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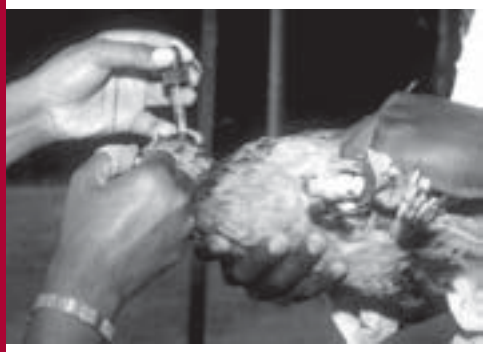

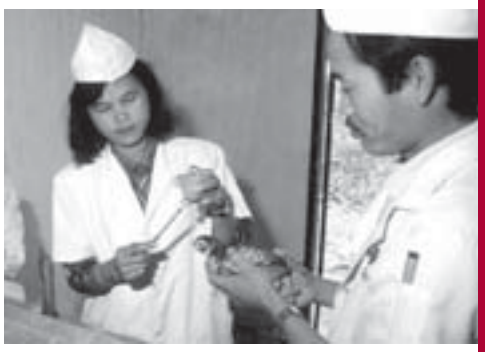

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A TECHNOLOGY REVIEW: NEWCASTLE DISEASE

with special emphasis on its effect on village chickens

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TECHNOLOGY REVIEW: NEWCASTLE DISEASE

with special emphasis on its effect on village chickens

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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FOREWORD

FAO Technology Review: Newcastle Disease

Keeping poultry makes a substantial contribution to household food security throughout in the developing world. They help diversify incomes as well as providing quality food, energy, fertilizer and a renewable asset in over 80 percent of rural households. Poultry keeping also helps to sustain the village economy and contributes to the prevention of urban migration. The benefits derived from keeping poultry go directly to the rural poor and, in most cases, to the women in their capacity as active caretakers.

Small-scale poultry production however suffers from number of constraints including poor animal health, especially Newcastle Disease, as well as insufficient nutrition and poor housing. Together, these factors result in high losses and corresponding low levels of productivity. Overcoming these constraints could substantially increase productivity would result in real and direct benefits of the farmers themselves.

One of the principal constraints to increasing small-scale poultry production is Newcastle Disease (ND). This acute viral disease can typically kill up to 80 percent of unprotected poultry in rural areas and is found throughout the developing world. The disease is spread by contact between birds and is exacerbated by birds being mixed together in rural markets, although many aspects of the epidemiology of the disease in the village situation is not yet fully understood.

This technical review is written by three of the foremost experts in the field of Newcastle Disease control in the developing world. It presents the latest understanding of Newcastle Disease, its characteristics, epidemiology, symptoms and control. It will be of practical value to state and private veterinarians and to all those involved with rural poultry production and wishing to control this disease.

FAO acknowledges and commends the effort that the authors have put into making this such a comprehensive and valuable reference for those involved in the control of Newcastle Disease in the developing world. The views expressed are, however, those of the authors and do not necessarily reflect those of FAO.

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CHAPTER 1
NEWCASTLE DISEASE VIROLOGY AND EPIDEMIOLOGY

INTRODUCTION

History

The first outbreaks to be recognized and termed Newcastle disease (ND) occurred in poultry in 1926, in Java, Indonesia (Kraneveld, 1926), and in Newcastle-upon-Tyne, England (Doyle, 1927). However, there are earlier reports of similar disease outbreaks in Central Europe before this date. (Halasz, 1912). In particular, Macpherson (1956) attributes the death of all the chickens in the Western Isles of Scotland in 1896 as being due to Newcastle disease. It is possible, therefore, that ND did occur in poultry before 1926, but its recognition as a specifically defined disease of viral aetiology dates from the outbreaks during this year in Newcastle-upon-Tyne.

The name "Newcastle disease", (after the geographical location of the first outbreaks in Great Britain), was coined by Doyle as a temporary measure because he wished to avoid a descriptive name that might be confused with other diseases (Doyle, 1935). The name has, however, continued to be used although when referring to the ND virus (NDV), the synonym 'avian paramyxovirus type 1' (APMV-1) is now often employed.

Later it became clear that other less severe infections were caused by viruses almost identical to the original virus. In the United States, a relatively mild respiratory disease, often with nervous symptoms, was first reported in the 1930s and subsequently termed *pneumoencephalitis* (Beach, 1942). It was shown to be due to a virus indistinguishable from NDV in serological tests (Beach, 1944). Since then, numerous NDV isolations of viruses that produce an extremely mild disease or no evidence of disease in chickens, have been made around the world and it is now accepted that pools of such viruses are perpetuated in waterfowl and other wild birds.

The pattern of outbreaks which are due to virulent NDV throughout the world suggest that several panzootics have occurred in poultry since 1926. The first appeared to have spread very slowly across the globe, apparently from the Far East. It probably took over 20 years to become a true panzootic and probably never reached poultry in the USA. The beginning of the second ND panzootic was first recognised at the end of the 1960s and within four years had reached all corners of the earth. (Hanson, 1972). The reasons for the different spreading rates of the two panzootics appear to be the development of the world poultry industry and the commercialisation of poultry food production both of which lead to greater contact between separate farms because food delivery vehicles move from one to another. Another factor is the revolution that has occurred in world transport. Air transportation especially has led to a huge and growing trade in captive caged birds. There is no doubt that imported caged birds were responsible for introducing the panzootic virus into poultry in California (Hanson, 1972; Francis, 1973) and Walker *et al.*, (1973) were able to link most of the outbreaks occurring in the USA during 1970-1972 to importations of exotic birds.

Antigenic and genetic evidence (Alexander *et al.*, 1997; Lomniczi *et al.*, 1998; Herczeg *et al.*, 2001) has indicated that there was probably a worldwide spread of a third virulent virus during the late 1970s,

the start and spread of which is unclear, presumably due to the masking of disease by the almost universal use of vaccines since the mid-1970s.

Another ND panzootic occurred in the 1980s, but in racing and show pigeons (*Columba livia*) rather than in poultry, although spread of the responsible virus did occur to poultry. The world population of racing or show pigeons is enormous and at the end of the 1970s these birds were still largely unvaccinated and fully susceptible to infection with NDV. Infections in pigeons with this variant NDV strain probably began in the Middle East in the late 1970s (Kaleta *et al.*, 1985), and by 1984-5 had become a true panzootic. In many countries where outbreaks occurred there was also spread to feral pigeons and doves. The way pigeons are kept and raced has meant that this panzootic has proven difficult to control and in several countries it probably remains endemic in racing and possibly also in feral pigeons.

The effect panzootics of ND have had on the poultry populations of different countries has not always been well recorded. Alexander (2001) documented the history of ND in Great Britain in detail and considered it a good example of the effect ND may have on the poultry industry in a developed Western country where eradication policies have been employed.

Aetiology

The three virus families *Rhabdoviridae*, *Filoviridae* and *Paramyxoviridae* form the order Mononegavirales; i.e. viruses with negative sense, single stranded and non-segmented RNA genomes. ND is caused by avian paramyxovirus serotype 1 [APMV-1] viruses, which, with viruses of the other eight APMV serotypes [APMV-2 to APMV-9], have been placed in the genus *Avulavirus*, sub-family *Paramyxovirinae*, family *Paramyxoviridae*, in the current taxonomy (Lamb *et al*., 2000; Mayo 2002).

Antigenic variation of ND viruses [APMV-1] detectable by conventional haemagglutination inhibition [HI] tests has been reported, although only rarely (Arias-Ibarrondo *et al*., 1978; Hannoun, 1977, Alexander *et al*., 1984). One of the most noted variations of this kind has been the virus responsible for the panzootic in racing pigeons. This ND virus, referred to as ‘pigeon APMV-1 [PPMV-1]’, was demonstrably different from standard strains in haemagglutination inhibition tests, but not sufficiently different antigenically that conventional ND vaccines were not protective (Alexander and Parsons, 1986). In recent years antigenic variations detected by monoclonal antibodies and genetic variations detected by nucleotide sequencing of the virus genome have proved invaluable in understanding the epidemiology of ND (Alexander *et al*., 1997; 1999; Herczeg *et al*., 1999; 2001).

CURRENT WORLD SITUATION

In many respects, it is extremely difficult to assess the prevalence of ND in the world at any given time. In some countries or areas disease is not reported at all or only if it occurs in commercial poultry, while its presence in village chickens or backyard flocks is ignored. Even in poultry reared commercially, estimations of the geographical distribution of NDV are confused by the use of live vaccines in all but a few countries throughout the world. In some countries the distribution is especially complicated by using, as live vaccines, viruses that are considered sufficiently virulent in other countries to warrant the current definition of ND.

When countries or areas are declared free of ND, further complications are caused by the definition of the type of ND virus described as harmless although this is being be addressed by the new definitions and codes to be adopted by the Office International des Epizooties. Even in countries that have long been recognised as free of ND, monitoring surveys often reveal symptomless infections with avirulent viruses which have presumably spread from waterfowl or other wild birds. However, there can be little doubt that the highly pathogenic form of ND is a serious problem, either as an enzootic disease or as a cause of regular, frequent epizootics throughout Africa, Asia, Central America and parts of South America (Copland, 1987; Spradbrow, 1988; Rweyemamu *et al.*, 1991; Alders & Spradbrow, 2001a). In other areas such as Europe, the situation appears to be one of sporadic epizootics occurring despite vaccination programmes (Kaleta & Heffels-Redmann, 1992).

In Western Europe there was a marked increase in reported outbreaks during the early 1990s, peaking with 239 outbreaks in European Union [EU] countries in 1994. The distribution overtime suggests a single epidemic from the early to mid-1990s, but, in fact, antigenic and phylogenetic evidence indicates that several strains of virus were responsible for these outbreaks. During 1991-1995 the majority of outbreaks in the EU occurred in the Benelux countries and Germany, predominantly in backyard poultry and most of the outbreaks since 1995 have been in these types of birds. One of the most extensive epidemics in Western Europe occurred in Italy in 2000 when 254 outbreaks of ND were confirmed, again mainly in backyard poultry.

One notable aspect of the outbreaks during the 1990s concerned those that occurred in countries that had been free of the disease for many years. Between 1995 and 1999, there were 18 outbreaks in Denmark, 2 in Finland and 27 in Northern Ireland. There was also 1 in Sweden, 1 in Norway and 1 in the Republic of Ireland.. These were all areas of Western Europe that had been declared free of ND and which were monitored regularly by serological testing and had no evidence of ND virus infections other than occasional incursions of avirulent viruses typical of spread from wild birds.

From the time of the 1932 outbreak (Albiston & Gorrie, 1942) to 1998, Australia had been free of virulent ND virus. Since 1966, however, it has been recognised that viruses similar to those placed in the "asymptomatic enteric" pathotype group (Westbury, 1981; Spradbrow, 1987) are present in wild birds in Australia and on occasions have spread to commercial poultry flocks. Two outbreaks of virulent ND occurred in Australia in 1998 and further outbreaks were reported in 1999 and 2000 (Kirkland, 2000; Westbury, 2001).

DISEASE AND PATHOGENICITY

Newcastle disease

The clinical signs seen in birds infected with NDV vary widely and are dependent on factors such as: the virus, the host species, age of host, infection with other organisms, environmental stress and immune status. In some circumstances infection with the extremely virulent viruses may result in sudden, high mortality with comparatively few clinical signs. Although none of the variable clinical signs can be regarded as pathognomonic, certain signs do appear to be associated with particular viruses. This has

resulted in the grouping of viruses into five "pathotypes" on the basis of the predominant signs in affected chickens (Beard and Hanson, 1984):

- **Viscerotropic velogenic**: viruses responsible for disease characterised by acute lethal infections, usually with haemorrhagic lesions in the intestines of dead birds.
- **Neurotropic velogenic**: viruses causing disease characterised by high mortality which follows respiratory and neurological disease, but where gut lesions are usually absent.
- **Mesogenic**: viruses causing clinical signs consisting of respiratory and neurological signs, with low mortality.
- **Lentogenic**: viruses causing mild infections of the respiratory tract.
- **Asymptomatic enteric**: viruses causing avirulent infections in which replication appears to be primarily in the gut.

These groupings are by no means clear-cut, and even in experimental infections of specific pathogen-free [SPF] chickens, considerable overlapping occurs (Alexander & Allan, 1974). In addition, in the field exacerbating factors may result in the clinical signs induced by the milder strains mimicking those of the more pathogenic viruses.

In general terms, ND may consist of signs of depression, diarrhoea, prostration, oedema of the head and wattles, nervous signs, such as paralysis and torticollis, and respiratory signs (McFerran & McCracken, 1988). Fall in egg production, perhaps leading to complete cessation of egg laying, may precede more overt signs of disease and deaths in egg-laying birds. Virulent ND strains may still replicate in vaccinated birds, but the clinical signs will be greatly diminished in relationship to the antibody level achieved (Allan *et al*., 1978).

As with clinical signs, no gross or microscopic lesions can be considered pathognomonic for any form of ND (McFerran & McCracken, 1988). Carcasses of birds dying as a result of virulent ND usually have a fevered, dehydrated appearance. Gross lesions vary with the infecting virus. Virulent panzootic ND viruses typically cause haemorrhagic lesions of the intestinal tract. These are most easily seen if the intestine is opened and may vary considerably in size. Some authors have reported lesions most typically in the proventriculus, while others consider them to be most prominent in the duodenum, jejunum and ileum. Even in birds showing neurological signs prior to death, there is usually little evidence of gross lesions in the central nervous system. Lesions are usually present in the respiratory tract when clinical signs indicate involvement. These generally appear as haemorrhagic lesions and congestion; airsacculitis may be evident. Egg peritonitis is often seen in laying hens infected with virulent NDV.

Microscopic lesions are not considered to have any diagnostic significance. In most tissues and organs where changes occur, they consist of hyperaemia, necrosis, cellular infiltration and oedema. Changes in the central nervous system are those of nonpurulent encephalomyelitis.

Molecular basis of pathogenicity of ND

During replication, NDV particles are produced with a precursor glycoprotein, F0, which has to be cleaved to F1 and F2 for the virus particles to be infectious (Rott and Klenk 1988). This post translation

cleavage is mediated by host cell proteases (Nagai *et al*. 1976a). Trypsin is capable of cleaving F0 for all NDV strains and *in vitro* treatment of noninfectious virus will induce infectivity (Nagai *et al*., 1976b).

The cleavability of the F0 molecule was shown to be related directly to the virulence of viruses *in vivo* (Rott, 1979; Rott, 1985). It would appear that the F0 molecules of viruses virulent for chickens can be cleaved by a host protease or proteases found in a wide range of cells and tissues. This allows these viruses to spread throughout the host, damaging vital organs. In contrast F0 molecules in viruses of low virulence appear to be restricted in their sensitivity to host proteases resulting in restriction of these viruses to growth only in certain host cell types.

Since the initial studies comparing the deduced amino acid sequences at the cleavage site of the F0 precursor of a number of virulent and avirulent ND strains (Collins *et al*, 1993), a large number of studies has confirmed the presence of multiple basic amino acids at that site in virulent viruses. Usually the sequence has been ^{113}RQK/RR ↓ F^{117} in virulent viruses and most have had a basic amino acid at position 112 as well. In contrast, viruses of low virulence usually have the sequence ^{113}K/RQG/ER ↓ L^{117}.

The major influence on the pathogenicity of NDV is therefore the amino acid motif at the F0 cleavage site, the presence of basic amino acids at positions 113, 115 and 116 and phenylalanine at 117 in virulent strains means that cleavage can be effected by protease or proteases present in a wide range of host tissues and organs. For viruses of low virulence, cleavage can occur only with proteases recognizing a single arginine, i.e. trypsin-like enzymes. Such viruses are therefore restricted in the range of sites where they are able to replicate to areas with trypsin-like enzymes, such as the respiratory and intestinal tracts, whereas virulent viruses can replicate in a range of tissues and organs resulting in a fatal systemic infection (Rott, 1979).

Definition of Newcastle Disease

Although it is likely that the vast majority of birds are susceptible to infection with ND viruses of both high and low virulence for chickens, the disease seen with any given virus may vary enormously from one species to another. Many other factors also affect the course of disease (see above). ND viruses show a considerable range of virulence for susceptible hosts such as chickens. Generally, variation consists of clusters around the two extremes in tests used to assess virulence, but, for a variety of reasons, some viruses may show intermediate virulence [mesogenic]. Equally, the very virulent viruses may infect and replicate in vaccinated birds without causing clinical disease (Parede & Young, 1990; Guittet *et al*., 1993; Capua *et al*., 1993). This enormous variation in virulence and clinical signs means that none can be regarded as pathognomonic and that it is necessary to define carefully what constitutes ND for the purposes of trade, control measures and policies.

The current OIE definition (OIE, 2000a) is:

Newcastle disease 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' refers 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 characterisation of the isolated virus by an ICPI test.*

In this definition, amino acid residues are numbered from the N-terminus of the amino acid sequence deduced from the nucleotide sequence of the F0 gene, 113–116 corresponds to residues –4 to –1 from the cleavage site."

Origins of Virulent ND Viruses

The emergence of ND as a highly pathogenic disease in poultry since 1926, (initially predominantly in South East Asia), suggests that some sudden major change has occurred either in the virus or in its hosts. Hanson (1972) considers that the various hypotheses which have been put forward can be grouped into three categories:

- The virulent virus has always existed in poultry in South East Asia, but it was not until the beginning of the commercialisation of the industry in that part of the world that the disease, with its enormous economic impact, was noticed as a significant problem.
- The virus is enzootic in different species, possibly inhabiting tropical rain forests, and spread to domestic poultry because of the incursion of man into that habitat.
- There is a major mutation of a precursor virus of low virulence.

The first explanation remains a possibility. Some consider it unlikely that the disease would have gone unreported if it was enzootic in village chickens, but even today village chickens throughout Africa, Asia and the Americas often show high levels of mortality, either regularly or as large die-offs every few years which go largely undiagnosed. Similarly, there have been occasional descriptions of disease outbreaks prior to 1926 that are very similar to ND.

The second explanation has, until recent years, been generally accepted as the most likely. The reason is mainly the discovery that during the 1970-73 panzootic, movement of captive caged birds, particularly psittacines which may be resistant excreters of NDV, was, to some extent, responsible for the introduction and spread in some countries, particularly California (Francis, 1973, Walker *et al*., 1973). However, as discussed above, viruses isolated from feral birds are usually of low virulence and it has been suggested that caged birds are most probably infected after they have been trapped. Maintenance of the virus in any feral bird species seems unlikely because of the effect that infection is likely to have on the bird's survival.

The third explanation has usually been dismissed out of hand as probably representing a mutation too big to be within the bounds of probability, especially without any apparent evolutionary advantage that would result from such selection. However, viruses isolated from ND outbreaks in Ireland and Australia during the 1990s have suggested that this may be how some virulent ND viruses emerge.

In 1990 in Ireland two outbreaks of ND occurred in egg laying birds. The viruses isolated were highly virulent and apparently identical (Alexander *et al*., 1992). They were very closely related antigenically and genetically (Collins *et al*., 1998) to viruses of low virulence normally isolated from feral waterfowl but known to have infected chickens in Ireland in 1987 (McNulty *et al*., 1988). The group formed by these viruses was both antigenically and genetically distant from all other ND viruses. Collins *et al* (1993) has shown that the virulent virus had four nucleotide differences at the site coding for the F0 cleavage site compared to the related viruses of low virulence (Table 1.1.), which would explain the higher virulence for chickens. However, the distinctiveness of this group of viruses from other ND viruses support the theory that the virulent viruses arose by mutation from those of low virulence.

Phylogenetic studies have shown all the virulent viruses responsible for the outbreaks in Australia from 1998 to 2000 are extremely closely related to each other and to the endemic virus of low virulence. This suggests their emergence by mutation which, in this instance, required only two point mutations (Table 1.2., Westbury, 2001).

If mutations to virulence do occur, it is not clear whether these take place in feral birds and are then passed to poultry or whether they occur once the virus has been introduced to poultry. The lack of virulent isolations from feral birds, however, suggests that the latter is the more likely.

If virulent ND strains can emerge from those of low virulence by mutation, this may have important repercussions on the current methods of control of ND – mainly because of the enormous amounts of live vaccines used throughout the world.

Table 1.1. Nucleotide/amino acid sequences at F0 cleavage site of virus of high virulence [34/90] isolated from poultry in Ireland compared to antigenically and genetically closely related virus of low virulence isolated from ducks

Virus	Virulence	Nucleotide/amino acid sequence at F0 cleavage site
MC110	low	GAA CGG CAG GAG CGT CTG ^{112}ERQER*L^{117}
34/90	high	**A**AA CGG CAG **A**AG CGT **T**T**T** ^{112}**K**RQ**K**R*L^{117}

Table 1.2. Nucleotide/amino acid sequence at F0 cleavage site of virus of high and low virulence isolated in Australia in 1998

Virus	Virulence	Nucleotide/amino acid sequence at F0 cleavage site
1154/98	low	GGA AGG AGA CAG GGG CGT CTT ^{111}GRRQGR*L^{117}
1238/98	high	GGA AGG AGA CAG **A**GG CGT **T**TT ^{111}GRRQ**R**R*L^{117}
1249/98	high	GGA AGG AGA CAG **A**GG CGT **T**TT ^{111}GRRQ**R**R*L^{117}

EPIDEMIOLOGY

Host Range

ND viruses have been reported to infect animals other than birds, ranging from reptiles to man (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. It seems probable that all birds are susceptible to infection but, as stressed by Kaleta and Baldauf, the disease seen with any given virus may vary enormously from one species to another.

Wild birds

NDV isolates have been obtained frequently from migratory feral waterfowl and other aquatic birds. Most of these isolates have been of low virulence for chickens and similar to viruses of the "asymptomatic enteric" pathotype. The most significant outbreaks of virulent NDV in feral birds have been those reported in double-crested cormorants (*Phalacrocorax auritus*) in North America during the 1990s. Earlier reports of ND in cormorants and related species had been in the late 1940s in Scotland (Blaxland, 1951) and in Quebec in 1975 (Cleary, 1977). Recent outbreaks in cormorants in North America were first seen in 1990 in Alberta, Saskatchewan and Manitoba in Canada (Wobeser *et al*., 1993). In 1992 the disease re-appeared in cormorants in western Canada, around the Great Lakes and North mid-west USA, in the latter case spreading to domestic turkeys (Mixson & Pearson, 1992; Heckert, 1993). Antigenic and genetic analyses of the viruses suggested that all the 1990 and 1992 viruses were very closely related despite the geographical separation of the hosts. Disease in double-crested cormorants was observed again in Canada in 1995 and in California in 1997 and in both instances NDV was isolated from dead birds; as before, these viruses appear to be closely related (Kuiken, 1998).

Thirty-eight outbreaks of ND in commercial poultry were confirmed in 1997 in the United Kingdom (Alexander *et al*., 1998). There were also outbreaks caused by genetically similar viruses in Scandinavian countries in 1996 (Alexander *et al*., 1999). These, linked to the unusual patterns of movement of migratory birds at the end of 1996 and the beginning of 1997, suggest that migratory birds may have been responsible for the primary introduction of the causative virus into Great Britain (Alexander *et al*., 1998).

Caged "pet birds"

Virulent NDV isolates have often been obtained from captive caged birds (Senne *et al.*, 1983). Kaleta and Baldauf (1988) thought it unlikely that infections of recently imported caged birds resulted from enzootic infections in feral birds in the countries of origin. They considered that the infections probably originated at holding stations before export, either as a result of enzootic NDV at those stations or of spread from nearby poultry such as backyard chicken flocks. Panigrahy *et al.*, (1993) described outbreaks of severe ND in pet birds in six states in USA in 1991. Illegal importations were assumed to be responsible for the introductions of the virus.

Domestic poultry

Virulent NDV strains have been isolated from all types of commercially reared poultry, ranging from pigeons to ostriches.

Racing and show pigeons

In the late 1970s, an NDV strain, PPMV-1, showing some antigenic differences from classical strains, appeared in pigeons. It, , probably arose in the Middle East and subsequently produced a true panzootic, spreading in racing and show pigeons to all parts of the world (Alexander, 1997).

Introduction and spread

Transmission between birds

Apart from predatory birds or the practice of feeding poultry with untreated swill containing poultry meat, spread from bird to bird appears to occur as the result of either inhalation of excreted droplet particles or the ingestion of infective material such as faeces. Although it is clear from the administration of live vaccines by aerosol that infection may be established via the respiratory route, there is remarkably little experimental evidence that infected birds pass on the virus to susceptible birds in this way, even over short distances. The success of this route of transmission depends on many environmental factors, such as temperature, humidity, and stocking density. In contrast, it is easily demonstrated that virus infection can be passed from one bird to another via contaminated faeces. It seems most likely that the pigeon variant virus, the "asymptomatic enteric" viruses, and other viruses which fail to induce significant respiratory signs in infected birds, are transmitted primarily in this way (Alexander *et al*., 1984).

Several reviews have dealt with the way in which the ND virus may be introduced into a country or area and then subsequently spread from flock to flock (Lancaster, 1966; Lancaster and Alexander, 1975; Alexander 1988b, 1995). In summary, the main methods by which virus can be spread are:

Movement of live birds

Migratory feral birds may be responsible for the primary introduction of infection, but nearly all NDV isolates obtained from feral birds have been of low virulence. A more significant role of such birds may be the spread within an area once NDV infections have already occurred in poultry. Exceptions to the presence of the virus of low virulence in migratory birds have been discussed in the Host Range section above.

World trade in captive caged birds is enormous and in many countries virulent NDV has been isolated frequently from such birds held in quarantine. For example, 147 virulent NDV isolations were made from 2 274 lots of captive birds held in quarantine in the USA during 1974-1981 (Senne *et al*,. 1983). Some infected psittacines have been shown to excrete virulent virus intermittently for extremely long periods, in some cases for more than one year (Erickson *et al*., 1977). This further emphasises the potential role these birds may have in the introduction of NDV to a country or area.

There is also considerable international trade in game birds, which are often imported for immediate release.

The potential for racing pigeons to carry and introduce ND into a country or area has been highlighted by the panzootic in such birds over the last ten years.

Trade in backyard flocks and other birds kept for recreational purposes (hobby birds) has been implicated in the introduction and spread of ND in the outbreaks in European Union countries during 1991-1994.

Modern methods of slaughter of commercial poultry, marketing of poultry meat and veterinary inspection, have reduced the movement of live commercial poultry (with the exception of day-old chicks) in many European and other developed countries. However, in many countries, the normal method of trade is by live poultry markets. Such markets, where birds of many different species may be placed in close contact with each other, represent ideal opportunities for viruses to be disseminated. The movement of village chickens from one village to another, whether directly or through live bird markets, is the main method of spread of ND [see below].

Movement of people and equipment

Secondary spread during most epizootics of ND in recent times has been the result of the movements of personnel or equipment. Human beings may be infected with NDVs, but their most likely role is the transfer of infective poultry faeces from one site to another via clothing, footwear, crates, feed sacks, egg trays or vehicles.

Movement of poultry products

In the past, poultry meat has been seen as the main vehicle for the introduction and spread of NDV. Modern methods of poultry carcass preparation as well as legislation on the feeding of untreated swill to poultry have greatly diminished the risk from poultry products, but the possibility of spread in this way still remains.

Contaminated poultry food or water

In the British Isles, outbreaks of ND in commercial poultry have been associated with food contaminated with infective faeces from feral pigeons infected with the ND virus (Alexander *et al.*, 1985; O'Reilly *et al*., 1994). Similarly, water contaminated with infective faeces may introduce NDV to a flock.

Airborne spread

In recent years, the significance of airborne transfer of viruses has been the subject of some debate. During the 1960s and 1970s, this was considered a major method of spread and Smith (1964) considered it the most logical explanation of spread in outbreaks occurring in 1960 and 1962 in Great Britain. In the same country, Dawson (1973) considered windborne spread to be of major significance during the 1970-1972 outbreaks that were noted for the severe respiratory signs and unusual patterns of spread. But in the 1971-1973 epizootic in California, with ostensibly the same virus, respiratory signs were not especially prominent and Utterback and Schwartz (1973) considered airborne spread to be of little significance.

There have been few attempts to assess the survival of airborne virus, but Hugh-Jones *et al*., (1973) were able to detect virus 64 metres but not 165 metres downwind of infected premises. These authors stressed the importance of environmental conditions, particularly relative humidity, with regard to the likelihood of airborne spread.

It is possible that when climatic conditions have been right and poultry farms sufficiently concentrated, as in Northern Ireland in 1973 (McFerran, 1989), airborne spread may have played a significant role in epidemics of ND. But in recent years, airborne spread has not been an issue in reported outbreaks and there has nearly always been an alternative and more likely cause, particularly the movement of poultry and humans.

VACCINES

Good manufacturing practices should ensure that vaccines are highly unlikely to be carriers of virulent ND virus. However, in the past, birds have become infected by vaccines for other diseases being contaminated with ND and also as a result of failure to properly inactivate vaccines prepared from virulent ND virus. In 1996-1997, a series of ND isolates of low virulence were obtained from poultry flocks in Denmark, a country which pursues a non-vaccinating policy for ND. It was demonstrated that these viruses were the result of contamination of avian virus vaccines with vaccinal ND viruses (Jorgensen *et al*., 2000). This episode further emphasises the potential of spread of ND in this way.

Non-avian hosts

This is likely to be by mechanical transfer of infective faeces, for example, by insects, rodents or scavenging animals. In hot countries, reptiles may enter poultry houses and should not be ignored as potential spreaders of NDV, as their susceptibility to infection has been reported.

BIOSECURITY AND HYGIENE IN THE CONTROL OF ND

In countries or areas that are free of virulent NDV, the primary aim should be to prevent the introduction of the virus. Because migratory and other feral birds frequently carry NDV strains of low virulence, which spread from time to time to domestic poultry, it is usual to exclude such viruses from control policies. Vaccinal viruses are somewhat different, as some are sufficiently virulent to cause disease in fully susceptible birds.

In some countries there is legislation designed to reduce the likelihood of outbreaks from specific sources. For example, on the island of Ireland there has been a legal requirement to heat treat poultry feed to reduce the possibility of introduction of NDV by this route.

On commercial farms, control measures should attempt to prevent viruses from infecting the flock. It is of paramount importance that good hygiene and biosecurity measures aimed at preventing the introduction of viruses by the routes described above are practised at all times on poultry farms.

Biosecurity aimed at preventing disease should begin at the planning stage of commercial poultry farms. Farms and flocks should be well separated, hatcheries should be isolated from poultry farms, different species should be reared on different sites, and there should be an adequate fresh water supply,

preferably one that does not draw on surface water. Often in developed countries such practices are difficult to impose as the poultry industry may already be established in areas with high concentrations of poultry flocks and with little opportunity to change due to limitations of available land. But in countries where the commercialisation of poultry farming is at a developmental stage, these points should be adopted.

On the farms the following points should be observed:

- Houses, food stores and water tanks should be bird-proofed.
- Movements on and off the farm should be kept to a minimum.
- All equipment, especially vehicles, should be disinfected before access to the site is permitted.
- Movements between different farms for egg collection, carcass collection, food delivery and the like should be confined to a specified collection and delivery point away from the poultry flocks. Unfortunately, as poultry farming has become industrialised and its profits marginalised, the move has been away from such precautions which are often considered an expensive luxury.

Visits by personnel such as bleeding or vaccination teams, inseminators and veterinarians are the most likely method of introduction of ND and if such visits are unavoidable, regimens of clothing change, equipment disinfection and other basic hygiene controls must be enforced.

Possibly the greatest aid to implementing biosecurity and hygiene measures that will assist in the prevention or control of ND is the education of farmers and those working with poultry with respect to the spread of virus and measures to avoid it. The best tool for the control of ND at any level, international, national or local farm, may well be an efficient, well-manned poultry extension service.

CHAPTER 2
DIAGNOSIS OF NEWCASTLE DISEASE

The methods used in the diagnosis of ND are detailed in the OIE Manual of Standards (OIE 2000a) and prescribed for European Union countries in Directive 92/66/EEC (CEC, 1992). Detailed descriptions of the tests which are outlined below can be obtained from these publications.

CLINICAL SIGNS AND LESIONS

For a definitive diagnosis of ND, both virus isolation and laboratory characterisation are necessary. Nevertheless, if the disease is known to be present in a given area, signs and lesions may be considered highly suggestive, especially for village chickens. Typical clinical signs are: a state of prostration and depression in the birds, with ruffled feathers; greenish white diarrhoea; and, in survivors, the head turned to one side, a condition known as torticollis is very often seen, as are paralysis of the legs, wings or other neurological signs. Other typical characteristics of the disease include: rapid spread; death within 2-3 days; a mortality rate of over 50 percent in naïve populations; and an incubation period of 3-6 days or, on rare occasions, 2-15 days (Beard and Hanson, 1984).

On necropsy, typical lesions are mucus in the trachea, and usually haemorrhages in the intestine, particularly in the proventriculus. It should be borne in mind that all the preceding signs and lesions can be caused by other diseases.

SEROLOGICAL DIAGNOSIS

In the absence of vaccination, the presence of specific antibodies against the ND virus indicates that the bird has been infected by the virus at some time, but not necessarily that it was suffering from the disease at the time of sampling. In practice, a high antibody titre is indicative of a recent infection. Two methods are used to measure antibody titres: the haemagglutination inhibition (HI) test, and the enzyme-linked immunosorbent assay (ELISA). For both, it is necessary to collect blood samples from the chickens. Catching village chickens for this purpose can present a problem. There are two approaches: where overnight housing is used, they can be retained in the morning; or children can be persuaded to catch them. Blood samples are taken from the wing veins – a detailed description of the method is given in Alders and Spradbrow (2001a). Cocks are usually harder to sample than hens. Blood can be drawn directly into a syringe, or collected into a tube after piercing the vein with a needle. In both cases, the sample is subsequently placed nearly horizontally to allow clotting and to permit separation of the serum sample, which should be straw coloured. The serum sample should be kept cool until it can be frozen in the laboratory.

The haemagglutination inhibition test

The HI test is based on the principle that the haemagglutinin on the viral envelope can bring about the agglutination of chicken red blood cells and that this can be inhibited by specific antibodies. V-bottomed microtitration plates are used. The serum samples are diluted in serial twofold dilutions in phosphate

buffered saline and then a fixed quantity of viral antigen is added to each well. Usually 4 Haemagglutination Units are used, according to the method of Allan and Gough (1974). Following incubation, a suspension of red blood cells is added to each well and the plate is incubated again. In the absence of any antibody against the virus, haemagglutination occurs, appearing as a diffuse red colour at the bottom of the well. In the wells where the antibody against the virus is of a sufficient level, haemagglutination is inhibited and the red blood cells sediment and appear as a small pellet at the bottom of the well. The presence or absence of agglutination is accurately assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (containing RBCs and PBS only) should be considered to show inhibition. The HI titre is the reciprocal of the highest dilution of serum which completely inhibits haemagglutination and is usually and most conveniently expressed as the logarithm to the base 2. Although the test is difficult to standardise between laboratories, the HI titre gives an indication of the immune status of the bird. A titre of $\log_2 3$ is indicative of protection and a titre of $\log_2 6$ or more suggests a recent infection by the virus. If no vaccination has taken place, diagnosis of the infection can be made on this basis, although it cannot be determined exactly when it took place. Sequential samples taken at different times can indicate whether the titre is rising – indicative of a recent infection – or declining.

When the HI titre is used as a measure of immunity (for example, when testing flock immunity following vaccination), it is recommended that an avirulent strain such as V4 or Ulster 2C be used as the viral antigen. The La Sota antigen has been found to be unsuitable for this purpose when vaccination is done by the same strain as it results in an overestimation of protective serum antibody titres (Maas, *et al*. 1998).

The ELISA works on the principle of recognition of anti-NDV antibodies, attached to a plate coated with viral antigen, by antibodies produced in another species against chicken antibodies. This anti-chicken antibody is conjugated to an enzyme that catalyses a reaction, causing a change of colour which can then be read quantitatively on a photo spectrometer designed to read microtitration plates. The Animal Production & Health Section of the Joint FAO/IAEA Division has produced an ELISA kit for antibodies against NDV which is designed to be easily transportable and give uniform results under widely varying ambient temperatures. HI and ELISA titres show a good degree of correlation and ELISA titres can be interpreted in a similar way to HI titres (Bell, *et al*., 1991).

VIRUS ISOLATION

The definitive diagnosis of ND is done through isolation and identification of the virus (Alexander, 1998). Tracheal and cloacal swabs are good sources of virus for isolation from living birds without having to kill them. A cotton-covered stick is inserted into the trachea or cloaca, and then put into a vial containing phosphate buffered saline plus penicillin and streptomycin. It is important to ensure that cloacal swabs are coated with faeces. These samples must be kept cool during transport to the laboratory where they should be stored at 4^{o}C if they are to be processed within 48 hours or frozen at least at -20°C until the isolation attempt. Although cloacal swabs or faeces should always be sampled, virus can also be isolated from homogenised organs from dead birds, chosen to reflect the clinical signs. Nine-day-old embryonated

fowls' eggs are injected with 0.1mL of the suspension into the allantoic cavity and returned to incubation. The eggs are candled twice daily. As dead eggs occur, they are chilled, together with d all eggs after 5-7 days incubation, are chilled at 4°C at which point the allantoic fluid is then harvested and tested for its ability to haemagglutinate chicken red blood cells. Diagnosis is based on the inhibition of haemagglutination by specific anti-NDV serum. This proves infection of the bird by the virus, but does not indicate whether the virus is a pathogenic or avirulent strain.

PATHOGENICITY TESTS

The full diagnosis of ND requires an assessment of the virulence of the virus. As indicated above (Section 1.4), the current definition allows either molecular characterisation by nucleotide sequencing and deduction of the F0 cleavage site amino acid sequence or an *in vivo* estimation of virulence.

The recommended *in vivo* test is the intracerebral pathogenicity index (ICPI) test in day-old chicks (OIE 2000a). This involves the inoculation of virus derived from fresh infective allantoic fluid into the brain of ten day-old chicks from specific pathogen-free parents. Each bird is examined at 24-hour intervals for eight days and graded zero if normal, one if sick and two if dead. The index is the mean score per bird per observation over the 8-day period. The most virulent viruses give ICPI values approaching the maximum score of 2.0, while lentogenic viruses give values of, or close to, 0.0.

Where day-old chicks are not available, the mean death time (MDT) in eggs (i.e. the mean time in hours for the minimum lethal dose to kill all the inoculated embryos), can be used as a guide to virulence. The MDT has been used to classify ND virus strains into velogenic (taking under 60 hours to kill); mesogenic (taking 60 to 90 hours to kill); and lentogenic (taking more than 90 hours to kill).

CHAPTER 3
VACCINATION

INTRODUCTION

Vaccination, initially with inactivated virus, was considered a possibility for the control of ND at the time of the apparent emergence of the virus. However, after the 1933 outbreak in England, an attenuated live vaccine was produced which was called strain H. Later, the naturally occurring USA isolates of low virulence, Hitchner B1 (HB1) and La Sota, became the most used veterinary vaccines throughout the world. Fifty years or more have passed since vaccine was first used to protect village poultry against ND. (Placcidi and Sentucci, 1952). During this time, a wide variety of types of vaccine have been developed. Many, but not all, have been tested on village poultry. It is the purpose of this chapter to present an overview of the different kinds of vaccine available. It is not the intention to recommend a particular vaccine, but rather to try and outline the relative advantages and limitations of each, with particular reference to its use in the village situation and giving examples of how the different vaccines are employed.

The principle of vaccination against a viral disease is well-known: to elicit an immunological response against the virus in a way that does not cause the disease. The simplest way to do this is to take the virus, kill it, and then inject it into the bird. This is an inactivated vaccine. Another approach is to select a naturally occurring virus that is not virulent enough to cause serious disease, and infect the birds with this virus. This is a live vaccine. This latter approach can be taken further by taking a non virulent natural virus and selecting a clone from the virus population with desirable properties, such as lack of vaccinal reactions, or heat tolerance. This is a cloned live vaccine. Finally, it is possible to genetically engineer a vaccine by, for example, taking part of the genetic material of the virus that codes for a surface antigen, and inserting this into another, different, virus to produce a recombinant vaccine.

These different approaches to vaccination have been applied to ND. There are three types of vaccines 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 HB1, La Sota 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.

INACTIVATED VACCINES

Inactivated vaccines are produced by growing a ND virus in eggs, and then treating the infective allantoic fluid with an inactivating agent, such as formalin or betapropiolactone. An adjuvant, such as mineral oil, is usually then added to make the inactivated virus more immunogenic. Since the vaccine is no longer capable of replication or spread, it has to be injected individually into every bird needing vaccination. It is normally injected into the back of the thigh muscle (sometimes the breast muscle is used), using 0.3 or 0.5 ml per bird. This requires some training, and cannot be done by every keeper of chickens without prior

demonstration. Inactivated vaccines produce very high levels of antibodies against NDV, and provide good protection against the virulent virus.

In intensive poultry production, inactivated vaccines are usually applied after an initial priming vaccination with a live vaccine. In village poultry, however, good results in the absence of an initial vaccination with live vaccine have been reported (Bell *et al*., 1990). The reason for this is probably, as serological surveys have shown where they have been carried out (Bell and Mouloudi, 1988), that antibodies to the virus are already present in the village poultry as a result of previous infection by the wild virus.

Inactivated vaccines have been used extensively in village poultry, for example, in a successful project in Burkino Faso (Verger, 1986). Although inactivated vaccine gives good protection, it is relatively expensive to produce. It also carries a slight risk to the user of accidental self-injection. While inactivated vaccines are, to some extent, heat sensitive, they are much less so than conventional live vaccines which makes transporting them to villages more feasible.

LIVE VACCINES

Live vaccines differ from inactivated vaccines in that they can replicate in the host. This is both an advantage and a disadvantage. It is an advantage in that it is not necessary to vaccinate every bird individually; the vaccinal virus can spread on its own from one bird to another. It is, however, a disadvantage in that, since an infection with a live virus is involved, this may result in clinical signs because of the innate virulence of the vaccine virus or by exacerbating other organisms that may be present, especially in the respiratory tract. The severity of this reaction depends therefore on the particular vaccinal strain used (Westbury *et al*., 1984) and the presence or otherwise of concurrent infection with other pathogens.

Another advantage of live vaccines compared to inactivated vaccines, is their ease of application as they can be applied to the drinking water or with an eye-dropper.

Although NDV has essentially only one serotype, there is a wide difference in the pathogenicity of different strains, ranging from those that cause virtually no signs to those that kill within a few days. These have been classified, in order of increasing pathogenicity, into asymptomatic enteric, lentogenic, mesogenic and velogenic strains. The majority of live vaccines are derived from asymptomatic enteric or lentogenic strains, although some vaccines derived from mesogenic strains are still in use.

Conventional lentogenic vaccines

The level of vaccine reaction is an important consideration for intensive commercial poultry and because HB1 has very mild vaccinal reactions, it has been widely used for initial vaccination of intensive poultry. In a controlled trial in village poultry, HB1 provided effective protection against ND (Bell *et al*., 1990). La Sota produces moderate vaccinal reactions, especially in immunologically naive birds and is not usually recommended for primary vaccination. In theory, La Sota would also be unsuitable for vaccinating a multi-age population, including young chicks which is inevitably seen in the village situation. This is because the virus spreads and it is not practical to isolate the adults from the chicks. In

practice, the degree of reaction from La Sota as a primary vaccine depends on the residual level of antibodies, which could protect the birds from vaccinal reactions, and on the extent of other concurrent infections, such as *Mycoplasma* spp, pathogenic *E. coli*, or infectious bursal disease virus and other respiratory viruses. In intensive systems, vaccination using spray delivery systems which produce small particle sizes, may also exacerbate the vaccine reaction.

Some lentogenic vaccines have been cloned by taking a single infectious virus and growing a homogenous population from it, with the aim of selecting a virus which gives less vaccinal reactions than a La Sota-like virus, while retaining its superior immunogenicity compared to a HB1-like virus. An example of this kind of vaccine is “clone 30”.

All conventional live vaccines have the disadvantage of needing to be kept at low temperatures to maintain their efficacy. This is not a problem for intensive poultry production in an industrial setting, but the maintenance of the “cold chain” during distribution can be very difficult in village settings, particularly where there is high ambient temperature.

Another problem that is often encountered when using commercial vaccines in the village situation is that they are sold in vials containing 1 000 or 500 doses, many more than the average village farmer needs. In fact, the packaging is a major component of the cost of manufacturing them, because a vial containing a smaller number of doses would not necessarily reduce the cost proportionally.

Oil adjuvant, normally used with inactivated vaccines to improve immunogencicity, has also been tested with live vaccines and found to improve immunogenicity (Peleg *et al*., 1993), but this combination has not been tested with village chickens.

Heat tolerant vaccines

Some asymptomatic enteric viruses have been noted for their greater heat resistance than more conventional lentogenic viruses. This property has been enhanced by selection and cloning in the laboratory to produce heat tolerant vaccines. These have a distinct advantage in the village situation because it is possible to transport the vaccine without a cold chain. The most extensively used vaccine has been the NDV4-HR vaccine, which was pioneered in Malaysia, where a significant proportion of the village poultry was eventually covered by this vaccine (Ibrahim *et al*., 1992). The application was in feed, which, because of its thermostability, it was possible to pre-coat with the vaccine. Theadvantage of this method is that it is not necessary to catch the chickens before vaccinating them. The same vaccine has also been tried in other countries in South East Asia, but not always with the same success as in Malaysia. Tests of its application on a variety of foodstuffs have produced variable results (Spradbrow, 1992). The vaccine was also tested in some African countries, but applied by eye-drop and gave good protection against the virulent virus (Saglid and Spalatin, 1982; Bell *et al*., 1995). Given the difference between African and Asian feeds, the variety of feeds within Africa, and the variable results with some feedstuffs in Asia, it seems that application of this type of vaccine is best done by eye-drop. It can also be argued that the additional security provided by the vaccine is an incentive to invest in some form of housing, in which case catching the chickens is no longer a problem.

More recently, a similar vaccine to NDV4-HR, called I-2 (Bensink and Spradbrow 1999), has been made available for local production in non-industrialised countries, which has the significant advantage of low cost. In trials in Ghana, Mozambique, Tanzania and Vietnam village chickens, vaccinated with strain I-2, were protected against artificial and field challenge with virulent virus (Amakye-Anim *et al*., 2000; Dias *et al*. 2001; Tu *et al*., 1998; Wambura *et al*., 2000).

Mesogenic vaccines

Mesogenic strains have long been used for vaccination in the village situation. These produce severe vaccinal reactions in an immunologically naïve population, and the use of this kind of vaccine is not advisable in situations where chickens are without any immune protection against the virus. Normally mesogenic vaccines, such as Komarov (Saifuddin *et al.*, 1990) and Mukteswar (Alexander, 1997) are used as secondary vaccines after a primary vaccination with a lentogenic vaccine.

RECOMBINANT VACCINES

NDV has two surface glycoproteins, fusion [F] and haemagglutinin/neuraminidase [HN]. The genes coding for either of these can be inserted into a different kind of virus to make a recombinant vaccine. For example, the fusion gene inserted in herpes virus of turkeys produced a vaccine which gave good protection against virulent NDV (Morgan *et al*., 1993). One advantage of this technique is that the host virus may have better stability than NDV. Another advantage is that antigens for multiple different pathogens can be inserted into the same host virus to produce a single vaccine against several different diseases. Perhaps the most significant advantage for field use is that it is possible to monitor the response to the vaccine independently of the wild virus but in its presence, and conversely, it is possible to detect antibodies against the wild virus in the presence of vaccination. This is done by using an enzyme-linked immunoabsorbent assay (ELISA) that uses a purified antigen, and comparing the results with those of an ELISA using a whole virus antigen. For example, Makkay *et al.* (1999) prepared an ELISA using only nucleocapsid protein of NDV as antigen. This detected antibodies against wild virus, but not antibodies against a recombinant fowl pox virus expressing HN glycoprotein. A parallel ELISA using whole virus as antigen detected antibodies against the vaccine.

A disadvantage of recombinant vaccines is that where they have been developed commercially the cost is high.

VACCINE APPLICATION

Mass administration methods

In intensively developed commercial poultry industries, an important cost of vaccination is the administration. For this reason, mass application methods have been developed, primarily for live vaccines. Various forms of equipment are manufactured to generate coarse sprays, which allow mass application with minimum adverse reaction, although in some circumstances, mass application by fine sprays and aerosols are employed (Kouwenhoven, 1993).

Application of live vaccines via drinking water is still employed in some areas, although in the commercial sectors, this gives some problems in preparing and cleaning the drinking water system, with a tendency for a less than uniform uptake (Kouwenhoven, 1993).

Administration to village chickens

Vaccine administration to village chickens is not comparable to intensively reared commercial chickens as the birds are rarely housed and seldom in large numbers. However, labour is usually available at little or no cost which means that individual methods of vaccine administration are feasible.

Eye-drop administration

Application of the vaccine by eye-drop methods is probably the most effective for live lentogenic vaccines (Fig. 1). It ensures that the vaccine reaches the individual bird and, as a consequence, titres obtained are usually uniform throughout the flock.

Correct dilution of the vaccine is critical. If eye-droppers are being used, they should be calibrated beforehand (see Alders and Spradbrow 2001a). In the absence of suitable eye-droppers, it is also possible to use the tip of a feather or a syringe (preferably a 1 mL syringe) to administer the drop. However, these two options should be seen as last resorts as they are inaccurate and cause considerable wastage of vaccine. Most live ND vaccines require re-vaccination at 3-4 monthly intervals.

Eye-drop administration provides good protection because the vaccine passes to the Harderian gland just behind the eye, which in chickens is a key organ in the development of the immune response.

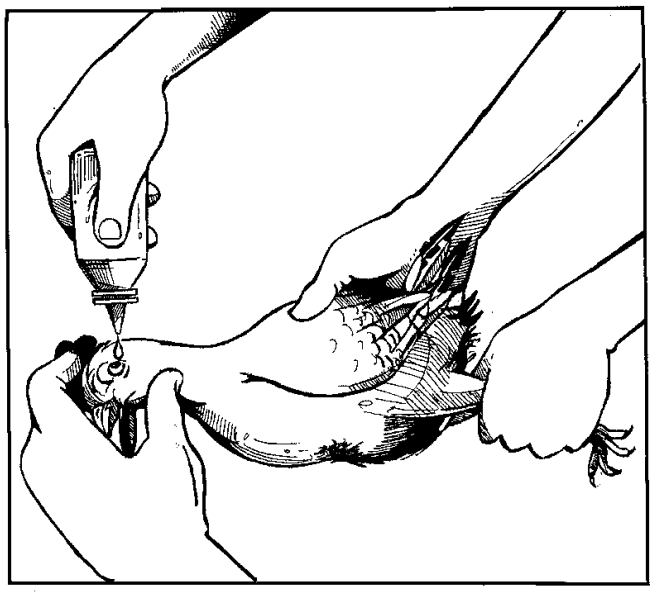

Figure 1 Eye-drop administration. When using an eye-dropper, hold it in a vertical position. Eye-droppers are calibrated according to the size of the drop that forms when the dropper is held in a vertical position (Alders and Spradbrow 2001a).

Administration of the vaccine via drinking water

Vaccination by placing the live vaccine virus in the drinking water is easier than application to individual birds, but it provokes a lower level of immunity than eye-drop administration, has less uniform uptake and requires more frequent application. The vaccine should be given twice, initially 2-3 weeks apart, with re-vaccination at least every three months.

It is important to:

- remove drinking water from the chickens for 1-2 hours before the administration of the vaccine.
- mix the vaccine with a volume of water that the chickens will be able to drink during one hour (usually 5-7 mL of water per bird).
- always use fresh and clean water.

It is important **not** to:

- use metal water receptacles.
- use disinfectants to clean water receptacles as they will inactivate the vaccine virus.
- use treated tap water. If there is only access to treated tap water, it is advisable to let the treated tap water stand overnight to allow the chlorine to dissipate, or add one teaspoon of powdered milk per 10 litres of water to neutralise the effects of the chlorine.
- place water receptacles containing vaccine directly in sunlight or in hot areas.
- allow other animals access to the vaccine. It should be restricted to chickens.

In rural areas, it is best to give the drinking water in the morning just as the chickens are released from the chicken house. In areas with abundant surface water, chickens find their own source of drinking water and vaccination via water is not appropriate.

Administration via feed

Oral vaccination of chickens with thermostable vaccines (i.e. NDV4-HR and I-2) has been successful in some developing countries. Good veterinary services, local availability of suitable grains and recovery of virus from the grain are important considerations for successful oral vaccination. One problem with food-based ND vaccination is the low recovery of virus from some grains (especially maize), a consequence of either binding or inactivation. This method should be thoroughly tested before being used widely in the field. The vaccine must be given more often when administered via feed, making it more expensive, and survival rates in the face of an outbreak are lower than those achieved by eye-drop administration. Food used in any vaccination campaign should therefore be recommended by the Veterinary Authority. 7-10 grams of food per bird should be well mixed with the corresponding number of doses of appropriately diluted vaccine. With most grains, 1 ml. of fluid will efficiently moisten 10 grams of grain. The treated food is best given in the morning as the birds are leaving the roost. The vaccine should be given twice, initially 2-3 weeks apart, with re-vaccination at least every 2-3 months.

Administration via injection

Inactivated ND vaccines are administered by intramuscular or subcutaneous injection only (in the breast or the leg). Inactivated vaccines should be allowed to reach ambient temperature (approximately 28°C) and the contents should be well shaken prior to use. If stored in a cool, dark location, an inactivated vaccine may retain its activity for 1-2 weeks outside a refrigerator.

Inactivated vaccines are more effective in chickens which have previously received a living vaccine. Re-vaccination is usually done every 6 months.

Accidental injection into the vaccinator of inactivated vaccines based on emulsions formed with mineral oil can cause a serious localised reaction. These usually require incision and washing. Expert medical advice should be sought at once, and the doctor must be informed that the vaccine was a mineral oil emulsion.

In many parts of Asia, mesogenic strains (for example, Mukteswar) of the ND virus are used and can be administered by injection only. This vaccine should be used in birds over eight weeks of age and following a primary vaccination with a lentogenic strain such as F strain.

DISCUSSION

The inactivated and recombinant vaccines have the advantage of not inducing vaccinal reactions. The heat tolerant clones produce almost no vaccinal reactions whereas the other live vaccines produce slight to moderate reactions, depending on the vaccine strain and the immune status of the population vaccinated.

Inactivated vaccines are, however, the most difficult to apply and whether using needle and syringe or automatic equipment, training is necessary before any injection technique is mastered.

For transportability, heat tolerant vaccines appear to be best and can be transported to even remote villages under high ambient temperatures without a cold chain. The inactivated vaccine is second best, having a better heat tolerance than the conventional live vaccines.

In choosing a vaccine for use in the village situation (Bell, 2001), one factor to take into consideration is previous experience with that type of vaccine. There has been extensive village experience in the use of both heat tolerant and inactivated vaccines. Live mesogenic vaccines have also been used in villages, particularly in Asia. The other live vaccines, with the possible exception of some clones, have, at least, been formally tested in villages.

Finally, cost is an important factor. All the live vaccines are relatively cheap, and can be even cheaper if they are produced locally. Inactivated vaccines are more expensive and recombinant vaccines are likely to be very expensive when produced commercially.

The choice of vaccine and how to administer it depends not only on the preceding factors, but also on the conditions in each region, such as the structure of veterinary services, previous experience, the population distribution, the communication infrastructure and the climate.