Newcastle disease and other avian paramyxoviruses

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Summary
Newcastle disease (ND), caused by avian paramyxovirus serotype 1 (APMV-1) viruses, is included in List A of the Office International des Epizootics. Historically, ND has been a devastating disease of poultry, and in many countries the disease remains one of the major problems affecting existing or developing poultry industries. Even in countries where ND may be considered to be controlled, an economic burden is still associated with vaccination and/or maintaining strict biosecurity measures. The variable nature of Newcastle disease virus strains in terms of virulence for poultry and the different susceptibilities of the different species of birds mean that for control and trade purposes, ND requires careful definition. Confirmatory diagnosis of ND requires the isolation and characterisation of the virus involved. Assessments of virulence conventionally require in vivo testing. However, in vitro genetic characterisation of viruses is being used increasingly now that the molecular basis of pathogenicity is more fully understood. Control of ND is by prevention of introduction and spread, good biosecurity practices and/or vaccination. Newcastle disease viruses may infect humans, usually causing transient conjunctivitis, but human-to-human spread has never been reported.

Eight other serotypes of avian paramyxoviruses are recognised, 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. Both types of viruses cause respiratory disease and egg production losses which may be severe when exacerbated by other infections or environmental stresses. No reports exist of natural infections of chickens with APMV-3 viruses.

Keywords
Aetiology - Avian diseases - Avian paramyxoviruses - Control - Diagnosis - Epidemiology - Newcastle disease - Public health.

Introduction
Two poultry diseases are considered to be sufficiently serious to be included in List A of the Office International des Epizootics (OIE), namely: highly pathogenic avian influenza (HPAI) and Newcastle disease (ND) (85). While HPAI occurs relatively rarely, ND is enzootic in some areas of the world and a constant threat to most birds reared domestically. Very few commercial poultry flocks are not influenced in some way by measures aimed at controlling ND and spread of the virus (4). A large majority of the countries rearing poultry commercially rely on vaccination to control ND, but ND nevertheless represents a major limiting factor for increasing poultry production in many countries.

The greatest impact of ND may be on village or backyard chicken production. In developing countries throughout Asia, Africa, Central America and some parts of South America, the village chicken is an extremely important asset representing a significant source of protein in the form of eggs and meat. However, ND is frequently responsible for devastating losses in village poultry. For example, Spradbrow estimated that 90% of the village chickens in Nepal die each year as a result of ND (107). Social and financial restraints mean that the
control of ND in village chickens in developing countries is extremely difficult, if not impossible, and this situation impinges on the further development of commercial poultry production and the establishment of trade links.

Aetiology

Viruses

The three virus families Rhabdoviridae, Filoviridae and Paramyxoviridae form the order Mononegavirales, i.e. viruses with negative sense, single-stranded, non-segmented, ribonucleic acid genomes. Newcastle disease is caused by avian paramyxovirus serotype 1 (APMV-1) viruses, which together with viruses of the other eight APMV serotypes (APMV-2 to APMV-9), have been placed in the genus Rubulavirus, sub-family Paramyxovirinae, family Paramyxoviridae, in the current taxonomy (95). Recent studies involving the sequencing of the whole ND virus (NDV) genome have suggested that avian paramyxoviruses are sufficiently different from other rubulaviruses to warrant the creation of a separate genus (45).

The prototype strains of each of the nine serotypes of avian paramyxoviruses are shown in Table I.

Strains of NDV have been distinguished on the basis of the clinical signs produced in infected chickens. Beard and Hanson (29) defined the following five groups or pathotypes:

- 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 in which 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 occur primarily in the gut.

Although these categories are useful for descriptive purposes, some overlapping does occur and some viruses are difficult to place.

Antigenic relationships

Cross relationships in haemagglutination inhibition (HI) and other tests have been detected between some of the APMV serotypes (7, 8). However, use of a variety of serological and non-serological tests has tended to confirm the distinctiveness of the APMV serotypes. Antigenic relationships between APMV-1 and other serotypes are important as these may affect diagnosis of ND. Generally, such cross-reactions in serological tests are low, but APMV-3 viruses may show sufficiently high levels of cross reactivity with conventional APMV-1 antisera to cause problems. Apparent antibodies to APMV-3 virus detected in chickens have been attributed to high antibody levels to NDV as a result of vaccination (33).

Antigenic variation between viruses within APMV serotypes has been reported for most of the serotypes where more than a few isolates have been obtained. For NDV (APMV-1), differences detectable by conventional HI tests have been reported, although only rarely (12, 26, 54). One of the most noted variations of this kind has been the virus responsible for the panzootics in racing pigeons that occurred during the 1980s. The NDV responsible, referred to as pigeon APMV-1 (PPMV-1), was demonstrably different from standard strains in HI tests, although not sufficiently different that

Table I
Serotypes of avian paramyxovirus

<table>
<thead>
<tr>
<th>Prototype virus strain</th>
<th>Usual natural hosts</th>
<th>Other hosts</th>
<th>Disease produced in poultry</th>
</tr>
</thead>
<tbody>
<tr>
<td>APMV-1 (Newcastle disease virus)</td>
<td>Numerous</td>
<td>Chickens, psittacines, rails</td>
<td>Varies from extremely pathogenic to inapparent, depending on strain and host infected</td>
</tr>
<tr>
<td>APMV-2/chicken/California/Yucaipa/56</td>
<td>Turkeys, passerines</td>
<td>None</td>
<td>Mild respiratory disease or egg production problems; severe if exacerbation occurs</td>
</tr>
<tr>
<td>APMV-3/turkey/Wisconsin/68</td>
<td>Turkeys</td>
<td>None</td>
<td>Mild respiratory disease but severe egg production problems worsened by exacerbating organisms or environment</td>
</tr>
<tr>
<td>APMV-3/parakeet/Netherlands/449/75</td>
<td>Psittacines, passerines</td>
<td>Geese, rails</td>
<td>No infections of poultry reported</td>
</tr>
<tr>
<td>APMV-4/duck/Hong Kong/03/75</td>
<td>Ducks</td>
<td>Geese, rails, turkeys</td>
<td>No infections of poultry reported</td>
</tr>
<tr>
<td>APMV-5/budgerigar/Japan/Kunitachi/74</td>
<td>Budgerigars</td>
<td>None</td>
<td>Mild respiratory disease in turkeys</td>
</tr>
<tr>
<td>APMV-6/duck/Hong Kong/199/77</td>
<td>Ducks</td>
<td>Geese, rails, turkeys</td>
<td>No infections of poultry reported</td>
</tr>
<tr>
<td>APMV-7/dove/Tennessee/4/75</td>
<td>Pigeons, doves</td>
<td>Turkeys, ostriches</td>
<td>No infections of poultry reported</td>
</tr>
<tr>
<td>APMV-8/goose/Delaware/1035/76</td>
<td>Ducks, goose</td>
<td>None</td>
<td>None known</td>
</tr>
<tr>
<td>APMV-9/domestic duck/New York/22/78</td>
<td>Ducks</td>
<td>None</td>
<td>None known</td>
</tr>
</tbody>
</table>

* Serological tests may distinguish between turkey and psittacine isolates

APMV: avian paramyxovirus
conventional ND vaccines were not protective (16). Monoclonal antibodies (mAbs) raised against various strains of NDV have been used to establish the uniqueness of the variant NDV responsible for the panzootic of ND in pigeons and have proven particularly useful in identifying the spread of this virus around the world (14, 17, 91). Monoclonal antibodies to NDV have also been used to distinguish between specific viruses. For example, two groups have described mAbs that distinguish between the common vaccine strains, Hitchner B1 and La Sota (47, 77), while other mAbs can separate vaccine viruses from epizootic virus in a given area (108).

Antigenic variation detected by mAb typing has also been used in epidemiological studies. In a large study of over 1,500 viruses, Alexander et al. (21) used the ability of viruses to react with panels of mAbs, consisting initially of nine mAbs and later extended to twenty-six or twenty-eight mAbs, to place strains and isolates of NDV into groups on the basis of ability to react with the different mAbs. Viruses in the same mAb group shared biological and epizootiological properties. Use of such panels, particularly the extended panel, has indicated that viruses tend to remain fairly well conserved during outbreaks or epizootics and this often allows valuable assumptions to be made concerning the source and the spread of ND.

Variations within other serotypes have been reviewed by Alexander (7). Marked variations have been recorded between viruses placed in APMV-2, APMV-3 and APMV-7 serotypes. The variations recorded often showed two viruses with little relationship to each other, but each showing strong similarity with a third virus. Ozdemir et al. produced mAbs against APMV-2 virus and were able to class isolates from a wide range of hosts and geographical locations into four groups (88). The antigenic variations in the APMV-3 group seem to have greater significance, as these appear to separate turkey isolates from pittacinque isolates. Anderson et al. produced mAbs to APMV-3 virus and the use of these suggested that antigenic variation within the APMV-3 serogroup may be more complicated than suggested using polyclonal antisera (25).

Phylogenetic studies

Development of improved techniques for nucleotide sequencing, the availability of sequence data of NDV placed in computer databases and the demonstration that even relatively short sequence lengths could give meaningful results in phylogenetic analyses has led to a considerable increase in such studies in recent years. Considerable genetic diversity has been detected, but viruses sharing temporal, geographical, antigenic or epidemiological parameters tend to fall into specific lineages or clades and this has proven valuable in assessing both the global epidemiology and local spread of ND (23, 40, 57, 67, 68, 73, 102, 109).

Disease

Newcastle disease

The clinical signs seen in birds infected with NDV vary widely and are dependent on factors such as the virus, 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 the clinical signs are variable and influenced by other factors, so that none 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 (see above). These groupings are by no means clear-cut, and even in experimental infections of specific-pathogen-free (SPF) chickens considerable overlapping occurs (9). 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 (75). Decline 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 replicate in vaccinated birds, but the clinical signs will be greatly diminished in relationship to the antibody level achieved (24).

As with clinical signs, no gross or microscopic lesions can be considered pathognomonic for any form of ND (75). Carcasses of birds dying as a result of virulent ND usually have a fevered, dehydrated appearance. Gross lesions vary according to the infecting virus. Virulent panzootic NDVs 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 lesions to be most prominent in the duodenum, jejunum and ileum. Even in birds showing neurological signs prior to death, little evidence is found 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, changes consist of hyperaemia, necrosis, cellular infiltration and oedema. Changes in the central nervous system are those of nonpurulent encephalomyelitis.
Other avian paramyxoviruses

As summarised in Table I, of the other APMVs, only APMV-2 and APMV-3 viruses have been consistently shown to infect and cause disease in poultry, although APMV-6 and APMV-7 viruses have been associated with clinical disease in turkeys.

Uncomplicated infections of chickens or turkeys with APMV-2 viruses probably result in only mild respiratory disease. However, much more serious disease may ensue due to exacerbation by other organisms. Avian paramyxovirus serotype 2 viruses have been associated with respiratory and egg production problems in chickens and turkeys ranging from mild to severe with elevated mortality. Associated disease has usually been more severe in turkeys than chickens (7). Virus isolation and/or the presence of antibodies have also confirmed APMV-2 virus infections in chicken and turkey flocks that have experienced no clinical disease (34). The clinical outcome of infections of domestic poultry with APMV-2 viruses is probably dependent on exacerbation by other organisms or environmental conditions. Experimental infections of laying turkeys showed that egg production and hatchability were adversely affected although fertility was not (28).

Avian paramyxovirus serotype 3 viruses have been associated with respiratory disease and egg production problems in turkeys (27). To date, no natural infections have been recorded in chickens, although experimental infections have demonstrated that these birds are susceptible to the serotype. In uncomplicated infections of turkeys, the first sign is often a decline in egg production. However, early mild respiratory signs have been reported, which suggest the respiratory tract may be the initial site of infection (10). The decline in egg production varies considerably according to the age of the birds and the presence of secondary infections. In uncomplicated infections of birds a few weeks after beginning to produce eggs, the effective loss may be around 1-2 eggs per bird per week for five or six weeks, after which the production returns to expected levels (32). Production problems are associated with a high level of white-shelled eggs, and the hatchability and fertility of eggs are also reduced. Infection, or just before, the point of lay may result in more serious losses, with the flock failing to reach target production throughout the laying period. Far more serious respiratory disease and egg production problems have been recorded when dual infection with NDV (including live vaccines), influenza viruses, chlamydiae, mycoplasmas or other bacteria has occurred. No studies have reported on the lesions associated with APMV-3 infections of turkeys.

Avian paramyxovirus serotype 6 has been isolated on one occasion from turkeys with egg production and mild respiratory problems.

Saif et al. reported APMV-7 virus as the primary pathogen in natural outbreaks of respiratory disease with elevated mortality in turkeys. Furthermore, mild respiratory disease was demonstrated in turkeys infected experimentally (100).

Molecular basis of pathogenicity of Newcastle disease virus

During replication, NDV particles are produced with a precursor fusion glycoprotein, F0, which has to be cleaved to F1 and F2 for the virus particles to be infectious (98). This post translation cleavage is mediated by host cell proteases (79). Trypsin is capable of cleaving F0 for all NDV strains and in vitro treatment of non-infectious virus will induce infectivity (80).

The importance of F0 cleavage can be demonstrated by the ability of viruses normally unable to replicate or produce plaques in cell culture systems to do both if trypsin is added to the agar overlay or culture fluid. The cleavability of the F0 molecule was shown to be related directly to the virulence of viruses in vivo. Viruses pathogenic for chickens could replicate in a wide range of cell types in vitro with or without added trypsin, whereas strains of low virulence could replicate only when trypsin was added (96, 97). The F0 molecules of viruses virulent for chickens can apparently 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 have restricted sensitivity to host proteases, resulting in restriction of these viruses to growth only in certain host cell types.

Collins et al. compared the deduced amino acid sequences at the cleavage site of the F0 precursor of twenty-six APMV-1 isolates (38). All fourteen viruses that were virulent for chickens had the sequence 112K/R-Q-R-K/R-R116 at the C-terminus of the F2 protein and F (phenylalanine) at residue 117, the N-terminus of the F1 protein. The eleven viruses of low virulence had sequences in the same region of 112G/E-K/R-Q-G/E-R116 and L (leucine) at residue 117. Thus, a double pair of basic amino acids was apparently required at residues 112 and 113, and 115 and 116, plus a phenylalanine at residue 117, if the virus was to show virulence for chickens. The exception was the single pigeon variant virus examined (PPMV-1) which had the sequence 112G-R-Q-K-R-F117; in tests in vivo, this virus fell within the definition of viruses virulent for chickens (see below). Jestin and Cherbonnel demonstrated a similar motif at the F2/F1 cleavage site for two viruses isolated from chickens (61), which they grouped antigenically with PPMV-1 viruses (60). Further studies have indicated that this variation is usual for PPMV-1 viruses, but has no significance in the variability of pathogenicity for chickens recorded with these viruses (39).

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 in the presence of proteases recognising a single arginine, i.e. trypsin-like enzymes. The replication of such viruses is therefore restricted to areas where trypsin-like enzymes are present, 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 (96).

**Epidemiology**

**Host range**
The natural and occasional hosts of the different APMV serotypes are summarised in Table I.

**Host range of Newcastle disease**
Newcastle disease viruses have been reported to infect animals other than birds, ranging from reptiles to humans (71). Kaleta and Baldauf concluded that NDV infections have been established in at least 241 species of birds representing 27 of the 50 orders of the class (64). All birds are probably susceptible to infection, but, as stressed by Kaleta and Baldauf, the disease observed with any given virus may vary enormously from one species to another.

**Wild birds**
Newcastle disease virus 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 potential of such viruses to become virulent and the role of feral birds in the spread of ND are described below. The most significant outbreaks of ND in feral birds were those reported in double-crested cormorants (Phalacrocorax auritus) in North America during the 1990s. Newcastle disease in cormorants and related species had been reported earlier, in the late 1940s in Scotland (31) and in Quebec in 1975 (37). Recent outbreaks in cormorants in North America were first reported in 1990 in Alberta, Saskatchewan and Manitoba in Canada (114). In 1992, the disease re-appeared in cormorants in western Canada, around the Great Lakes, and in the northern Midwest of the United States of America (USA), in the latter case spreading to domestic turkeys (56, 78). 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. Since these outbreaks affected birds that would follow different migratory routes, the initial infection is thought to have occurred at a mutual wintering area in Central America. Disease in double-crested cormorants was observed again in Canada, in 1995 and in California in 1997. In both instances, NDV was isolated from dead birds, and as previously, the viruses appeared to be closely related (70).

**Caged 'pet birds'**
Virulent NDV isolates have often been obtained from captive caged birds (103). Kaleta and Baldauf suggested that infections of recently imported caged birds were unlikely to have resulted from enzootic infections in feral birds in the countries of origin (64). The infections were considered likely to have 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. Panigraphy et al. described outbreaks of severe ND in pet birds in six States of the USA in 1991 (89). 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 named PPMV-1, showing some antigenic differences from classical strains, appeared in pigeons, probably arising in the Middle East. In Europe, the strain was first reported in racing pigeons in Italy in 1981 (30) and subsequently produced a true panzootic, spreading in racing and show pigeons to all parts of the world (8).

**Host range of other avian paramyxoviruses**
The known host range of each of the other serotypes of APMV is more restricted than that of NDV Table I. Viruses of serotypes APMV-4, APMV-8 and APMV-9 appear to be restricted to infections of ducks and geese. Similarly, APMV-6 viruses have been isolated frequently from feral ducks and geese, in which viruses of this serotype appear to be enzootic. Avian paramyxovirus serotype 6 viruses have also been isolated from rails that presumably may have had contact with ducks and geese, spread to turkeys has also been recorded.

Avian paramyxovirus serotype 5 viruses were restricted to isolations made during a single epizootic in budgerigars in Japan between 1974 and 1976 (81), but more recently, similar viruses have been isolated from budgerigars in Great Britain (52). Species of psittacines closely related to budgerigars may also be infected.

The natural hosts for APMV-7 viruses appear to be pigeons and doves (species of the order Columbiformes). However, infections of turkeys (100) and ostriches (115) have recently been reported. Presumably, these infections resulted from contact with pigeons or doves.

Avian paramyxovirus serotype 2 viruses have been isolated from both chickens and turkeys in association with respiratory disease. However, the primary natural host appears to be small perching birds of the order Passeriformes. Other species have been reported to be infected, including captive caged psittacines that have usually been in contact with caged passerines.
Avian paramyxovirus serotype 3 viruses can be divided into two antigenically distinguishable groups. The first has only been reported to infect turkeys. In experiments, chickens have been demonstrated to be susceptible to infection, but no natural infections have been reported. Viruses of the second group have been isolated frequently from captive caged psittacines and passerines. The evidence suggests that these APMV-3 viruses primarily infect psittacine species but will spread to passerines placed in contact. No isolations of APMV-3 viruses have been reported from feral birds.

**Geographical distribution**

**Newcastle disease virus**

In many respects, estimations of the geographical distribution of NDV in domestic poultry are confused by the use of live vaccines in all but a few countries throughout the world. In addition, in many countries, a distinction is made between the disease situation in large scale commercial poultry and in village chickens. When countries or areas are declared free of ND, further complications are caused by the definition of the type of ND viruses described within the claim of 'freedom'.

Even in Australia, which was free of the virulent virus between the 1932 outbreak (2) and the 1998 outbreaks, commercial poultry flocks were frequently found to be infected with viruses similar to those placed in the 'asymptomatic enteric' pathotype group (105, 113). Similarly, on the island of Ireland, which had long been recognised as free of ND, monitoring surveys often reveal symptomless infections, presumably due to spread from waterfowl, and occasional epizootics of virulent infections occur (87).

The severe form of ND is undoubtedly 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 (43, 99, 106).

In other areas such as Europe, the situation appears to be one of sporadic epizootics occurring despite vaccination programmes (65).

**Other avian paramyxoviruses**

The countries in which each of the APMV serotypes has been isolated from the various types of avian hosts have been listed by Alexander (7). Viruses of the serotypes isolated regularly from migratory ducks and geese (i.e. APMV-4 and APMV-6) appear to be ubiquitous and distributed world-wide. Fewer isolations of APMV-8 viruses have been recorded, but the presence of this serotype in migratory waterfowl in the USA and Japan is an indication that these too may have a world-wide distribution. Isolation of APMV-7 viruses from feral and/or captive pigeons and doves in Japan, the USA and England (18) again suggests that infections of viruses of this serotype may occur throughout the world.

Avian paramyxovirus serotype 2 and APMV-3 viruses both appear to have a world-wide distribution in varying hosts. Viruses of both serotypes have been isolated from captive caged birds in all countries that monitor such birds. Avian paramyxovirus serotype 2 viruses have been isolated in surveys of passerine birds in countries of Eastern and Western Europe, the Middle East, South-East Asia, Africa and Central America, essentially wherever surveillance of passerine species has been undertaken. In poultry, APMV-2 viruses have been isolated from chickens and/or turkeys in countries of Europe (Eastern and Western), Asia, the Middle East, North and Central America. The APMV-3 subserotype infecting turkeys has been isolated only in countries of Western Europe and North America, but these countries represent the primary producers of turkeys.

**Current distribution**

**Newcastle disease**

Assessment of the prevalence of ND in the world at any given time is extremely difficult. In some countries or areas, disease is not reported at all or only reported if outbreaks occur in commercial poultry, while the presence of disease in village chickens or backyard flocks is ignored. Even in commercially reared poultry, 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 confused by the use as live vaccines of viruses that would be considered sufficiently virulent in other countries to be defined as ND when infecting poultry.

In Western Europe, reported outbreaks increased markedly during the early 1990s, peaking at 239 outbreaks in countries of the European Union (EU) in 1994. The distribution with time suggested a single epidemic during the early to mid-1990s, but in fact, antigenic and phylogenetic evidence indicates that several strains of virus were responsible for these outbreaks.

Between 1991 and 1995, the majority of outbreaks in the EU occurred in the Benelux countries and Germany, predominantly in backyard poultry. Most of the outbreaks since 1995 have also been in backyard poultry.

One notable aspect of the outbreaks in Western Europe during the 1990s was the occurrence of outbreaks in countries which had been free of the disease for many years. Since 1995, eighteen outbreaks have been reported in Denmark, one in Sweden, two in Finland, one in Norway, one in the Republic of Ireland and twenty-six in Northern Ireland, all areas of Western Europe that had been declared free of ND and which were monitored regularly by serological testing with no evidence of ND virus infections.

Although voluntary vaccination was permitted, Great Britain was also essentially free of ND, and the outbreak confirmed in pheasants in 1996 was the first in this country since 1984 (20). That outbreak and those in Great Britain in 1984 (15) were shown to be caused by the variant NDV responsible for the panzootic in racing pigeons (PPMV-1) that had reached
Great Britain in 1983 (13). This virus is still detected occasionally in racing and feral pigeons. However, in 1997, eleven outbreaks of ND were confirmed in Great Britain in commercial poultry, four in broiler chickens and seven in turkeys (22). Although the viruses isolated were highly virulent for chickens infected in the laboratory, the clinical disease signs seen in field infections were variable and not always associated with high mortality, especially in turkeys. Epidemiological investigations indicated that the majority of the outbreaks occurred as a result of secondary spread by human agency from two or more primary infected flocks. Outbreaks were caused by similar viruses in countries of Scandinavia in 1996 (23). These outbreaks, linked to the unusual patterns of movement of migratory birds at the end of Scandinavia in 1996 (23), suggested that migratory birds may have been responsible for the primary introduction of the causative virus into Great Britain (22).

Until 1998, Australia had been free of virulent NDV since the 1932 outbreak (2). Since 1966, viruses similar to those placed in the 'asymptomatic enteric' pathotype group (105, 113) had been recognised in wild birds in Australia, and on occasions had spread to commercial poultry flocks. Two outbreaks of virulent ND occurred in Australia in 1998 and further outbreaks were reported in 1999.

Other avian paramyxoviruses

As discussed above, the most recent developments concerning other APMVs were the isolation of APMV-7 viruses from ostriches (115) and turkeys (100). Until these reports, natural infections with APMV-7 viruses had been detected only in pigeons and doves. Recent reports of other APMVs are scarce, although investigations of poultry and other birds for other reasons suggest that the distribution and host range of the other APMVs remains constant.

Origins of virulent Newcastle disease viruses

The emergence of ND as a highly pathogenic disease of poultry in 1926, initially predominantly in South-East Asia, suggested that a sudden major change had occurred either in the virus or hosts, although earlier outbreaks may have been confused with HPAI. Hanson (55) considered that the various hypotheses proposed could be grouped in three categories, as follows:

a) the virulent virus has always existed in poultry in South-East Asia, but the disease with its enormous economic impact was only recognised as a significant problem when the commercialisation of the poultry industry commenced in that part of the world

b) the virus was enzootic in different species, possibly inhabiting tropical rain forests, and spread to domestic poultry due to the incursion of man into that habitat

c) a major mutation occurred in a precursor virus of low virulence.

The first explanation remains a possibility. Some consider that the disease is unlikely to have gone unreported if it was enzootic in village chickens, but even at present, 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 largely go undiagnosed. Similarly, occasional descriptions of disease outbreaks prior to 1926 are very similar to ND.

Until recent years, the second explanation has been the one generally accepted to be most likely. This was led by the finding, during the panzootic of 1970-1973, that movement of captive caged birds, particularly psittacines, which may be resistant excreters of NDV, was to some extent responsible for the introduction and spread of NDV in some countries, and most noticeably in California (50, 112). However, as discussed above, viruses isolated from feral birds are usually of low virulence and caged birds are most probably infected after trapping. Maintenance of virus in any species of feral bird that is at all affected by the virus seems unlikely due to the likely effect of infection on the survival of the bird.

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

In Ireland in 1990, two outbreaks of ND occurred in egg laying birds. The viruses isolated were highly virulent and apparently identical (19). Interestingly, these viruses were very closely related antigenically and genetically (40) to viruses of low virulence normally isolated from feral waterfowl, but known to have infected chickens on the island of Ireland in 1987 (76). The group formed by these viruses was both antigenically and genetically distant from all other ND viruses. Collins et al. had shown that the virulent 34/90 virus had four nucleotide differences at the site coding for the F0 cleavage site compared to the related viruses of low virulence (Table II), which would explain the higher virulence for chickens (38). However, while the distinctiveness of this group of viruses from other NDVs supports the theory that the virulent viruses originally arose by mutation from those of low virulence, the mechanism by which this may have occurred is not at all clear.

Phylogenetic studies have shown all the virulent viruses responsible for the outbreaks in Australia in 1998 and 1999 to be extremely closely related to each other and to the endemic virus of low virulence, suggesting emergence by mutation, which in this instance required only two point mutations (Table III).
If mutations to virulence do occur, it is not clear whether these take place in feral birds and then pass to poultry, or occur once the virus has been introduced in poultry. However, the lack of virulent isolations from feral birds suggests that the latter is more likely.

If virulent ND strains can emerge from those of low virulence by mutation, this may have important repercussions on the current philosophy on control of ND, not least in view of the enormous quantities of live vaccines used throughout the world. However, no evidence exists to suggest that infected birds will pass on the virus to susceptible birds in this way, even over short distances. The success of this route of transmission will depend on many environmental factors, such as temperature, humidity and stocking density. In contrast, transmission of virus infection from one bird to another via contaminated faeces can be easily demonstrated. The pigeon variant virus, the ‘asymptomatic enteric’ viruses, and other viruses which fail to induce significant respiratory signs in infected birds, are likely to be transmitted primarily in this way (11, 13).

**Primary and secondary introduction**

Several reviews have dealt with the way in which NDV may be introduced into a country or area and then subsequently spread from flock to flock (3, 8, 71, 72). The main methods by which virus can be spread are summarised below.

**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 are of low virulence. A more significant role of such birds may be the transmission of virus within an area following NDV infection of poultry. Exceptions to the presence of virus of low virulence in migratory birds have been discussed in the section entitled ‘Host range’.

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 from 1974 to 1981 (103). Some infected psittacines have been shown to excrete virulent virus intermittently for extremely long periods, in some cases for more than one year (48), which further emphasises the potential role these birds may have in the introduction of NDV to a country or area.

Game birds may also be implicated in the introduction of NDV to a country, since considerable international trade occurs in these birds, which are often imported for immediate release.

The potential for racing pigeons to carry and introduce NDV to a country or area has been highlighted by the panzootic in such birds since the early 1980s.

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 countries of the EU from 1991 to 1994.

Modern methods of slaughtering commercial poultry, marketing of poultry meat and veterinary inspection, have reduced the movement of live commercial poultry (excluding day-old chicks) in many developed countries. However, in many countries, the normal method of trade is by live poultry markets. Such markets, where birds of many different species

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### Table II

Nucleotide/amine acid sequences at F0 cleavage site of a virus of high virulence isolated from poultry in Ireland compared to an antigenically and genetically closely related virus of low virulence isolated from ducks

<table>
<thead>
<tr>
<th>Virus</th>
<th>Virulence</th>
<th>Nucleotide/amino acid sequence at F0 cleavage site</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC110</td>
<td>Low</td>
<td>GAA GGC CAG GAG CGT CTG 117 PERGER*F 117</td>
</tr>
<tr>
<td>34/90</td>
<td>High</td>
<td>AAA CGG CAG AAG CGT TTT 118 KNOKR*F 117</td>
</tr>
</tbody>
</table>

### Table III

Nucleotide/amine acid sequence at F0 cleavage site of viruses of high and low virulence isolated in Australia in 1998

<table>
<thead>
<tr>
<th>Virus</th>
<th>Virulence</th>
<th>Nucleotide/amino acid sequence at F0 cleavage site</th>
</tr>
</thead>
<tbody>
<tr>
<td>1154/98</td>
<td>Low</td>
<td>GGA AGG AGA CAG GGC GGT CTG 117 GRRGR*F 117</td>
</tr>
<tr>
<td>1238/98</td>
<td>High</td>
<td>GGA AGG AGA CAG GGC GGT TTT 117 GRRGR*F 117</td>
</tr>
<tr>
<td>1249/98</td>
<td>High</td>
<td>GGA AGG AGA CAG AGG CTG TTT 117 GRRGR*F 117</td>
</tr>
</tbody>
</table>

---

**Introduction and spread**

Investigation into the epidemiology of avian paramyxoviruses other than APMV-1 that infect poultry has been limited. The similarities to APMV-1 in infection and replication suggest that the same mechanisms and risks will apply.

**Transmission between birds**

Excluding predatory birds or the feeding of untreated swill containing poultry meat to poultry, 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 the administration of live vaccines by aerosol demonstrates clearly that infection may be established via the respiratory route, remarkably little experimental evidence exists to suggest that infected birds will pass on the virus to other birds.
may be placed in close contact, represent ideal reservoirs of virus from which disease may be disseminated.

Movement of people and equipment
Secondary spread during most epizootics of ND in recent years has been the result of the movements of personnel or equipment. Humans may be infected with NDV, but the most likely role of personnel is in the transfer of infective faeces from one site to another on hair, clothing, footwear, crates, feed sacks, egg trays or vehicles.

Movement of poultry products
In the past, poultry meat has been incriminated as the main vehicle for the introduction and spread of NDV. For example, in 1947, one-third of the first 542 outbreaks in England and Wales were considered to be directly attributable to feeding poultry waste to chickens (51). Sampling of batches of frozen poultry imported into Great Britain in the same year produced an isolation rate of up to 66% (94). Modern methods of poultry carcass preparation and 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 nevertheless remains.

Contaminated poultry food or water
In countries of the British Isles, outbreaks of ND in commercial poultry have been associated with food contaminated with infective faeces from feral pigeons infected with NDV (15, 87). Similarly, water contaminated with infective faeces may introduce NDV to a flock.

Airborne spread
In recent years, the significance of airborne transfer of virus has been the subject of some debate. During the 1960s and 1970s, this was considered to be a major method of spread and Smith considered it the most logical explanation of spread in outbreaks occurring in 1960 and 1962 in Great Britain (104). In the same country, Dawson considered windborne spread to be of major significance during the outbreaks of 1970-1972 that were noted for severe respiratory signs and unusual patterns of spread (44). However, in the epizootic of 1971-1973 in California, with ostensibly the same virus, respiratory signs were not especially prominent and Utterback and Schwartz considered airborne spread to be of low significance (110).

Few studies have attempted to assess the survival of airborne virus, but Hugh-Jones et al. were able to detect virus 64 m but not 165 m downwind of an infected premises (58). These authors stressed the importance of environmental conditions, particularly relative humidity, on the likelihood of airborne spread.

When climatic conditions are favourable and poultry farms sufficiently concentrated, as in Northern Ireland in 1973, it is difficult not to conclude that airborne spread may play a significant role in epidemics of ND (74). However, in the majority of outbreaks there has been no evidence that airborne spread has played a major role and in recent years airborne spread has not been an issue in reported outbreaks and an alternative, more likely, cause has nearly always existed, particularly the movement of poultry and the agency of humans.

Vaccines
Good manufacturing practices should ensure that vaccines are highly unlikely to be carriers of virulent NDV. However, in the past, birds have become infected when vaccines against other diseases have been contaminated with NDV, or as a result of failure to inactivate killed vaccines prepared from virulent NDV. In 1996 and 1997, a series of NDV isolates of low virulence were obtained from poultry flocks in Denmark, a country which pursues a non-vaccinating policy for ND. These viruses were demonstrated to be the result of contamination of avian virus vaccines with vaccinal NDVs (63). This episode further emphasises the potential for spread of ND in this way.

Non-avian hosts
Non-avian species are likely to introduce NDV by mechanical transfer of infective faeces, e.g. by insects, rodents or scavenging animals. In hot countries, reptiles which may enter poultry houses should not be ignored as potential transmitters of NDV, as susceptibility to infection has been reported in reptiles.

Diagnosis
The diagnostic methods described in this section are primarily those recommended by the OIE for ND. Diagnosis of other APMVs is essentially similar. Avian paramyxovirus infections have usually been diagnosed by serology or virus isolation. In common with ND, antibodies to APMVs may be detected by HI tests using the relevant antigens and controls. Avian paramyxoviruses can be isolated from tracheal or faecal swabs or tissue samples from infected birds by inoculation of eight- to ten-day-old embryonating chicken eggs via the allantoic cavity. Confirmation of the virus as belonging to the APMV serotype can be performed by HI tests with specific antiserum.

Definition of Newcastle disease
Although the vast majority of birds are likely to be susceptible to infection with NDVs of both high and low virulence for chickens, the disease seen with any given virus may vary enormously from one species to another.

The clinical signs seen in birds infected with NDV vary widely and are dependent on factors such as the virus, 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. Thus, the clinical signs are variable and influenced by other factors so that none can be regarded as pathognomonic. As discussed
above, virulent viruses have been divided into those in which
the predominant signs produced in infected chickens are
intestinal lesions (termed viscerotropic) and those that induce
above, virulent viruses have been divided into those in which
mainly neurological signs (neurotropic). However, in practice
these distinctions are not clear cut and may be affected by
other factors. Some viruses produce only mild respiratory
disease (lentogenic), and others replicate in the intestine with
little or no clinical signs (asymptomatic enteric). Viruses in
these last two categories are widely used as live vaccines
against ND.

Newcastle disease viruses show a considerable range of
virulence for susceptible hosts such as chickens. In tests used
to assess virulence, variation generally consists of clusters
around the two extremes, 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 (35, 53, 90).

This enormous variation in virulence and clinical signs means
that careful definition of what constitutes ND is necessary for
the purposes of trade, control measures and policies.

At the 67th General Session of the OIE held in Paris in May
1999, Resolution No. XIII was to amend the definition of ND
in the Manual of Standards for Diagnostic Tests and Vaccines,
Chapter 2.1.15. on Newcastle disease, as follows:

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' (86).

Diagnostic tests
Identification of the agent
Samples from dead birds should consist of oro-nasal swabs, as
well as samples collected from lung, air sac, intestine
(including contents), spleen, brain, liver and heart tissues.
These may be collected separately or as a pool.

Samples from live birds should include both tracheal and
cloacal swabs, the latter should be visibly coated with faecal
material. Swabbing may harm small delicate birds, but the
collection of fresh faeces may serve as an adequate alternative.

Where opportunities of obtaining samples are limited, it is
important that cloacal swabs (or faeces) and tracheal swabs
(or tracheal tissue) are examined in addition to organs or
tissues that are grossly affected or associated with clinical
disease.

The samples should be placed in phosphate buffered isotonic
saline (PBS) at pH 7.0-7.4 containing antibiotics. The
antibiotics can be varied according to local conditions but
could contain penicillin (2,000 units/ml), streptomycin
(2 mg/ml), gentamycin (0.05 mg/ml) and mycostatin
(1,000 units/ml) for tissues and tracheal swabs, but at
concentrations 5-fold higher for faeces and cloacal swabs.
The pH of the solution must be adjusted to pH 7.0-7.4 following
the addition of the antibiotics. Faeces and finely minced
tissues should be prepared as 10%-20% (w/v) suspensions in
the antibiotic solution. Suspensions should be processed as
soon as possible after 1 h-2 h at room temperature. Where this
is impracticable, samples may be stored for up to several days
at 4°C.

The supernatant fluids of faeces or tissue suspensions
obtained through clarification by centrifugation at 1,000 g
are inoculated into the allantoic sac of at least five embryonated
SPF chicken eggs of nine to eleven days incubation. After
inoculation, these eggs are incubated at 35°C-37°C for four to
seven days. Eggs containing dead or dying embryos as they
arise, and all eggs remaining at the end of the incubation
period, should first be chilled at 4°C and the allantoic fluids
tested for haemagglutination (HA) activity. Fluids that give a
negative reaction should be passaged into at least one further
batch of eggs.

Haemagglutination activity detected in bacteriologically sterile
fluids harvested from inoculated eggs may be due to the presence
of any of the fourteen haemagglutinin subtypes of
influenza A viruses or of the eight other APMV serotypes.
Newcastle disease virus can be confirmed by the use of
specific antisera in an HI test. Usually chicken antiserum is
used, prepared against one of the strains of NDV.

Cross-reactions in HI tests between NDV and APMVs,
particularly APMV-3 may cause some problems which can be
resolved by the use of suitable antigen and antiserum controls
(5).

Pathogenicity indices
The extreme variation in virulence of different NDV isolates
and the widespread use of live vaccines means that the
identification of an isolate of NDV from birds showing clinical
signs does not confirm a diagnosis of ND. As indicated in the
...
definition, an assessment of the virulence of the isolate by ICPI test or amino acid sequencing is also required.

The intracerebral pathogenicity index (ICPI) test should be done as follows:
- fresh infective allantoic fluid with an HA titre of greater than 2^4 (1/16) is diluted 1/10 in sterile isotonic saline, with no additives
- 0.05 ml of the diluted virus is injected intracerebrally into each of ten one-day-old chicks hatched from an SPF flock
- the birds are examined every 24 h for eight days
- the ICPI is the mean score per bird per observation over the eight-day period.

The most virulent viruses will give indices that approach the maximum score of 2.0, whereas lentogenic strains will give values close to 0.0.

**Serological tests**

Newcastle disease virus may be employed in a wide variety of serological tests as an antigen, enabling neutralisation or enzyme-linked immunosorbent assays (ELISA) to be used for diagnosis. At present, the HI test is most widely used. Chicken sera rarely give non-specific positive reactions in this test and any pre-treatment of sera is unnecessary. Sera from species other than chickens may sometimes cause agglutination of chicken red cells, so this property should first be determined and then removed by absorption of the serum with chicken red cells.

Variations of the procedures for HA and HI tests are practised in different laboratories. Examples are given in Chapter 2.1.15. of the *Manual of Standards for Diagnostic Tests and Vaccines*, as presented below (85).

The following examples apply in the use of V-bottomed microwell plastic plates in which the final volume for both types of test is 0.075 ml. The reagents required for these tests are isotonic saline buffered with phosphate (0.1M) to pH 7.0-7.2 (PBS) and red blood cells (RBC) taken from a minimum of three SPF chickens and pooled into an equal volume of Alsever's solution (if SPF chickens are not available, blood may be taken from unvaccinated birds regularly monitored and shown to be free of antibodies to NDV). Cells should be washed three times in PBS before use as a 1% (packed cell v/v) suspension. Positive and negative control antigens and antisera should be run with each test, as appropriate.

**Haemagglutination test**

The HA test should be done as follows:
- dispense 0.025 ml of PBS into each well of a plastic microtitre plate (V-bottomed)
- place 0.025 ml of serum into each well of the plate
- make two-fold dilutions of 0.025 ml amounts of the serum across the plate
- add 4 HAU of virus/antigen in 0.025 ml to each well and leave for a minimum of 30 minutes at room temperature or 60 minutes at 4°C
- add 0.025 ml of 1% v/v chicken RBC to each well and after gentle mixing allow RBC to settle for approximately 40 minutes (either at room temperature or 4°C if ambient temperatures are high) when control RBC should be settled to a distinct button
- haemagglutination is determined by tilting the plate and observing the presence or absence of tear-shaped streaming of the red blood cells. The titration should be read to the highest dilution giving complete haemagglutination (no streaming); this represents 1 HA unit (HAU) and can be calculated accurately from the initial range of dilutions.

The HI test should be done as follows:
- dispense 0.025 ml of PBS into each well of a plastic microtitre plate (V-bottomed)
- place 0.025 ml of serum into first well of plate
- make two-fold dilutions of 0.025 ml amounts of the serum across the plate
- add 4 HAU of virus/antigen in 0.025 ml to each well and leave for a minimum of 30 minutes at room temperature or 60 minutes at 4°C
- add 0.025 ml of 1% v/v chicken RBC to each well and after gentle mixing allow RBC to settle for approximately 40 minutes (either at room temperature or 4°C if ambient temperatures are high) when control RBC should be settled to a distinct button
- the HI titre is the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination is assessed more exactly by tilting the plates. Only those wells in which the red cells 'stream' at the same rate as the control wells (containing 0.025 ml RBC and 0.05 ml PBS only) should be considered as showing inhibition
- the validity of results should be assessed against a negative control serum, which should not give a titre >2^4 and a positive control serum for which the titre should be within one dilution of the known titre.

The value of serology in diagnosis is clearly related to the immune status of the affected birds. Haemagglutination inhibition titres may be regarded as positive if inhibition occurs at a serum dilution of 1/16 (2^4) or more against 4 HAU of antigen.

Haemagglutination inhibition titres may be used to assess the immune status of a flock. In vaccinated flocks that are being monitored serologically, anamnestic responses may be
observed as the result of a challenge infection with field virus (24), but great care should be exercised as variations may occur from other causes. For example, APMV-3 virus infections of NDV-vaccinated turkeys have been demonstrated to result in substantially increased titres to NDV (10).

Other diagnostic methods

Nucleotide sequencing

The knowledge that the virulence of NDV strains is primarily dependent on the amino acid sequence at the F0 cleavage site has led to several suggestions for in vitro tests based on detection of the nucleotide sequence coding for the F0 cleavage site to replace the in vivo pathogenicity tests. Studies have been performed using molecular techniques to determine the F0 cleavage site sequence by reverse transcription polymerase chain reaction either on the isolated virus or on tissues and faeces from infected birds (62, 66, 84, 102). In other studies, oligonucleotide probes tailored to the F0 cleavage site have been used to detect NDV in tissues and differentiate between viruses of high or low virulence by hybridisation techniques (59, 83). With both these approaches, problems relating to mismatching of the primers or probes or mixtures of virulent and avirulent viruses mean that, at present, these in vitro techniques cannot wholly replace the in vivo ICPI test.

Monoclonal antibodies

Mouse mAbs directed against strains of NDV have been used in HI tests to allow rapid identification of NDV without the possible cross reactions with other PMV serotypes that may occur with polyclonal sera (6).

Some workers have used mAbs to distinguish between specific viruses. For example, two groups have described mAbs which distinguish between the common vaccine strains, Hitchner B1 and La Sota (47, 77), while other mAbs can separate vaccine viruses from epizootic virus in a given geographical area (108). Panels of mAbs have been used to establish antigenic profiles of NDV isolates based on their ability to react or not with the viruses. This has proven to be a valuable method for grouping and differentiating isolates of NDV which has been particularly valuable in understanding the epizootiology of outbreaks (20, 21).

Control

Newcastle disease

Policies

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

In some countries, legislation is designed to reduce the likelihood of outbreaks from specific sources. For example, on the island of Ireland, legislation requires that poultry feed is heat-treated to reduce the possibility of introduction of NDV by this route.

On farms, control measures should attempt to prevent virus from infecting the flock. The practice of good hygiene and biosecurity measures aimed at preventing the introduction of virus by the routes described above is of paramount importance on poultry farms.

Biosecurity aimed at preventing disease should commence 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 an adequate fresh water supply should be available, preferably one that does not draw on surface water.

On poultry farms, the following points should be observed:

a) bird houses, feed stores and water tanks should be bird-proofed
b) movements on and off the farm should be kept to a minimum
c) all equipment, especially vehicles, should be disinfected before access to the site is permitted
d) movements between different farms for egg collection, carcass collection, feed delivery and similar should be to and from a specified collection and delivery point away from the poultry flocks.

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

Vaccination

Vaccination must be regarded as complementing good management, biosecurity and hygiene in rearing domestic poultry. Vaccination against ND must never be considered an alternative to these other measures.

Vaccinated birds challenged with virulent NDV may become infected and excrete virus, although in relatively small amounts, while remaining apparently healthy (53, 90, 110). This disadvantage must be fully understood when planning the role of vaccination in the control of ND.

Details of vaccination regimens, methods of administration and interpretation of antibody titres can be found in the review by Kouwenhoven (69), the text that follows considers
the factors leading to the decision to vaccinate and to the choice of vaccine.

**Decision to vaccinate**

National or international legislation or agreements that give the farmer and advisers little choice may control vaccination policies. For example, in the Netherlands vaccination is compulsory, but in countries such as Finland, Sweden and Norway vaccination is banned. In countries where legislation does not enforce vaccination under normal circumstances, ‘ring vaccination’ may be required should an outbreak occur.

In many countries, severe ND is enzootic, usually in village chickens at least, and in such high risk areas, vaccination of commercially reared birds is the only way to reduce the disease and losses resulting from the inevitable introduction of virus. In countries where disease is not enzootic, a decision to include prophylactic vaccination, as part of the control of ND, does not so clear cut, especially when the cost and the possibility of vaccine reactions are considered. In addition, the existence of a slaughter policy based on infection rather than disease, as in the EU (41), would seem a disincentive for routine vaccination.

**Choice of vaccine**

Choice of vaccine is governed by three main considerations, namely:

a) the immunogenicity of the vaccine

b) the type of vaccine (inactivated or live)

c) 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.

For live vaccines, the likely exacerbation of infections with other organisms or vaccines must also be considered.

Using modern techniques, considerable antigenic diversity has been detected in NDV isolates and strains (21). However, the vaccines commonly in use are capable of producing antibodies that protect against even those NDV isolates showing a high degree of antigenic divergence from the vaccine strain (16, 19). Thus, antigenic variation does not seem to be an important consideration in the choice of vaccine.

Although inactivated oil emulsion vaccines can be efficacious if used as primary vaccine (111), the additional cost of these vaccines, both direct and in administration, means that live vaccines are usually preferred. Vaccination programmes 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 maintenance of high antibody titres is important to prevent serious disease. The presence of enzootic disease is usually linked to severe economic restrictions, which exclude the use of oil emulsion vaccine. Mesogenic vaccines may have serious clinical effects if given to birds which have not been immunised previously and these vaccines are sufficiently virulent to fall within the new OIE definition of ND.

The most popular live vaccines have evolved from field isolates of low virulence. Most are based on the ‘lentogenic’ viruses Hitchner B1 or La Sota or similar viruses, although some ‘asymptomatic enteric’ viruses have also been used as live vaccines. A difficulty associated with live vaccines is that the immune response appears to be related to the virulence of the virus strain (93); La Sota, for example, gives better protective antibody titres than Hitchner B1, but is more likely to cause reactions. 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 La Sota virus, even high mortality.

The choice of vaccine may also be restricted by control policies, and the Commission of the EU has set criteria banning the use of live ND vaccines with an ICPI of greater than 0.4 and inactivated vaccine production from viruses with an ICPI exceeding 0.7 (42).

**Other avian paramyxoviruses**

The risk from APMV-2 virus may be minimised by prevention of introduction of the virus by bird-proofing poultry houses and good general hygiene practices. Some success has been reported in treatment of exacerbating secondary bacterial infections with antibiotics. Samberg et al. reported good antibody response in day-old turkeys to an inactivated oil emulsion APMV-2 vaccine (101).

Avian paramyxovirus serotype 3 viruses appear to spread slowly. However, good hygiene, including careful disinfection and allowing time between restocking, has not always prevented infection in subsequent flocks. Inactivated, oil-emulsion vaccines are available in the USA, the United Kingdom (UK) and other countries of Europe for use in turkey breeding flocks. These are usually injected twice, four weeks apart, before the birds begin to lay (32, 49). Duchatel et al. showed that subcutaneous inoculation of parakeets with APMV-3 vaccine afforded these birds protection from challenge with APMV-3 virus (46).

**Public health**

Newcastle disease virus is a human pathogen. In the UK, the virus is placed in Hazard group 2 of the Advisory Committee on Dangerous Pathogens (1). The virus is thus considered to
be: 'A biological agent that can cause human disease and may be a hazard to employees; it is unlikely to spread to the community...'.

A review of ND as a zoonosis recorded thirty-five published reports of ND virus infections of humans between 1948 and 1971 (36). Since that time, few additional reports have been published, which probably reflects the lack of serious, lasting effects resulting from such infections and the fact that such infections are commonplace.

Reported infections have not been life threatening and usually have not been debilitating for more than one or two days. The most frequently reported and best substantiated clinical signs in human infections have been eye infections, usually consisting of unilateral or bilateral reddening, excessive lachrymation, oedema of the eyelids, conjunctivitis and sub-conjunctival haemorrhage (36). Although the effect on the eye may be quite severe, infections are usually transient and the cornea is not affected.

Reports of other clinical symptoms in humans infected with NDV are less well substantiated, but suggest that a more generalised infection may sometimes occur, resulting in chills, headaches and fever, with or without conjunctivitis (36). Both vaccinal strains and strains virulent for poultry may infect and cause clinical signs in humans.

Human infections with NDV have usually resulted from direct contact with the virus, infected birds or carcasses of diseased birds. Human to human spread has never been reported, although spread by contagion is theoretically possible. The types of people known to have been infected with NDV include laboratory workers (usually as a result of accidental splashing of infective material into the eye), veterinarians in diagnostic laboratories (presumably as a result of contact with infective material during post-mortem examinations), workers in broiler processing plants and vaccination crews, especially when live vaccines are administrated as aerosols or fine dust. Pedersen et al. reported significantly higher antibody titres to NDV in people who had an association with poultry (92).

No reports exist of other APMV serotypes infecting humans. However, the potential may exist, and virus of APMV-2 serotype has been isolated from cynomolgus monkeys (Macaca fascicularis) (82).

La maladie de Newcastle et les autres paramyxoviroses aviaires

D.J. Alexander

Résumé

La maladie de Newcastle, conséquence d'une infection par des paramyxovirus de sérotype 1 (APMV-1), est inscrite sur la Liste A de l'Office international des épizooties. Très meurtrière dans le passé, la maladie de Newcastle pose toujours d'importants problèmes à l'aviculture industrielle existante ou naissante de nombreux pays. Elle a une incidence économique élevée, même dans les pays où elle est pratiquement maîtrisée, en raison des coûts liés à la vaccination et/ou au maintien de mesures rigoureuses de biosécurité. La virulence varie selon les souches du virus de la maladie de Newcastle, et la sensibilité des oiseaux diffère d'une espèce à l'autre, ce qui entraîne la nécessité de bien définir la maladie avant toute mesure prophylactique liée aux échanges. Pour confirmer le diagnostic, il faut isoler et caractériser le virus en cause. L'évaluation de la virulence se fait habituellement par des recherches in vivo. Cependant, depuis que la base moléculaire du pouvoir pathogène du virus est mieux connue, on
recourt de plus en plus à la caractérisation génétique in vitro. La prophylaxie de la maladie repose sur des méthodes visant à empêcher l'introduction et la propagation du virus ainsi que sur des bonnes pratiques de biosécurité, associées ou non à la vaccination. Les virus de la maladie de Newcastle peuvent infecter l'homme, provoquant le plus souvent des cas sporadiques de conjonctivite, mais aucun cas de transmission entre êtres humains n'a jamais été observé.

Huit autres sérotypes de paramyxovirus aviaires ont été reconnus (APMV-2 à APMV-9). La plupart de ces sérotypes semblent spécifiques à des espèces d'oiseaux sauvages qui leur servent de réservoir naturel, mais d'autres espèces hôtes sont également sensibles. Seuls les virus APMV-2 et APMV-3 ont eu un impact sanitaire et économique important sur la production aviaire. Ces deux types de virus provoquent des maladies respiratoires et une chute de ponte qui peuvent être graves lorsque d'autres infections ou des facteurs de stress liés à l'environnement viennent s'y ajouter. Aucune infection naturelle par les virus APMV-3 n'a été signalée chez les volailles.

Mots-clés

Enfermedad de Newcastle y otros paramixovirus aviares
D.J. Alexander

Resumen
La enfermedad de Newcastle, causada por paramixovirus aviares pertenecientes al serotipo 1 (APMV-1), figura en la Lista A de la Oficina Internacional de Epizootias. Esta enfermedad, que históricamente ha tenido efectos devastadores sobre las poblaciones de aves de corral, sigue constituyendo en muchos países uno de los principales problemas que afectan a las explotaciones avícolas, tanto jóvenes como consolidadas. Incluso en países donde la enfermedad puede considerarse bajo control, las campañas de vacunación y/o el mantenimiento de estrictas medidas de seguridad biológica siguen suponiendo una pesada carga económica. La desigual virulencia que presentan las cepas víricas para las aves de corral, junto con la distinta susceptibilidad de las diversas especies de aves, hace necesaria una definición muy cuidadosa de la enfermedad a efectos de control sanitario y de actividad comercial. Para el diagnóstico de confirmación de la enfermedad de Newcastle es preciso aislar y caracterizar el virus de que se trate. Para evaluar su virulencia suelen ser necesarias pruebas in vivo. Sin embargo, ahora que se comprenden más cabalmente los mecanismos moleculares en los que se funda el poder patógeno del virus, se viene usando cada vez más su caracterización genética in vitro. La lucha contra la enfermedad integra desde medidas para prevenir la introducción y propagación del virus hasta buenas prácticas en materia de seguridad biológica y/o vacunaciones. Aunque el virus de la enfermedad de Newcastle puede afectar al hombre, causando en general episodios pasajeros de conjuntivitis, no se ha descrito ningún caso de transmisión entre seres humanos.

Se conocen otros ocho serotipos de paramixovirus aviares, numerados del APMV-2 al APMV-9. Buena parte de esos serotipos parecen estar presentes en reservorios naturales de determinadas especies aviares silvestres, aunque hay
otras especies que suelen ser susceptibles y pueden ejercer de huéspedes. Sólo los serotipos APMV-2 y APMV-3 han tenido efectos sanitarios y económicos de cierta importancia para la producción avícola. Ambos tipos de virus provocan afecciones respiratorias y caídas de la producción de huevos, que pueden revestir cierta importancia cuando a sus efectos se suman los de otras infecciones o presiones ambientales. No se ha descrito ningún caso de infección natural de pollos por virus del tipo APMV-3.

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