine development are the immunity stimulation in the gastrointestinal tract and in certain other mucosa and the easy and safe administrations. However, oral vaccine need more and repeated antigen doses to achieve the protective immune response level because the antigen
degradation by gastric acid and proteases which present in the gastrointestinal tract.

Ahmad et al., (2007) studied the development of a vaccination program in order to improve the antibody response and give good protection against challenge with virulent virus of ND. They demonstrated that the efficacy of a lentogenic strain (LaSota) vaccine could be improved by subsequent administration of a mesogenic strain (Mukteshwer) vaccine.

In ovo vaccination: is used successfully and has been proved to be effective against Marek’s disease (MD) (Sharma and Burmester, 1982) and IBD (Riaz et al., 2004). However, studies have been performed to evaluate the efficacy of this kind of vaccination against ND. The live in ovo vaccine which was licensed but not marketed, caused considerable embryo mortality and reduced hatchability (Dilaveris et al., 2007) but, in ovo administration of lentogenic F-strain of ND virus replicated in embryos and newborn chicks, did not hamper hatchability, successfully induced antibody response and conferred protection against the disease in broiler birds (Manna et al., 2007).

2. 8- Immunity:

2. 8. 1- Overview of the Immune System:

Kuby, (1994) stated that the immune system provides protection against infectious diseases that are caused by various microorganisms including viruses, bacteria, pathogenic fungi and parasites, and it is broadly divided into two categories – namely innate or non-specific immune system and the acquired or specific immune response. Innate immunity is the basic defense mechanism for an animal provided by non-discriminatory barriers of anatomic, physiologic, endocytic and phagocytic, and inflammatory
mechanisms. The acquired immunity is a specialized response against infectious agents, and is characterized by specificity, diversity, memory, and self/non-self recognition.

The immune system of the chicken has been studied most extensively. Several researchers have reviewed and discussed the structure anatomy and functions of the avian immune system (Fleischer, 1981; Sharma, 1991 and Korver, 2006). There are many similarities between the general immune mechanisms of mammals and chickens. There are also important differences. Birds respond to antigenic stimulation by generating antibodies as well as cellular immunity (Sharma, 1997).

In birds as well as in mammals the lymphoid tissues can be divided into primary immune tissue and secondary lymphoid tissue. In birds, the primary includes bursa Fabricius (BF) and thymus while the secondary consists of the spleen, Peyer’s patches, cecal tonsils, Meckel’s diverticulum, lymph nodes, bone marrow, glandula pineale and the Harderian gland (Glick, 2000; Sharma, 1999 and Agger et al., 1995).

Although they have a lot in common, some anatomically differences do exist between the lymphoid tissues in mammals and birds. Thymus, the central organ for differentiation of T cells, is in birds divided into 12 lobi and bursa of Fabricious (BF) the central organ for B cell differentiation in birds does not even exits in mammals where the same process takes place in the bone marrow (Ambrosius and Hadge, 1987).

Qureshi et al., (1998) mentioned that the avian immune system is both structurally and functionally unique; it is directly influenced by physiological, genetic, nutritional, and environmental factors. Therefore, the immune system can serve as a sensitive indicator of management and production influences on avian health. Based on current developments in
Review of literature

Genetics, nutrition, and biotechnology, the immune system is an ideal candidate for multidisciplinary efforts in improving avian health.

Seal et al. (2000) reviewed the immune response against ND virus. They suggested that, although protection is measured by presence of antibodies to NDV, vaccinated B-cell depleted chickens are resistant to disease. Consequently, immune protection involves responses that are presently incompletely defined. They concluded consequently, there must be more subtle determinants that contribute to the virulence of NDV and its ability to cause differing pathogenesis in the host. Elucidation of these determinants and maintenance of monitoring programs for NDV remains a high priority for improvement of currently used ND control strategies.

Janeway et al., (2001) reported that the effector cells involved in the innate immune response include granulocytes and macrophages, while the adaptive immune response involves lymphocytes. However, there is not complete demarcation between these two types of immunities and cells involved in the innate immune response take part in effector actions of the adaptive immune response.

2.8.2 Innate immunity (non specific immunity):

Martin et al., (1972) explained that interferon is widely studied as nonspecific viral defense. Both exogenous and endogenous interferon has some inhibitory effects on NDV replication in chicken tracheal explants cultures.

Agger et al., (1995) stated that the innate immune response does not depend on previous infections with the pathogen and therefore it is the most important mechanism for protecting an individual infected for the first time with a pathogen.
Heller et al., (1997) observed that chickens express two types of interferon (IFN) and chicken embryo fibroblasts produce a mixture of type-1 and type-2 IFN which designated ChIFN1 and ChIFN2.

Jeurissen et al., (2000) stated that birds have well-developed innate defense mechanisms. Physical integrity of the skin, epithelial lining, cilia movement, mucus enzymes, low pH barriers, or normal mucosal microflora, prevents pathogens from entering the body. Nevertheless, some pathogens cross the barriers and enter the internal environment. Here, a variety of innate defense mechanisms are present again, such as phagocytic cells that include heterophils and macrophages (Collins et al., 1991) complement (Bennejean, 1988) and natural killer (NK) cells (Erdei et al., 1987).

Bose and Banerjee, (2003a) defined the non specific immunity as an innate characteristic of a host species or strain. It represents the hallmark of host defense against foreign pathogens, including viruses. Not only does this response combat viruses during initial stages of infection, but it shapes the adaptive immune response as well.

Erf, (2004) emphasized that non-specific immunity is comprised of a broad spectrum of defense mechanisms. These include: 1) physical and biochemical barriers designed to prevent invasion by both infectious and non-infectious agents (antigens) and 2) soluble and cellular components capable of eliminating foreign substances (antigens) which have successfully invaded the body tissues. The cellular components of the non-specific immune system include monocytes/macrophages, heterophils (neutrophils in mammals) basophils, eosinophils, and natural killer cells.

Bose and Banerjee, (2003) reviewed the innate immune response against the nonsegmented negative strand RNA viruses of the Paramyxoviridae family. They specifically focused on the two critical
transcription factors, nuclear factor-κB (NF-κB) and IFN, regulatory factor-3 (IRF-3) that play an important role in establishing an innate antiviral state. The antiviral cytokine IFN-α/β (IFN type I) produced following viral infection as a result of activation of NF-κB or IRF-3 or both exerts an antiviral state by inducing the Janus kinases/signal transducer and activator (Jak-Stat) pathway.

Lambrecht et al., (2004) confirmed that NDV is relatively insensitive to interferon action and the role of interferon in protection against ND generally is not considered important.

Genetic selection for increased non specific immunity has not been successfully exploited. It has been studied and genetic differences were reported to affect ND susceptibility. Variation of ND susceptibility among several avian species is another form of innate immunity (Cole and Hutt, 1961; Abplanalp, 1978; Sacco et al., 1994 and Boa-Amponsem, 1998).

2. 8. 3- Acquired immunity (specific immunity):

Beard and Brugh, (1975) defined the specific immunity as an adaptive response depends on the ability to recognize foreign substances respond to them and memorize the information in case of repeated exposure. The specific immunity expressed through the production of specific immunoglobulins (Igs) or the proliferation of sensitized cells that specifically interact with antigen or both.

Sharma, (1991) stated that, the avian immune system is governed by the same principles as the mammalian immune system in that cellular cooperation exists between macrophages and lymphocyte and the interplay between T-cells and B-cells is of critical importance. The T-lymphocytes
are components of cellular immunity while the B-lymphocytes constitute humoral immunity.

**Alexander, (1991)** stated that, like other viruses, ND virus may induce cell-mediated immunity (CMI) humoral immunity, local immunity and passive immunity. Immunosuppressive disease can influence the immune response induced by NDV infection, either naturally or by vaccines.

### 2.8.3.1 Local immunity:

Local immunity is an integral part of total immunity, in addition to cell mediated immunity (CMI) and other humoral factors, for early protection, it involves IgA and locally synthesized IgG and IgM in the Harderian glands. IgA is intimately associated with the mucosal surface secretion. It is associated with the mucosa of the upper respiratory tract, intestinal tract and Harderian gland (**Aitakent and Parry 1976; Zigteman et al., 1993 and Bouvet and Fischetti 1999**).

In ND virus infection, local immunity is induced naturally or with live ND virus vaccines incorporating lentogenic or pathogenic strains (**Russell, 1993; Russell and koch, 1993 and jayawardane and spradbrow, 1995**).

**Katz and Kohn, (1976)** compared local immunity of mammals and fowl; they found a similar system of local immunity against NDV, in the fowl. No antigenic differences were found between the serum IgA and the secretory IgA of airway washings and of bile. They compared the antibodies in the airway washings and in tears of chickens immunized by aerosol or i.m. NDV vaccine (attenuated or inactive) to serum antibodies produced by similar methods of administration. HI antibodies in secretions reached higher levels after aerosol vaccination than after i.m. administration, whereas in serum the situation was reversed. The antibody
activity in airway washings and in tears is associated with IgA, while in serum the main antibody is IgG.

**Malkinson and Small, (1977)** demonstrated effective local immunity when they found that birds may be susceptible to infection at one site but protected at another. They stated that the exact function of local immunity in protection is not clear, although a role in protection of the respiratory tract independent of humoral immunity has been proposed. They proved that, local immunity is responsible for prevention of infection, since birds were immune to reinfection at one site and simultaneously susceptible at the other site of infection.

**Ewert and Eidson, (1977)** suggest that IgA is not essential for the development of immunity in the chicken and that other locally produced immunoglobulins or transuded serum antibody may protect the tracheal mucosa in the absence of IgA.

**Holmes, (1979)** found a variable degrees of resistance induced to challenge with a virulent strain of NDV in the respiratory tract of chickens have been vaccinated with a live NDV, vaccine given either intranasally or intramuscularly and an inactivated NDV vaccine given by the respiratory route. An inactivated NDV vaccine given intramuscularly, however, induced little resistance, despite the high concentration of serum antibody that developed. When a live NDV vaccine was given to chicks with passively acquired antibody, serum antibody response was markedly inhibited, but no comparable inhibition of respiratory tract resistance could be demonstrated. They suggest that the direct or indirect exposure of the respiratory tract tissues to viral antigen was necessary for the induction of resistance to infection in the respiratory tract.
Parry and Aitken, (1977) and Powell et al., (1979) stated that in the upper respiratory tract, the immunoglobulins appear to be chiefly IgA with some IgG. Similar excretions occur in the Harderian gland following ocular, but not parenteral infection.

Ewert et al., (1979) found only immunoglobulin G (IgG) and IgA antibodies to NDV were detected in the salivas, whereas IgA and IgM antibodies were present in egg whites. The highest densities of plasma cells were in the Harderian glands; IgG was the predominant class, whereas IgA and IgM plasma cells were present in almost equal but lower numbers. The Harderian plasma cells were the most likely source of the antibody found in saliva. They reported that IgA was not essential for the development of immunity in chicken while the other locally produced immunoglobulins may protect the tracheal mucosa in the absence of IgA.

Russell (1993) recorded the first report of NDV replication in the Harderian gland (HG) and of virus-specific IgM in the lachrymal fluid. He found that the Eye-drop vaccination of chickens with Hitchner B1 resulted in replication of virus in the Harderian gland tissue without any accompanying virus in the faeces, which could be prevented by the presence of maternal IgG in lachrymal fluid (1/50 of serum levels). Replication of virus in the Harderian gland resulted in the production of lachrymal IgG, IgA, and IgM.

Russell and Koch, (1993) reported that in particular, the Harderian gland becomes the main site for IgA- antibody forming cells in the chickens.

Jayawardane and Spradbrow, (1995) detected a significant increasing in the numbers of plasma cells in sections of Harderian glands and NDV-
specific IgA which was detected in serum, lachrymal fluid, tracheal washing and intestinal washing after intracrop or eyedrop vaccination with V4 virus. They concluded that oral vaccination of chickens with V4 virus induces a mucosal immune response.

Russell and Ezeifeka, (1995) stated that the IgM response was seen first, at 5 days after vaccination, and antiviral IgM levels in the tears and serum were negatively correlated to the level of virus in Harderian gland (HG) over days 4 to 10 postinfection. The frequency of virus-specific IgM-antibody forming cells in the HG of 10-day-old birds that had been vaccinated at 1 day old could reach 1/30 of the total lymphoid cells prepared from the HG. Young chicks made an irregular or low response to inactivated virus by the intravenous route whereas 40-day-old birds made a high serum response to both live and inactivated virus. This emphasizes how day-old chicks respond well to live virus vaccination and that their IgM response is likely to have a role in the clearance of virus.

Takada and Kida, (1996) indicated that secretory antibodies induced on the respiratory mucosal surface by intranasal vaccination with inactivated NDV protected chickens from lethal infection by inhibiting virus replication at the portal of entry for the virus.

Sharma, (1998) confirmed that the bursa of Fabricius, an essential organ for B lymphocyte maturation and antibody production, is strategically located in the final section of gastrointestinal tract, allowing the contact between immune cells and antigens present in diet

Zhang et al., (2007) mixed with ND, vaccine with two novel compound adjuvants (cMIA I and cMIA II). They showed that two compound adjuvants could promote CD3+ T lymphocytes, IgA secreting cells,
intestinal intraepithelial lymphocytes (iIEL) and Mast cells formation and enhance serum and content antibody titre

2. 8. 3. 2- Humoral immunity:

Beard and Hanson, (1984) mentioned that all NDV strains are capable of provoking antibody response in chickens, rabbits, and other species into which they are introduced

Tizard, (1982) and Spradbrow and Samuel, (1991) defined the humoral immunity; by the resistance mediated by cellular immune system that (B-lymphocytes) is under control of the bursa of Fabricius. Activated B-lymphocytes become plasma cells and these secrete blood-derived immunoglobulins IgM and IgG, which constitute the principal component of humoral immunity.

Tizard, (1992) stressed that the humoral response involves interaction of B-cells with an antigen and the subsequent proliferation and differentiation into antibody-secreting plasma cells with or without the help of T-helper-cells. Two basic types of lymphocytes are involved in an antigen-specific response.

Russell and Ezeifeke, (1995) observed that the IgM response was first, at 5 days after vaccination, and antiviral IgM levels in the tears and serum were negatively correlated to the level of virus in HG over days 4 to 10 post infection. Young chicks made an irregular or low response to inactivated virus by the intravenous route whereas 40-day-old birds made a high serum response to both live and inactivated virus. They believed that the IgM response is likely to have a role in the clearance of virus.
Sayegh et al., (1999) reported that the B-lymphocytes express surface immunoglobulins that are specific to an epitope on the antigen and T lymphocytes that recognize processed antigens on antigen presenting cells. The antibody-secreting plasma cells produce soluble antibodies that are identical to the surface immunoglobulin on the original B-cell.

Jeurissen et al., (2000) antibodies form an essential component of the protection against potentially pathogenic microorganisms and are important for the clearance and neutralization of the virus infectivity by blocking the binding of the virus to its receptors on the cell surface.

Carlander et al., (2001) underlined three main classes of immunoglobulins in chickens: IgM, IgG, and IgA. A typical immune response of a chicken begins with IgM production. After some time, IgM production switches over to IgG production. IgG is also the principal antibody produced after secondary immunization and is the predominant Ig class in chicken blood. Because avian (and also amphibian, reptile, and piscine) IgG is larger than its mammalian counterpart, the chicken IgG is often called IgY.

Al-Garib et al., (2003a) demonstrated that the Ig classes (IgM, IgG and IgA) were captured using Ig capture ELISA. The antibodies of the IgM and IgG classes were mainly found in serum and the three classes were demonstrated in tracheal wash and bile after inoculation of ND live virus.

As stated by many studies, Mazanec et al., (1992); Alexander, (2003) and Anon, (2006) antibodies may be present in many body fluids but are most readily detected in the serum or the plasma fraction of blood. They explained three mechanisms by which antibodies contribute to defense against pathogens: A) Neutralization antibodies bind to and neutralize specific pathogens, particularly viruses. Neutralized viruses are unable to
attach to surface receptors of target cells and are thus prevented from replication.

**B)** Opsonization-bacterial pathogens, that can replicate extracellularly, are more readily internalized and destroyed by phagocytes if the pathogens are coated with antibodies.

**C)** Complement activation-antibodies bound to the surface of pathogens can activate complement and produce new complement proteins.

Kapczynski and King, (2006) Studied the duration of immunity of ND vaccines in specific-pathogen-free (SPF) chickens, to a lethal challenge with the California 2002-03 virulent NDV (vNDV). Their results indicated that, in the absence of maternal antibodies, single vaccination with either live or inactivated NDV Hitchner B1 vaccines prevented most all morbidity and mortality following lethal vNDV challenge.

**2. 8. 3. 3- Cellular immunity:**

Factors other than serum antibodies must be invoked to explain the resistance to challenge that is noted soon after conventional application of ND vaccine, or in some circumstances with oral ND vaccine. In these instances, the levels of HI antibody may be very low or undetectable. CM1 could contribute to protection under these circumstances. The initial immune response to infection with NDV is cell mediated and detected as early as 2-3 days after infection with live vaccine strains. CM1 can be regarded as a usual part of the complex immune response to vaccination with NDV. It has been demonstrated by various methods in response to a variety of NDV vaccines. This has been thought to explain the early protection against challenge that has been recorded in vaccinated birds before a measurable antibody response is seen (Allan and Gough, 1976;

Timms and Alexander, (1977) explained that the CMI induced by the live ND vaccine could not be induced again using a virulent NDV infection but only by revaccination. The age of the chicken, type of vaccine and the number of vaccinations before challenge also has a great influence on the degree of the CMI.

Agrawal and Reynolds, (1991) found a significant cell-mediated immunity (CMI) response in chickens vaccinated with ND vaccine by using the under-agarose leukocyte-migration-inhibition (LMI) assay. Their results also indicated that, the presence of the CMI response always correlated to the presence of the serologic immune response and vice versa. However, the quantitative level of the CMI response did not always correspond to the quantitative level of the serologic immune response. They were unable to predict humoral antibody level on the basis of a CMI response and vice versa.

Jayawardane and Spradbrow (1995) used the leukocyte migration inhibition assay to demonstrate antigen-specific CMI, in chickens vaccinated with the V4 strain of NDV. In some chickens, they found a temporary suppression of CM1 to NDV, especially after the initial application of vaccine to the crop. There was no obvious correlation between titres of HI antibody and CM1 responses in their study.

Russel et al., (1997) made an experiment using chickens with chemically suppressed T and B cell populations. After exposing the birds to virus in form of a ND-vaccination there was an increase in the number of T cells with a relatively higher increase of CD8+ cells than CD4+ cells indicating a T cell dominated response.
Reynolds and Maraqa (2000a) investigated the role of CMI, in protection of birds from ND in which only NDV-specific CMI was conveyed without neutralizing antibodies. They found that, birds that had NDV-specific CMI response but did not have VN or HI antibodies were not protected from challenge. Their results were indicating that specific CMI to NDV by itself is not protective against virulent NDV challenge. The presence of virus neutralization (VN) or HI antibodies is necessary in providing protection from ND.

Al-Garib et al., (2003b) speculated that the local lymphoid infiltrates are involved in first defense and that cytolytic cells clear virus by directly lysing infected target cells at the site of NDV inoculation. Secondly, various cell types, mainly T-lymphocytes and macrophages, may be equipped to produce a range of cytokines with antiviral activity and cytokines that stimulate B-lymphocytes to proliferate and differentiate into antibody-forming cells responsible for the local antibody production against NDV.

Lambrecht et al., (2004) used a live ND-vaccine as a substitute for a true infection it has been shown that the first response was a CMI a rising only a few days post infection. This response was followed by a humoral immune response after seven days. A rise was seen in the CMI until the second week and of the antibodies until the third week. The CMI declined after approximately four weeks

2. 8. 3. 4- Maternal immunity:

Richey and Schmittle, (1962) summarized the effect of maternal antibody levels on the response of chicks to ND vaccination in four points as following:
1) Individual vaccination of 1-day-old chicks with a single application of the B₁ strain of NDV resulted in a serologic response inversely related to the quantity of congenital passive antibodies. 2) A serologic "level of significance" titre for the HI, or neutralization indices (NI) tests could be correlated with the resistance to challenge only in chicks with the least amount of congenital immunity during the 8-week test period. 3) A transitory infection of 1-day-old chicks possessing increasing amounts of congenital immunity was evident in that HI and NI antibodies of the control chicks decreased more rapidly than those of the vaccinated birds. 4) The susceptibility to challenge at weekly intervals post vaccination was proportional to the degree of congenital ND immunity in 1-day-old chicks. Cumulative challenge mortality during the 7 to 8 weeks period was 0%, 10% and 27% among groups of vaccinated birds having increasing amounts of congenital antibodies.

Chu and Rizk, (1975) compared the response of groups of chicks from vaccinated and non-vaccinated hens after vaccination at various ages with ND vaccines of different pathogenicity (lentogenic and mesogenic strains). Their results show that: 1. maternal immunity has a great effect on the pathogenicity of the vaccine viruses and the subsequent immune response; 2. the older the chicks are at the time of vaccination, the better is their immune response; 3. for vaccines of low pathogenicity the immune response can be enhanced by increasing the dose of the virus irrespective of the presence of maternal immunity.

Malkinson and Small, (1977) reported that low levels of passively administered antibody did not prevent infection of the eye or air sac but greatly reduced the mortality rate after inoculation of either the vaccine or the challenge viruses. Passively administered antibody also suppressed hemagglutination-inhibiting and virus-neutralizing antibody formation.
stimulated by air-sac infection but not antibody formation stimulated by ocular infection

**Eissa, (1979)** found that the maternal immunity in chicks decreased gradually throw three weeks of age.

**Partadiredja et al., (1979)** concluded that maternal antibody levels in one day old chicks were related to the antibody titres in their dams. They added that the maternal antibody titres in chicks from breeder vaccinated with oil emulsion vaccine remained higher than other hatches.

**Tizard, (1979)** mentioned that the failure of antibody response to ND vaccine in newly hatched chicks may be due to the presence of maternal immunity.

**Kohama et al., (1987)** mentioned that chickens hatched from eggs of vaccinated hens with live attenuated ND vaccine, possessed maternal antibodies will resist the challenge with virulent ND virus strain.

**Koh et al., (2001)** measured the antibody titres against ND in breeder chickens and their progeny. They reported that, breeders serum antibody titres ELISA and HI were 30,200 GMT and (8.7) HI titre respectively. On one day old chicks, antibody levels were decrease to half in ELISA (16,270) compared with those of breeders, but HI titre was 7.4. The protective antibody level of progeny was maintains until 14 day old by ELISA but by 11 day old by HI titres. After then, ND antibody titre was continuously decreased under defense level.

**Kowalczyk et al., (1985) and Carlander, (2002)** explained that the transport of IgY from the hen serum to the offspring is a two-step process. First IgY is transported from the oolemma into the maturing oocyte in the ovarian follicle to the egg yolk in analogy to the crossplacental transfer of
antibodies in mammals. The second step is the transmission of IgY from the yolk sac to the developing embryo circulation. The concentration of IgY in the yolk is essentially constant through the oocyte maturation

2. 8. 3. 5- Transport of IgY from maternal serum to the offspring:

Rose et al., (1974) reported that the concentration of IgY in the yolk is essentially constant through the oocyte looking at the egg. IgY is not present in the egg white while IgA and IgM is not present in the yolk. The lack of a specific and well-developed humoral response during the first days of life leads chick to be very dependent of maternal antibodies. The antibodies IgM and IgA of amniotic fluid, which corresponds to colostrum in mammals, are ingested by embryo and has local role instead of the little transference

Linden and Roth, (1978) confirmed that the IgG is transferred by receptor-mediated endocytosis across the yolk sac epithelium. IgA and IgM are transferred via the amniotic fluid. The developing embryo swallows IgA- and IgM-containing amniotic fluid.

Ewert et al., (1979) reported that, in its subsequent passage down to the oviduct, the ovum (yolk) becomes surrounded by albumin which contains maternal IgM and IgA with the result that the newly lied egg possesses all three immunoglobulin classes. Maternal antibody primarily of the IgA and IgM classes may be acquired by offspring by ingestion rather than by direct absorption into the fetal circulation. Maternal IgA and IgM present in the oviduct and incorporated in the egg whites mix with the amniotic fluid and bathe the external and internal surfaces of the embryo, providing transient protection for the emerging chick.
Gordon and Jordan (1982) stated that during the greater period of embryonic development, the immunoglobulins remain in their respective location but with breakdown at mid-incubation of the barrier between the albumin and amniotic sacs, their fluid mingle and some is eventually swallowed by the embryo. In this way IgM and IgA gain access to the gut of the late embryo life in which traces of yolk-derived IgG are also found. So far, no antibody function has been attributed to IgM or IgA transferred in albumin but they may have an antibacterial role in the gut, as albumin is rich in lysosome with which IgA may act synergistically. They stated that absorption of IgG from the yolk sac into the circulation begins about day 15 and is not complete until the yolk sac is fully resorbed two to three days after hatching.

Eidson et al., (1982b) reported that Chicks from breeder flocks vaccinated with live virus vaccine had a low geometric mean hemagglutination-inhibition antibody titre (GMT) for Newcastle (low maternal antibody titre) at 1 day of age, whereas those chickens derived from breeder flocks vaccinated with inactivated oil emulsion ND vaccine had a high GMT (high maternal antibody titre). Even though they were protected against ND, there appears to have been an interference phenomenon in chicks derived from breeder flocks vaccinated with the live ND vaccine.

Allan et al., (1978); Gordon and Jordan, (1982) and Loeken and Roth, (1983) confirmed that the amount of IgY transported is independent of egg size and known to be proportional to the maternal serum IgY concentration. The concentration of passively acquired IgG in the serum of the newly hatched chicks are approximates as in the adult values and it can be equated with that of the hen 5 days before the egg was laid.
Kowalczyk et al., (1985) stated that the density of yolk is about 1.1 g/ml. About 50% of the yolk is non-aqueous material. The total amount of IgY in the hatched chick has been estimated to be only 2-3 mg, compared to the 100-400 mg present in the yolk, however, the major part of the IgY probably serves only as nutrition for the developing embryo. On the other hand, there is suggestion that IgG uptake occurred in the last few days before hatching.

Tressler and Roth, (1987) said that the immunoglobulin binds to an Fc receptor similar to mammalian FcRn and is actively transferred into the yolk and Eight-day embryos appear to have only a low affinity receptor but 18-day embryos have an additional high affinity receptor. Radiolabeled chicken IgG binds specifically to yolk sac tissue from day 7 or 8 of embryogenesis through at least day 18. This binding is saturable, Fc specific, pH-dependent, and reversible.

Rao et al., (1987) demonstrated that the antibody titres in egg yolks and chicks were lower than the titre of corresponding hen. A definite relationship was shown to exist between the egg yolk titres and the maternal antibody titres as the quantum of antibodies transferred to the chicks depended upon the egg yolk titres. The maternal antibodies in chicks persisted up to 10th day and no detectable levels of antibody were observed in 15-day-old chicks.

Van Eck, (1990) reported that, If maternal antibody titres are high, passive protection is provided for at least the first 3 weeks of life, and vaccination can occur after this period in low-risk areas, protocols based on an initial vaccination at 3–4 weeks after hatching are suitable if an inactivated vaccine is used for breeders.
Woolley and Landon, (1995) stated that a delay of three to four days is found between the appearances of IgY in serum until it is found in the yolk. The concentration of IgY in the yolk is by a factor 1.23 to the serum maturation, and at maturity the yolk will contain about 10-20 mg/ml IgY concentration.

Bailey et al., (1998) confirmed that PMV-1 antibodies were transferred to eggs and chicks derived from Korean bustard hens immunized with inactivated vaccine 5 to 8 months previously. Chicks hatched from dams with high HI, antibody titres had high titres of maternal derived immunity (MDI). They found that mean antibody levels in seropositive chicks were log2 6.3 and log2 2.9 on days 14 and 42, respectively. The rate of decline of detectable antibodies (1 log2) was estimated to be 5.50 to 6.25 days and 12.25 days in 14 to 21 and 28 to 42 day old chicks, respectively

Gelb and Jackwood (1998) and Cardoso et al., (2005) observed that the control group presented antibody titres against NDV that decreased from geometric titre (GMT) 4500 ELISA titre in the first day to basal levels at 45 days of age. They concluded that the maternal antibody titres against NDV progressively decreased until approximately zero after few weeks.

Alexander, (1991) and Tizard, (2002) as the egg passes down the oviduct, IgM and IgA from oviduct secretions are acquired with the albumin. The maternal IgM and IgA from the albumin diffuse into the amniotic fluid and are swallowed by the embryo, so that when the chick hatches it possesses IgY in its serum and IgM and IgA in its intestine. (This IgM seems to be in a monomeric rather than polymeric formY). The hatched chicks absorbs yolk immunoglobulins within 3 days of hatching when its serum IgY levels can reach those of adults (5 to 10 mg/ ml).
Sharma, (2003) established that the transfer from the yolk continues after hatch. Peak levels of maternal IgG in the circulation of the newly hatched chick are reached around 2-3 days of age. Maternally derived antibodies decline linearly in the recipient and become undetectable after 2-5 weeks.

Wakenell, (2006) stated that egg yolk begins to be absorbed by about 24 hours after hatch and failure of absorption can adversely affect transfer of maternal immunity. The half-life of maternal IgG is twice as long as adult IgG probably to allow enough time for proper absorption. The amount of antibody transferred from hen to chick can vary with the age of the hen and the point of time in lay as well as with the titre level in hen's serum. IgM will appear in the chick’s serum at about 4 days of age and IgA at about 10 days of age.

Hamal et al., (2006) established that the IgY levels, total or antigen-specific in the dams' plasma or eggs were found to be a direct indicator of maternal antibody transfer to the chicks' circulation, with an expected percentage transfer of approximately 30%. This knowledge, together with the observed time course of endogenous antibody production in broiler chicks, may find direct application in formulating strategies for protecting chicks, especially during the first few weeks of age when their immune system is not yet fully functional.

2. 8. 3. 6- Comparison of Egg yolk and serum for antibodies titration:

Piela et al., (1984) tested serum and yolks from commercial flocks and from hens exposed to NDV, IBV, and Mycoplasma gallisepticum (MG) for immunoglobulin G antibody by ELISA and HI test. They concluded that the yolk is suitable alternative to serum for antibody determination by the ELISA for NDV, IBV, and MG and by HI test for NDV.
Sulochana, (1988) used HI to test antibody of ND virus both in the serum and egg yolk to study the suitability of the latter for monitoring the immune status of birds. They found that before the third week of vaccination the HI titres in the yolk were lower than serum titres but from the third week onwards comparable results were obtained with both the samples. They noticed that no significant reduction in antibody titre in yolk when the eggs were incubated at 38°C degree C for six days. They concluded that, egg yolk for antibody titration to NDV by HI test is considered as an easy and safe method for monitoring the immune status of large number of laying hens compared to conventional serum method.

Silim and Venne, (1989) assayed the serum and egg-yolk extracts for antibody titres to IBDV, IBV, NDV, and Reo virus (RV) by a commercial ELISA kit. They compared between egg-yolk and serum antibody titres by a regression analysis and they observed a high correlation between serum and yolk antibody titres to all the viruses tested (r = 0.9 for IBDV, 0.84 for IBV, 0.84 for NDV, and 0.91 for RV). They concluded that antibody monitoring of commercial breeder flocks using egg yolk instead of serum with commercial ELISA plates is thus feasible and is recommended.

Keck et al., (1993) used an ELISA kits to detect antibody in egg yolk and to determine the adequacy of yolk samples to replace serum samples. They found that, the mean titres were consistently larger for serum than for yolk, but the size of the difference varied with the virus. The variation of mean egg titre was comparable to that of the serum titre and they concluded that the correlations between a hen's serum titre and the mean titre from hen eggs were only moderate, ranging from 0.35 to 0.85 across viruses and systems.
Ling et al., (1998) explained that the determining of the serum and egg yolk antibody titres in immunized laying hens to Pasteurella multocida. Regularly, the growth-decline trend of the egg yolk antibody levels was found to be similar to that of the serum antibody levels ($r = 0.94$) but the growth and decline of the egg yolk antibody seemed to be delayed 3-6 days compared with that of the serum antibody, and the egg yolk antibody titres were generally lower than those of the serum antibody ($P < 0.01$).

Beck et al., (2003) compared the utility of egg yolk vs. serum for determining AI status in laying hen flocks. Antibody levels were determined by the AGID test, HI test and ELISA. They stated that currently used serological tests can detect antibodies in serum and yolk samples from hens exposed to live AI virus or from those that have been vaccinated. Antibody was detected earlier in the serum than in the yolk and antibody was detected earlier from birds exposed to a live infection compared to birds vaccinated with an inactivated oil emulsion vaccine.

Yeo et al., (2003) attempted to establish methods for indirect prediction of HI, antibody titres to NDV, in sera of laying hens and day-old chicks by determining if these are correlated to HI titres in egg yolks. They stated that immunity to NDV in hens and their offspring can be maintained effectively, and the proper time for the vaccination or booster can be determined by reference to HI titres predicted from the linear regression in resulted. They concluded that, the approach of testing egg yolk for HI titres provides a feasible alternative to determining HI titres from blood samples and eliminates stress in birds during blood sampling.

Rauber et al., (2004) compared IBV antibody titres in serum and egg yolk samples from laying hens by indirect ELISA (commercial kit). Titres were compared by a correlation test ($P=0.05$). They found a high
correlation (r=0.62) between the two kinds of samples, which means that
titres of IBV antibodies in the egg yolk and in serum samples are quite the
same.

**Msoffe et al., (2006)** studied the HI dynamics of six local chicken
ecotypes of Tanzania and in their egg yolks and offspring to ND. They seen
that the mean HI titres in chicks were significantly higher than those in
hens and eggs (P<0.05) and they concluded that the comparable HI titre
means between eggs and hens provides the possibility of using eggs to test
the flock immunity instead of the invasive blood serum method.

**2.9- Yolk extraction:**

**Piela et al., (1984)** used the extracted egg yolks in serological tests by
chloroform method. They mixed 1ml of yolk 1:1 (vol/vol) with PBS and
then mixed 1:2 (vol/vol) with chloroform, and harvested the supernatant
after incubation for 1 hour at room temperature and centrifugation for 20
minutes at 1500 rpm.

**Piela et al., (1985)** compared between three methods of egg yolk
extraction used in the serological tests: 1)-dilution in phosphate buffer
saline (PBS). 2)- extraction of 1:1 mixture of the water – soluble fraction
(yolk / PBS ) with 2 volume chloroform 3)- freezing and thawing (PBS)
diluted yolks and testing the supernatant fluids by HI test with a final
dilution of 1:8 to use as the starting dilution in the HI test. They found that
yolks diluted in PBS or extracted with chloroform give titres similar to
those of sera, with a maximum difference of two folds. They concluded
that chloroform extracted yolks were suitable material for the HI, ELISA
and ID tests.
Mohammed et al., (1986) compared between saline and chloroform extraction methods for yolk antibodies monitoring against MG and Mycoplasma Synoviae (MS). They found that in HI test, the saline extracted samples given a high proportion of false positive, but not with chloroform extracted samples. In ELISA test, the correlation coefficient between the two procedures was 0.96 for the MG ELISA and 0.98 for the MS ELISA.

Sulochana, (1988) compared between the serum and egg yolk by HI test for monitoring the immune status of birds. They found that no difference in the pattern of agglutination of antibody titre could be observed when the diluents used was normal saline or phosphate buffered saline (pH 7.2). Similarly no difference in titre was noticed when the yolk was frozen overnight or titrated immediately after collection. They concluded that chloroform extraction of the yolk was unsuitable for antibody titration by HI test as such samples produced nonspecific agglutination.

Stone et al., (1992) harvested the yolk from eggs laid by hens' hyperimmunized with killed ND virus was inoculated into the yolk sac of 1- day specific- pathogen- free (SPF) chickens. Serum HI antibody titres reached maximum levels 1 to 4 days after yolk inoculation and declined at a rate similar to that reported for normally acquired maternal antibody. They concluded that yolk sac inoculated with yolk antibody is a suitable approach for producing maternally immune chickens for laboratory studies.

Akita and Nakai, (1992) employed a simple water dilution for the separation of water –soluble plasma proteins from egg yolk granules. An optimum recovery of IgY (93-96%) in water soluble fraction was obtained.
by six fold water dilution at pH 5.0-5.2 with incubation time of 6 hours at 4 °C.

Larsson et al., (1993) mentioned that crude egg yolk may be used as an antibody source, but the lipid in the yolk may interfere with the antibody activity. Therefore, antibodies are usually purified from the yolk. There are several published purification methods that can be used for the extraction of IgY from chicken egg yolk.

Keck et al., (1993) detected antibodies in egg yolks for four chicken viruses depending on a simple dilution method with no mixing or extraction. They used a 1:10 dilution of yolk PBS solution for detection the antibodies of NDV and other viral disease by commercial available ELISA kits.

Akita and Nakai, (1993) stated that the major problem in isolation of IgY- antibodies of egg yolk is the removal of lipids, which are present in high concentration. They had developed method by employing water dilution to separate the yolk plasma proteins from the granules and lipids. Comparatively evaluated for better results had been conducted between the water dilution and three purification methods namely polyethylene glycol (PEG) dextran sulphate (DS) and xanthan gum (Xan). They reported that the water dilution method provided a simple, rapid and efficient means of purifying IgY-antibodies with high activity.

Yeo et al., (2003) followed a simple method for extraction of antibodies against ND from egg yolk by adding 2.5 ml of sterile physiological saline to 1ml of yolk. After mixing vigorously, the mixture was incubated overnight and centrifuged at 3000 × g for 15 minutes. The supernatant containing antibodies was harvested to be analyzed by HI test.
Bizhanov et al., (2004) compared five methods to separate egg yolk IgY antibodies. The yolks were pooled, mixed and a mixture of TBS and egg yolk (4:1, v/v) was prepared. From this mixture aliquots were processed according to the five different protocols:

1. Precipitation with PEG-6000 (Fluka) following the procedure described by Polson et al., (1985);
2. Chloroform extraction as described by Ntakarutimana et al., (1992);
3. By the water dilution method described by Akita and Nakai, (1993) but replacing sodium sulfate by sodium citrate
4. As method 3 but replacing sodium sulfate by lithium sulfate
5. As method 3 but replacing sodium sulfate by ammonium sulfate.

In methods 3 - 5 the yolk was diluted 1:9 with distilled water. They found that the total protein and IgY contents when purified by chloroform were 1.4-2.8 times and 1.3-2.3 times higher, respectively than in corresponding preparations purified by the other methods. However, the proportion of nonsense proteins was approximately 10% higher in the IgY preparation purified by chloroform than in those purified by salt precipitation.

Hamal et al., (2006) used a chloroform-based method to extract immunoglobulin from the egg yolk. Twice the volume of Dulbecco’s PBS (Sigma-Aldrich Inc.) was added, and the chloroform equal to the volume of egg yolk and PBS was then added, mixed vigorously, centrifuged at 1,000xg for 30 min at room temperature, the watery phase on the top containing the Ig was removed, for analysis.
2. 10- Serology:

2. 10. 1- Serological Monitoring:

_Zander et al., (1997)_ established that serologic monitoring of layer and breeder flocks should be performed before the flock is placed in the layer building, with periodic serologic monitoring throughout the production cycle. In certain instances, breeders can be revaccinated during production to boost the maternal antibody titres of their progeny if they are found to be low.

_Alexander, (2003)_ stated that to assure that a vaccination program is effective, it must undergo regular evaluation. Methods for evaluating the effectiveness of a vaccination program, varies widely and generally involves evaluating and monitoring overall health. Frequently, absence of morbidity and mortality is used as a criterion for success. The important method is the regular serologic monitoring.

2. 10. 1. 2- HI test:

_Allan and Gough, (1974)_ confirmed that, it has not been possible to record the HI titre in a standard method which can be reproduced in different laboratories and the variation in the expression of HI titres has led to sum degrees of confusion. They described two standard methods of conducting HI, tests for antibody to ND virus: A micro method using an automated titrator and a macro method involving manual titrations in WHO plates. The two methods were compared by repeat testing of bulked serum samples and the HI levels recorded compared to the international reference preparation of ND antisera.

_Majiyagbe and Hitchner, (1977)_ investigated the differences in antibody response to three NDV strains, Hitchner B-1, LaSota, and Ulster,
using the HI microtitre test in chickens hatched from ND-immune and unimmune flocks.

Max Brugh et al., (1978) observed that the most marked effect on magnitude of HI titres was incubation time of twofold serum dilutions in antigen-saline; the average titre increase after incubation of the serum-antigen mixture for 1 hr at 37 C was \( \log_2 2.3 \) (fivefold). Twofold increases in virus concentration of the antigen-saline diluents caused an average titre reduction of \( \log_2 0.8 \). Shifts in HI titres were only minor with twofold changes in erythrocyte concentration (\( \log_2 0.3 \)) with variations of test reading times from 0.5 to 2.0 hr (\( \log_2 0.1 \)) and with variations in the period between preparation of the initial 1:10 serum dilution in antigen-saline and the subsequent serum dilutions (\( \log_2 0.3 \)).

Beard and Wilkes, (1985) described the results of two comparative trails using the ND HI test in 17 laboratories (Phase 1) and 16 laboratories (Phase 2) in 10 southeastern states. They found that there is considerable variation in ND HI results among the laboratories even when the same antigen preparation and incubation time are used.

Alexander, (2000b) explained that usually for HI tests, the problem to be faced with serum samples from avian species is one of agglutination of red blood cells, i.e. false negative results. This can be avoided by routine pre-treatment with the red blood cells to be used in the test or testing for agglutination by the samples under test and then adsorbing if necessary.

Kapczynski and Tumpey, (2003) studied the protection efficacy of existed commercial live and inactivated NDV, vaccines against the 2002–2003 END viruses. They found that protection from disease correlated with the presence of antibody titres (determined by ELISA or HI) at day of challenge.
Adebayo, (2004) standardized some mammalian erythrocytes as alternative indicator systems for ND HA and HI tests and determine the differential sensitivity of such erythrocytes to vaccinal and field NDV induced antibodies. He showed that 0.5 and 1% of goat and guinea-pig red blood cells (RBC) suspensions gave similar results with tests conducted with standard or control 0.5% chicken RBC as indicator systems. They found that the HI titres with the guinea-pig indicator systems was selectively and consistently higher than titres with the standard chicken RBC by 5log in flocks with confirmed ND history. However, the titres for flocks without ND, 2 outbreaks were generally similar with the three indicators thus showing the ability of the guinea-pig RBC indicator for the selective detection of high titre in ND infected flocks.

Alexander, (2004) described the procedure of micro titre HI test and he stated that HI titres may be used to assess the immune status of a flock. In vaccinated flocks that are being monitored serologically and may be possible to identify anamnestic responses as the result of a challenge infection with field virus. The value of serology in diagnosis is clearly related to the expected immune status of the affected birds. HI titres may be regarded as being positive if there is inhibition at a serum dilution of 1/16 ($2^4$ or log$_2$ 4 when expressed as the reciprocal) or more against 4 HAU of antigen. Some laboratories prefer to use 8 HAU in HI tests. While this is permissible, it affects the interpretation of results so that a positive titre is 1/8 ($2^3$ or log$_2$ 3) or more. Back titration of antigen should be included in all tests to verify the number of HAU used.

Msoffe et al., (2006) observed that the day seven value of overall HI titre means shows in the first immunization was significantly lower than other values. The mean HI titre for day 28 was significantly higher than mean day 7, day 14 and day 63 but not day 21. Following the second
immunization on day 70, the mean HI titres for day 1 were significantly lower (P<0.05) compared to other days. The mean HI titres on days 14, 21 and 28 were statistically similar.

Sakai et al., (2006) studied the prevalence of antibodies to NDV and AIV in ostriches in Japan. They reported that The HI test for NDV seemed not to be suitable for ostriches because of non-specific agglutination of chicken red blood cells.

2.10.1.3-ELISA:

A number of methods have been published for the detection of antibodies against NDV, by means of ELISA (Snyder et al., 1983; Wilson et al., 1984; Adair et al., 1989) and commercial kits have also been produced.

Wilson et al., (1984) developed an ELISA to measure antibodies to NDV, in chickens. Chickens 6 to 33 weeks old, with a variety of vaccination histories, were bled before challenge with a velogenic strain of NDV. Fourteen days post-challenge, 63 of the 73 challenged birds had survived. They found that, ELISA results of pre-challenge sera corresponded directly with survival rates of birds challenged with NDV.

Thayer et al., (1987) tested the sera for HI activity against NDV, and IBV, and VN activity against IBDV. They found good correlation of mean Flockchek® ELISA titres or EIA systems SP ratios with specific HI or VN titres. Flockchek ELISA profile group 3 and EIA systems mean S/P ratio of 1.12 corresponded to what were considered in their lab. to be minimum protective titres for each antigen against virulent challenge in area.
Adair et al., (1989) developed a quantitative single well ELISA for estimation of ND virus antibodies in chickens and turkeys using purified antigen form PMV-1/chicken/Ulster 2C/71. They found that, absorbance values for negative sera in chickens increased with the age of the birds but overall was lower than the cut-off for the test.

Bell et al., (1991) stated that the Animal Production and Health Section of the Joint FAO/IAEA Division have developed an ELISA kit for the screening of poultry sera for ND antibodies. Based on the design of a kit for bovine antibodies, the simple indirect method uses a single serum dilution, peroxidase conjugate and orthophenylenediamine as substrate. The reagents are stable and incubation steps are carried out at 37°C, so the assay is easy to transport and suitable for widely fluctuating temperatures. ELISA titres were higher than HI titres and showed a high degree of correlation with them.

Horvath et al., (1999) determined the potency of 27 different inactivated ND vaccines by immunization and challenge tests. Serum samples were tested by the HI test and by a monoclonal antibody (MAB) blocking ELISA (B-ELISA). They found a strong positive correlation (0.934) between actual protection (protection against challenge) and estimated protection (protection calculated from the B-ELISA results). Based their results, the B-ELISA seems to be suitable for replacing the challenge test in the potency control of inactivated ND vaccines in the future.

Al-Garib et al., (2003a) developed capture ELISA using Ig-class specific monoclonal antibodies (MAbs) (Ig-capture ELISA). The antibody specificity of the captured Ig was confirmed by binding of NDV. The Ig-capture ELISA assay developed in this study can be useful for evaluating
various strategies to improve the efficacy of ND vaccines and to study the evoked immune mechanisms.

Alexander (2003) stated that ELISA is by far the most common serologic assay used under commercial settings. Automated technology allows rapid processing of large numbers of serum samples. Computerized data transmission facilitates flock profiling and provides useful information on environmental exposure to pathogens and response to vaccination. Optimum vaccination strategies then may be designed. ELISA kits that can be used to detect antibodies against most of the common viral and bacterial pathogens of poultry are available commercially.

Manoharan et al., (2004) used an immunocomb-based dot-ELISA, employing specially designed apparatus to measure the antibody status for the three major poultry diseases, ND, IBDV and IB in single test sera. They observed that the simultaneous dot-immunobinding assay gave reproducible results and allowed considerable savings on the cost of reagents compared to liquid ELISA. They concluded that the antigen-coated immunocomb can be stored under refrigeration and the test can be performed rapidly under field conditions by trained personnel.

Mohan et al, (2006) developed a recombinant HN antigen-based single serum dilution ELISA to measure the specific antibody in sera of chickens against ND virus. They found that the assay proved to be sensitive, specific and accurate as compared to the standard HI test.

2. 10. 2- HI and ELISA comparison:

Piela and Yates, (1983) compared ELISA with HI and ID for detection of antibodies. They found that ELISA was a sensitive and reliable method for detecting antibody, although positive titres were not always in
agreement with HI and ID results at 1 week post inoculation, probably reflecting the different classes of antibody being detected.

Snyder et al., (1983) developed an ELISA, to measure a specific antibody activity in sera of chicken exposed to ND virus. They found a correlation (P<0.01) between ELISA and HI antibody titres to NDV. They concluded that, ELISA titres were as much as 160 times greater than the HI titres and ELISA was also able to detect much lower levels of antibody activity than the HI test.

Adair et al., (1989) performed a comparison between ELISA and HI for quantitative estimation of NDV antibody level in chicken and turkeys. They found that positive field sera were always positive by ELISA and the mean was significantly higher than that of negative population. They reported a significantly positive correlation between ELISA and HI titres for chicken and turkey sera.

Brown et al., (1990) studied the relationship between the HI test and the ELISA for the detection of antibody to NDV and found that a commercial ELISA had 98.2% sensitivity and 91.7% specificity relative to the HI test. The correlation coefficient was 0.85 and the kappa between the ELISA and HI test calculated to be 0.84 (z = 7.74, P = 0.00001) which indicates a highly significant agreement between the two tests.

Cvelic-Cabrilo et al., (1992) showed a good correlation between ELISA and HI test as commercial NDV ELISA, the correlation coefficient was 0.85.

Folitse et al., (1998 a) developed a method of dot blot ELISA for the detection of antibody against NDV in sera of experimentally infected
chickens with comparison to the HI test. They found that the ability of the ELISA to detect antibodies 2 days earlier than the standard HI test.

Koch et al., (1998) used a B-ELISA test, a VN test and the HI test (without pre-treatment of sera) to examine 211 ostrich sera for antibodies to ND virus. They reported high sensitivity (86%) and specificity (87%) values for the HI test relative to the VN, and similar values (83 and 91%, respectively) for the B-ELISA relative to the VN test. The specificity and sensitivity values for the B-ELISA relative to the HI test were 91 and 96%, respectively. They concluded that the good agreement between the b-ELISA and the HI test suggested that the two methods are interchangeable for the detection and measurement of ND virus-specific antibodies in ostrich sera.

Sousa et al., (2000) adapted a liquid phase blocking ELISA (LPB-ELISA) for the detection and quantification of antibodies to ND virus in vaccinated and unvaccinated commercial flocks of ostriches (Struthio camels) and rheas (Rhea Americana). The HI test was regarded as the reference method. They determined the cutoff point for the LPB-ELISA by a two-graph receiver operating characteristic analysis. They found that the LPB-ELISA titres regressed significantly \( (P < 0.0001) \) on the HI titres with a high correlation coefficient \( (r = 0.875) \). They concluded that the two tests showed good agreement \( (\kappa = 0.82; P < 0.0001) \) relative sensitivity (90.91%) and specificity (91.18%) and accuracy (91.02%) suggesting that they are interchangeable.

Koh et al., (2001) compared serum antibody titres using commercial ELISA kit and HI test. They detected the ELISA method was more sensitive than titration to detect serum antibody level to NDV and IBV.
Aydin et al., (2001) determined and compared the results of serum antibody levels against the ND virus in vaccinated chicken flocks and in experimental animals by HI test and ELISA. In addition to the 50 experimental animals, they tested a total of 504 chicken sera (broiler, layer and breeder flocks) collected from 21 farms (by HI and ELISA). They found that the results of HI and ELISA were positively correlated, and the t-test was significant in both experimental and test groups (r=0.97 and 0.87 respectively) upon statistically evaluation (p<0.001).

Merino et al., (2002) studied the correlation between the HI and the ELISA tests using the Synbiotics NDV ELISA kit (NDV+ ProFLOCK ELISA) when measuring anti-NDV serum antibodies. The titre value correlation between the HI and ELISA tests was determined by linear regression analysis. The results showed a significant correlation between ELISA titres and previously obtained HI titres (p<0.01) they concluded that there is a linear and proportional relationship between the HI test and the NDV ELISA test over a wide HI titre range.

Tabidi et al., (2004) compared the antibody (Ab) titres using HI test and an indirect ELISA, to the intermediate NDV, vaccine (Komorov strain) in broiler chicks. They compared the titres following vaccination of chicks via the aerosol, intranasal and drinking water routes. For all routes of the vaccine administration, higher Ab titres were detected using ELISA technique than HI test. For both serological assays, the highest Ab titres detected when the vaccine was administered via the aerosol route with significant level (p< 0.05) compared to the control group. Non-consistent pattern in the Ab levels between the two tests was observed for intranasal and drinking water routes. They concluded that, ELISA proved more
accurate, sensitive and rapid but less economic than HI test when used for
detection of Ab titres against NDV vaccines.

Bell et al., (2006) reported the correlation of the ELISA titres with the
HI titres, using the same serum samples. They found a positive high degree
of correlation between the HI titres and ELISA titres. The coefficient of
correlation was (r = 0.61).