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REVIEW ARTICLE

Newcastle disease in the European Union 2000-2009.

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Abstract

Newcastle disease (ND) is a devastating disease of poultry that has to some extent been neglected by those working in the field in the last 10-15 years while attention has been focused on the emergence and spread of highly pathogenic avian influenza caused by a H5N1 subtype virus. During 2000-2009 in European Union (EU) member states ND viruses virulent for chickens have been detected in wild birds, domesticated pigeons and poultry. Based on these isolations it appears that the epizootic in racing pigeons caused by the variant viruses termed pigeon avian paramyxovirus type 1 (PPMV-1), which form the genetic group 4b(VIb) first seen in Europe in 1981, continued during 2000-2009 and the virus is probably enzootic in racing pigeons in some EU countries. This virus appears to have spread regularly to wild birds, especially those of the *Columbidae* family and has been the cause of significant outbreaks in poultry. Other APMV-1 viruses responsible for ND outbreaks in the EU during 2000-2009 have been those from genetic groups 5b(VIIb) and 5d(VIId). There is evidence that the former may well represent spread from a wild bird source and these viruses have also been isolated from wild birds, while the latter represents continuing spread from the East. Future legislation or recommendations aimed at the control and eradication of ND will need to encompass these three sources of virulent ND viruses.

Introduction

Newcastle disease (ND) is enzootic in some areas of the world and a constant threat to most birds reared domestically. Every commercial flock of poultry is influenced in some way by ND infections or measures aimed at controlling ND and the spread of the causative virus. A large majority of the countries rearing poultry commercially rely on vaccination to keep ND in check, but nevertheless endemic ND represents a major limiting factor for increasing poultry production in many countries, while other countries, including most of those in the European Union (EU), experience sporadic outbreaks. Because of this worldwide impact, and as recently as 1997, most of those working in the field would have considered ND as the single most significant disease of poultry. However, since that time it has been overshadowed by the emergence and spread of highly pathogenic avian influenza (HPAI) virus of subtype H5N1 across Asia, Europe and into Africa (Alexander 2007). Although the HPAI H5N1 virus has become endemic in some countries and still causes spontaneous outbreaks in others, the acute alarm caused throughout the veterinary and medical fields has subsided to some extent and attention is once more being given to the other devastating disease of poultry, ND. It is worthwhile examining the impact this disease has had in countries of the EU during the period 2000-2009, a period when attention may have been deflected by the HPAI H5N1 virus concerns, and at a time when there are severe financial restraints and the control measures for ND in the EU are being reconsidered.

Aetiology

Taxonomy. In the current virus taxonomy ND virus (NDV), or avian paramyxovirus type 1 (APMV-1), is classified, with the viruses of the other 9 avian paramyxovirus subtypes, in the genus *Avulavirus*, sub-family *Paramyxovirinae*, family *Paramyxoviridae*, order *Mononegavirales* (Lamb

et al., 2005) i.e. it has a negative sense, single-stranded, filamentous RNA genome and a glycoprotein/lipid membrane.

Separation of avian paramyxoviruses into different groups was initially based mainly on antigenic variation, detected by a variety of serological tests, but more recently full genome sequences have become available for a number of APMV-1 strains (Czegledi *et al*., 2006) and at least one representative of the other serotypes: APMV-2 (Subbiah *et al*., 2008); APMV-3 (Kumar *et al*., 2008; 2010); APMV-4 (Nayak *et al*., 2008); APMV-5 (Samuel *et al*., 2010); APMV-6 (Chang *et al*., 2001); APMV-7 (Xiao *et al*., 2009); APMV-8 (Paldurai *et al*., 2009); APMV-9 (Samuel *et al*., 2009); APMV-10 (Miller *et al*., 2010). Phylogenetic analysis has confirmed the validity of the original avian paramyxovirus groupings.

The terms APMV-1 and NDV are synonymous, although the term pigeon paramyxovirus type 1 (PPMV-1) has been used to distinguish the variant APMV-1 virus responsible for the continuing panzootic in racing and other types of pigeons (see below).

*Antigenic variation***.** For APMV-1 viruses, differences detectable by conventional haemagglutination inhibition (HI) tests have 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 with the PPMV-1 viruses, which are demonstrably different from standard strains in HI tests, but not sufficiently different that conventional ND vaccines are not protective (Alexander & Parsons, 1986).

Monoclonal antibodies (mAbs) raised against various strains of ND virus have been used to establish the uniqueness of the variant PPMV-1 responsible for the pigeon panzootic and have proven particularly useful in identifying the spread of this virus around the world (Alexander *et al*., 1985a; Alexander *et al*., 1987; Pearson *et al*., 1987). mAbs to ND virus 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 (Erdei *et al*., 1987,

Meulemans *et al*., 1987), while other mAbs can separate vaccine viruses from epizootic virus in a given area (Srinivasappa *et al*., 1986).

Antigenic variation detected by mAb typing has also been used in epidemiological studies. In a large study of over 1500 viruses Alexander *et al*., (1997b) used the ability of ND viruses to react with panels of mAbs, consisting initially of 9 mAbs and later extended to 26 or 28 mAbs, to place strains and isolates of NDV into groups on the basis of their 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. However, in recent years this antigenic approach in epidemiology has tended to be replaced by methods aimed at detecting genetic differences and similarities.

Genetic variation. Genetic variation amongst APMV-1 viruses was established in an early study by Ballagi-Pordany *et al.*, (1996) using restriction enzyme analysis, in which six distinct genetic lineages (I to VI) were identified. The development of nucleotide sequencing techniques and the demonstration that sequences of as little as 250 base pairs give meaningful phylogenetic analyses, comparable to those obtained with much longer sequences (Seal *et al*., 1994; Lomniczi *et al.*, 1998) led to further studies with many more strains and isolates of APMV-1. Essentially these further studies confirmed the six lineages and have gradually extended the number of proposed lineages to 10 (I-X) with several sub-lineages within them (Ballagi-Prodany *et al*., 1996; Herczeg *et al*., 2001; Czegledi *et al*., 2006; Kim *et al*., 2007). Using a slightly different approach Aldous *et al*., (2003) studied the nucleotide sequences of a 375-nucleotide fragment at the 3' end of the fusion protein gene of 338 isolates of NDV representing a range of viruses of different phenotypes and temporal, geographical and host origins. They divided the isolates into six broadly distinct genetic groups (lineages 1 to 6). Lineages 3 and 4 were further subdivided into four sub-lineages (a to d) and

lineage 5 into five sub-lineages (a to e). Lineages 1, 2, 4 and 5 corresponded to the earlier defined lineages I, II, VI and VII, with comparable sub-lineages, but the earlier genetic groupings III, IV, V, VIII corresponded to their sub-lineages 3a to 3d. In addition lineage 6 represented a new group, which was genetically quite distant from the other viruses. Aldous *et al*., (2003) also stressed that while the NDV isolates placed in genetic lineages 1-5 (or I-VIII) are genetically quite close, viruses that were placed in lineage 6 are genetically very different from all the other NDV isolates.

Czegledi et al., (2006) investigated the evolution of APMV-1 viruses genetically and concluded that two clades of viruses probably arose in the primordial waterfowl reservoir, which could be divided into Class I viruses (corresponding to lineage 6 of Aldous *et al*., 2003) with a genome size of 15,198 and Class II viruses with a genome size of 15,186, representing the ancestors of all the other lineages. They further considered that a major branch in the Class II tree had occurred much more recently resulting in a clade of viruses with a genome size of 15,192 (lineages V-X).

The vast majority of APMV-1 viruses isolated and certainly all those responsible for major outbreaks of ND have been class II (lineages 1-5 or I-X) viruses. In a number of the class II subdivisions viruses showing marked differences in virulence for chickens are grouped together, including some of the viruses used as live vaccines. Class I viruses have been isolated worldwide, mainly from wild waterfowl, but significant spill over into poultry has been recorded and Class I viruses are frequently present in birds in live bird markets in the Americas (Kim *et al*., 2007). Most Class I viruses have proved to be of low virulence for chickens, but an outbreak caused by a Class I virus of high virulence occurred in Ireland in 1990 (Alexander *et al*., 1992) and there is evidence that very few point mutations are required for the low virulence viruses to become virulent (Collins *et al*., 1998).

Virulence variation. Different strains and isolates of NDV cause quite distinct clinical signs and severity of disease, even in the same host species. Based on the disease produced in chickens under laboratory conditions NDV isolates have been placed in five pathotypes (Beard & Hanson, 1984):

(1) viscerotropic velogenic: NDV strains that cause a highly virulent form of disease in which haemorrhagic lesions are characteristically present in the intestinal tract;

(2) neurotropic velogenic: NDV strains that cause high mortality following respiratory and nervous signs;

(3) mesogenic: NDV strains that cause respiratory and sometimes nervous signs with low mortality;

(4) lentogenic: NDV strains that cause mild or inapparent respiratory infections;

(5) asymptomatic enteric: NDV strains that cause inapparent enteric infections.

However, such groups should be regarded only as a guide as there is always some degree of overlap and some viruses are not easily placed in a specific pathotype (Alexander & Allan, 1974).

In addition to variation in severity of disease with virus strain, the species of bird, the immune status, age and conditions under which they are reared may also greatly affect the disease signs seen, while the presence of other organisms may greatly exacerbate even the mildest forms of disease. As a consequence, no disease signs may be regarded as pathognomonic (McFerran & McCracken, 1988).

The highly virulent viruses may produce peracute infections of fully susceptible chickens in which the first indication of disease is sudden death. Typically, disease signs such as depression, prostration, diarrhoea, oedema of the head and nervous signs may occur, flock mortality may reach 100%, but is usually lower in older birds. The appearance of shell-less or soft-shelled eggs, followed by complete cessation of egg laying, may be an early sign in adult domestic fowl. Virulent NDVs may still replicate in vaccinated birds (Alexander *et al*., 1999; Parede & Young, 1994; Utterback & Schwartz, 1973) but the clinical signs will be greatly diminished in relationship to the antibody level achieved (Allan *et al*., 1978).

The moderately virulent, or mesogenic, viruses usually cause severe respiratory disease, followed by nervous signs, with mortality up to 50% or more. The variant PPMV-1 virus produced no respiratory signs in infected chickens (Alexander *et al*., 1985b). In PPMV-1 infections diarrhoea and nervous signs were the main presentation of the disease, preceded by catastrophic drops in egg production in laying hens.

The viruses of low virulence may cause no disease, or mild respiratory distress for a short time in chickens and turkeys. However, the presence of other organisms, poor husbandry or environmental conditions may cause disease comparable to that seen with more virulent virus. Even infections where clinical signs are absent may result in loss of weight gain in broiler chickens and small reductions in egg yield in laying birds (Leslie, 2000).

Molecular basis for virulence. An understanding at the molecular level of the main mechanism that controls the virulence of NDV strains (Rott & Klenk, 1988) has meant that it is now possible, using nucleotide sequencing techniques, to assess whether or not an isolate has the genetic potential to be virulent for chickens. The viral F protein brings about fusion between the virus membrane and the cell membrane so that the virus genome enters the cell and replication can begin. The F protein is therefore essential for replication, but during replication, NDV particles are produced with a precursor glycoprotein, F0, that has to be cleaved to F1 and F2 polypeptides, which remain bound by disulphide bonds, for the virus particles to be infectious. This post translation cleavage is mediated by host cell proteases.

The cleavability of the F0 molecule has been shown to be related directly to the virulence of viruses *in vivo.* A large number of studies have confirmed the presence of multiple basic amino acids at the F0 cleavage site in virulent viruses (e.g. Collins *et al*., 1993). Usually the sequence has been ^{113}RQK/RR*F^{117} in virulent viruses, although most also have a basic amino acid at position 112. In contrast, viruses of low virulence usually have the sequence ^{113}K/RQG/ER*L^{117}.

Thus there appears to be the requirement of a basic amino acid at residue 113, a pair of basic amino acids at 115 and 116 plus a phenylalanine at residue 117 if the virus is to be virulent for chickens. The presence of these basic amino acids at these positions means that cleavage can be effected by a protease or proteases present in a wide range of host tissues and organs, but for lentogenic viruses, cleavage can occur only with proteases recognizing a single arginine, i.e. trypsin-like enzymes. Lentogenic viruses are therefore restricted in infected hosts to the sites where they are able to replicate i.e. those with trypsin-like enzymes, such as the respiratory and intestinal tracts, whereas virulent viruses can replicate and cause damage in a range of tissues and organs resulting in fatal systemic infections.

Although it appears that a multiple basic amino acid cleavage site sequence of the F0 protein is essential for ND viruses to be virulent in chickens, other factors associated with other virus genes and proteins have been shown to cause variation in virulence. Using reverse genetic techniques it has been demonstrated that the HN protein may influence the virulence of viruses (Huang et al., 2004; Romer-Oberdorfer *et al*., 2006). Similarly, the V protein has been shown to be an alpha interferon antagonist and to significantly influence virus virulence (Huang *et al*., 2003). Numerous PPMV-1 viruses have been isolated, which despite have a multiple basic amino acid cleavage site motif have shown low ICPI (<0.7) values (for examples see table 2), the difference[s] responsible for this reduced virulence has not yet been determined.

ND Definition. If countries are to impose trade embargoes on animal products from other countries where disease is endemic or experiencing outbreaks it is important that the disease is carefully defined. Equally, clear definition is needed for legislation imposing control measures. For many diseases merely the demonstration of the presence of the causative organism is sufficient. However, because of the variability of ND viruses described above, especially in virulence, and the worldwide presence of viruses of low virulence in wild bird reservoirs and in poultry when used as live vaccines, definition of ND becomes far more difficult. It is the role of the World Organisation for

Animal Health (OIE) to facilitate trade in animals and animal products between member countries and for ND this necessitates formulating a definition that can be accepted by those countries. The OIE definition for ND virus infections requiring notification (Alexander, 2008) has evolved considerably over the last 15 years and is currently:

'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 and 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.'

This definition takes account of the advances in understanding the molecular basis of virulence and thus allows molecular techniques to be used, which means confirmation of disease can be done rapidly without the need of protracted *in vivo* tests. However, it is important to understand that while the demonstration of the presence of virus with multiple basic amino acids at the F0 cleavage site confirms the presence of virulent or potentially virulent virus, the failure to detect virus or

detection of ND virus without multiple basic amino acids at the F0 cleavage site using molecular techniques does not confirm the absence of virulent virus. Primer mismatch, or the possibility of a mixed population of virulent and avirulent viruses mean that virus isolation and an *in vivo* assessment of virulence is still required. It is also worth noting that since it states "an infection of birds" the presence of virus fulfilling the criteria in wild birds also requires notification.

In EU countries control measures for ND were introduced by Council Directive 92/66/EEC in 1992 (CEC 1992) and have not been formally updated since then. In this Directive ND was defined:

'..an infection of poultry caused by any avian strain of paramyxovirus 1 with an intracerebral pathogenicity index (ICPI) in day-old chicks greater than 0.7.'

In practice, since 1998 the definition used in the EU has been that recommended by a Scientific Committee on Animal Health and Animal Welfare working group which was asked to review the definition of ND (Alexander *et al*., 1998) which was:

*'"Newcastle Disease" is defined as an infection of poultry caused by a virus of avian paramyxovirus serotype 1 (APMV-1) which has an intracerebral pathogenicity index (ICPI) in day-old chicks (*Gallus gallus*) of 0.7 or greater.*

- As an alternative to the ICPI test, the presence of "Newcastle Disease" virus can also be confirmed by the demonstration (either directly or by deduction) of multiple basic amino acids [at least three arginine or lysine residues between residues 113 and 116] at the C-terminus of the F2 protein and phenylalanine [F] at residue 117, which is the N-terminus of the F1 protein. Failure to demonstrate the presence of multiple basic amino acids or F at 117 would require characterisation of the isolated virus in an ICPI test.*

** 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.'*

Although this is essentially the same as the current OIE definition there is one important deviation in that it refers to an infection of poultry rather than an infection of birds. This in turn requires a definition of poultry and the most recent definition (CEC, 2006) is:

‘ "poultry" means all birds that are reared or kept in captivity for the production of meat or eggs for consumption, the production of other products, for restocking supplies of game birds or for the purposes of any breeding programme for the production of these categories of birds.’

This definition does result in some anomalies. For example, pigeons reared for racing infected with virulent APMV-1 viruses (such as the PPMV-1 variant) do not fall within the definition of ND, while pigeons reared for meat, infected with the same virus do. Equally there is some debate as to whether or not reared game birds that have been released are wild birds or not.

Virulent ND virus infections of wild birds. From the available literature, Kaleta & Baldauf (1988) concluded that in addition to the domestic avian species, natural or experimental infection with NDV had been demonstrated in at least 241 species from 27 of the 50 orders of birds. These authors stressed the variation in severity of clinical signs, even with different species of a genus. Since that review, the number of species from which NDV has been isolated, with or without clinical signs, has greatly increased. It seems reasonable to conclude that the vast majority of, if not all, birds are susceptible to infection, but the disease seen with any specified strain of virus may vary considerably with host. Ducks and some other water birds may become infected with ND viruses that are virulent for chickens, but show few, if any, clinical signs. Wild birds have often been considered as potential reservoirs for NDV, but despite the fact that virulent ND viruses are endemic in poultry in many parts of the world only exceptionally have wild birds been infected and shown to spread ND. Most isolates of virulent viruses from wild birds have been the result of sampling wild birds (usually dead) found on or nearby infected poultry premises. In contrast, viruses of low virulence for chickens have been obtained frequently from migratory feral waterfowl

and other aquatic birds. However, this may represent more of a threat than it appears at face value as there have been at least two instances where there is very strong evidence that low virulence viruses originating from wild birds have mutated to virulence in poultry (Alexander, 2001).

There have been three recorded exceptions to the absence of endemic virulent ND viruses in wild birds, these are: 1. the continuing panzootic in pigeons, 2. the apparent presence in cormorants in North America, 3. the circumstantial evidence of wild bird spread in Europe.

Pigeon panzootic. The first descriptions of this disease in domesticated pigeons, caused by a virulent NDV, were in the Middle East in the late 1970s (Kaleta *et al*., 1985). By 1981, the causative virus had reached racing pigeons in Europe (Biancifiori & Fioroni, 1983) and then spread rapidly to all parts of the world, largely as a result of contact between birds at races and shows and the large international trade in such birds. The variant nature of the virus enabled unequivocal demonstration of infection with this strain and for pragmatic purposes it became known as pigeon paramyxovirus type 1 (PPMV-1), although if this virus infects poultry, including pigeons reared for food, it fulfils the definitions of notifiable ND currently in use by OIE and in the EU discussed above.

Spread of PPMV-1 virus to chickens has occurred in several countries including Great Britain where there were 20 outbreaks in unvaccinated chickens in 1984 as a result of using feed that had been contaminated by infected pigeons (Alexander *et al*., 1985b). Three outbreaks in game birds in Great Britain were also caused by infections with the PPMV-1 virus; these covered the period 1995-2006 and all appeared to be the result of spread from pigeons as there were no outbreaks in other poultry at the time they occurred (Alexander *et al*., 1997a; Irvine *et al*., 2009).

The disease in pigeons has now been recognized for over 30 years but still seems to remain enzootic in racing pigeons in many countries, with regular spread to wild pigeons and doves and a continuing threat to poultry. The recent outbreaks in the EU due to PPMV-1 infections are described and discussed below.

*Cormorants in North America***.** Outbreaks in double-crested cormorants (*Phalacrocorax auritus*) 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 *et al*., 1998). Since these outbreaks covered cormorants that would follow different migratory routes it seemed most probable that initial infection occurred at a mutual wintering area in south USA or Central America. Further studies resulted in the isolation of virulent ND virus from cormorants on their wintering grounds in Florida, in 2002 (Allison *et al*., 2005). Nucleotide sequencing of a portion of the F gene of this virus showed 100% similarity in the deduced amino acids for that part of the F protein with the 1992 North US cormorant virus and the isolate from turkeys. It has also been demonstrated that although cormorants may exhibit disease signs following natural infection, in cormorants infected experimentally with NDV virulent for chickens excretion of virus occurred in the absence of clinical signs (Kuiken *et al*., 1998). As recently as January 2011 ND has been reported in cormorants in Florida (Poultry News, 2011).

Wild bird spread in Europe? In 1997 11 outbreaks of ND were confirmed in chickens and turkeys in Great Britain between early January and late April (Alexander *et al*., 1998). Epidemiological investigations suggested that most of the outbreaks were the result of secondary spread by human agency from one or two primary introductions. Nucleotide sequencing and phylogenetic analysis not only showed very close similarity between the British isolates and the viruses responsible for ND outbreaks in Scandinavian countries in 1996 but also with an isolate from a feral goosander

(*Mergus merganser*) in Finland (Alexander *et al*., 1999), all viruses were genetically closely related and placed in the genetic subgroup 5b(VIIb). There were also unusual patterns of movement of migratory birds at the end of 1996 and beginning of 1997 and it was suggested they may have been the vehicle for the primary introduction of the causative virus into Great Britain. Subsequently, very closely related viruses were obtained from a cormorant in Denmark in 2001 and poultry in Finland, Denmark and Sweden during 2002-2004. An outbreak in pheasants in Great Britain in 2005 was also the result of infection with a very similar virus (Aldous *et al.*, 2007) and while the virus was thought to have been introduced with pheasants imported from France, where they were known to have become infected, the close proximity of the French farm supplying the birds to a lake led to speculation that wild birds may have been the source of the virus. Although still circumstantial, there is mounting evidence that there may be a wild bird reservoir for this virulent ND genotype 5b(VIIb) virus in Europe, which is discussed further below.

*Infections of pigeons in the EU***.** The reported isolations of the variant APMV-1 termed PPMV-1 from pigeons in EU countries during the 10 year period 2000-2009 are listed in Table 1. It is worth noting that most countries do not distinguish between racing and other domesticated non-meat pigeons and wild pigeons, some may include other species of *Columbidae.* However, the majority of the 1364 isolates would have been obtained from racing pigeons. Twenty-three of the 27 current EU countries are represented in Table 1, only Lithuania, Luxembourg, Malta and Romania are absent. The isolations are likely to represent only a minor proportion of the true infections of pigeons with PPMV-1 since many, even those presenting marked clinical signs, would not be tested by virus isolation. Some countries such as Belgium, Italy and the UK have reported isolations every year over the 10 years, whereas in others, isolations, and presumably outbreaks, seem to be more sporadic. In the Netherlands there was a widespread epizootic amongst racing pigeons in 2009 hence the large number of isolations made in that year.

As already mentioned above PPMV-1 outbreaks have been occurring in pigeons in EU countries for over 30 years and although Directive 92/66/EEC (CEC, 1992) does deal with infections of racing pigeons with ND virus the control requirements are considerably less than those for poultry, and in practice the measures imposed are that infected birds are required to be kept in captivity for 60 days after recovery from clinical signs and all birds must have certification of vaccination if taking part in races or shows. However, the apparent endemic status of PPMV-1 in pigeons in the EU, which is implicit in the data recorded in Table 1, suggests these control measures are not having the required impact and this is not helped by a general but erroneous acquiescence amongst many of those working in the field that PPMV-1 virus is not an ND virus. As discussed in the next two sections the continued presence of PPMV-1 in pigeons has a considerable impact on wild birds and poultry.

Isolations of ND viruses virulent for chickens from wild birds in the EU 2000-2009. In the ten years 2000-2009 there were a significant number of isolations of ND viruses virulent for chickens from wild birds in EU countries and these are listed in Table 2. The large majority of these isolates has been identified as PPMV-1 viruses and have come from other *Columbidae* species that are likely to share habitats with pigeons. It seems likely that, in addition to racing and feral pigeons, PPMV-1 virus has become enzootic in these other species in parts of Europe. A study of the isolates obtained from the outbreaks in collared doves in Italy in 2000-2001 showed that the viruses infecting these birds were genetically distinguishable from contemporaneous isolates from pigeons (Terregino, *et al*., 2003). Whether or not this applies to isolates from collared doves and turtle doves in other countries is not clear. Isolates of PPMV-1 virus were reported from a robin in Italy in 2005, 'wild waterfowl' in Germany in 2006 and a goshawk in Belgium in 2007 demonstrating the threat to other species.

The isolations of viruses of genetic group 5b(VIIb) in Denmark from free-living pheasants in 2000 and a cormorant in 2001 may well be considered significant in view of the isolation of

genetically very similar viruses in the preceding 5 years from poultry and wild birds in Scandinavian countries and the United Kingdom (Alexander *et al*., 1999; Jørgensen *et al*., 1999) and in EU countries after 2001 (Table 4). Few surveillance studies of wild birds for ND viruses, or even for avian influenza viruses that may have revealed the presence of ND viruses, have included cormorants or similar birds. But, in view of the historical associations between such birds and NDV (Blaxland, 1951; Cleary, 1977), the reports for North America discussed above and the more recent findings in Europe, surveillance for NDV aimed specifically at cormorants may be worthwhile.

Ourbreaks in poultry in the EU.

PPMV-1 outbreaks in poultry. It is inevitable that if a large reservoir of ND virus is present in racing and feral pigeons and other wild birds, spread to poultry will occur and, as listed in Table 3, infections of poultry with PPMV-1 virus were reported regularly in EU countries during 2000-2009. Most of the outbreaks occurred in small backyard or ornamental flocks in which biosecurity was probably minimal and contact with wild birds likely. Nevertheless such infections are notifiable ND outbreaks and may have damaging consequences for trade. More significant outbreaks due to PPMV-1 viruses occurred in Sweden in 2001 (6,370 broiler breeders), France 2005 (1,500 pheasants), UK 2006 (13,396 game birds) and Estonia 2007 (5,122 layers). It is also worth drawing attention to the two outbreaks in pigeons reared for meat in France, in 2005 a flock of 8,500 pigeons was affected and in 2006 a flock of 5,250. In contrast to racing pigeons, pigeons reared for meat fall within the definition of poultry. Possibly more outbreaks in poultry due to PPMV-1 virus may have been expected, especially due to the increase in free range flocks in recent years. It is feasible that introductions do occur but go unnoticed due to the lower virulence of these isolates for poultry (Tables 2 & 3), as discussed above, and the widespread use of prophylactic vaccination. Vaccination may be especially pertinent since, although the vaccination status of the poultry listed in Table 3 is largely unknown, the birds are mainly described as backyard or ornamental, which

may mean that vaccination, if used, was not optimal, or commercial birds that were in countries (Sweden and Estonia) which at the time had non-vaccination policies. It is worth noting that in December 2010 there were a further two outbreaks in meat pigeons in France due to PPMV-1 infections; while in February 2011 there were two outbreaks in commercial poultry in Sweden due to PPMV-1 infections (OIE, 2011). None of the birds in these four outbreaks had been vaccinated and in each case spread of the virus from wild birds was suspected.

*Other ND outbreaks in poultry***.** The virulent ND viruses isolated from poultry in EU countries during 2000-2009 are listed in Table 4. With the exception of the uncharacterised viruses from Portugal in 2007 and 2008 all viruses have been placed in either genetic group 5b(VIIb) or 5d(VIId). There is a marked geographical demarcation for the isolates, those of genetic group 5b(VIIb) occurring primarily in countries in the western and northern parts of the EU (Denmark, Finland, Germany, Sweden, France, Italy, UK and Spain), while 5d(VIId) viruses have been obtained from countries in the eastern part of the EU (Hungary, Greece, Romania and Bulgaria).

Viruses that are genetically closely related within group 5b(VIIb) have caused sporadic outbreaks in Europe since the mid-1990s and, as discussed above, the isolation of similar viruses from wild birds, particularly cormorants has led to speculation that there may be an unusual reservoir of virulent NDV in wild birds. If this is the case, and birds such a cormorants are an important factor, it may well be significant that a number of the isolations from poultry came from flocks situated close to the coast (San Miguel & Sánchez, 2010; OIE, 2011) or inland water (Aldous *et al.*, 2007). It was speculated that the large number of outbreaks due to a similar virus in Italy in 2000 (Cattoli *et al*., 2001) may have resulted from the importation of hatching eggs with secondary spread linked to a hatchery (Capua *et al*., 2002).

In contrast, infections of poultry with 5d(VIId) viruses appear to be the result of the ongoing eastern spread of genetically closely related, but evolving, ND viruses, which were first recorded in

the Far East in the 1990s (Aldous *et al*., 2003; Liu *et al*., 2003; Lee *et al*., 2004) and continue to be isolated across Asia (Berhanu *et al*., 2010; Ke et al., 2010; Zhang Rui *et al*., 2010).

Conclusions

It is clear from the data presented in Table 1 that if the objective of the control measures for ND in pigeons laid down in Council Directive 92/66/EEC (CEC 1992) was to eradicate the virus that had been responsible for the panzootic in racing pigeons first isolated in Europe in 1981, they appear to have failed. Essentially the variant PPMV-1 virus is enzootic in racing and other domesticated pigeons in the majority of countries in the EU and for the past 30 years its control has been ineffective. The presence of PPMV-1 virus in wild birds (Table 2) and causing a series of ND outbreaks in poultry (Table 3) is indicative of the threat posed by this reservoir in domesticated pigeons. It would appear to be prudent for any new Directive for the control of ND to address this problem with a view to eradication of PPMV-1 infections in domesticated pigeons, especially racing pigeons.

Other ND outbreaks have been caused by APMV-1 viruses from genetic groups 5b(VIIb) and 5d(VIId). The former may well represent spread from a wild bird source, while the latter represents continuing spread from the East. While these sources continue it is inevitable that further outbreaks will occur. Hopefully, any future outbreaks of ND will be detected early and eradicated rapidly. However, current control measures for ND in the EU (CEC, 1992) allow prophylactic vaccination, which is in marked contrast to control measures for AI (CEC, 2006). Since vaccination may prevent clinical signs, but not infection and virus replication (Parede & Young, 1990; Capua *et al*., 1993; Alexander *et al*., 1999), it is possible that, following introduction, spread of virulent ND virus in vaccinated birds may go unnoticed. This could result in an endemic situation that only becomes apparent when immunity is not achieved due to not vaccinating, vaccination failure or

immune suppression due to infections with other agents. Capua *et al*., (2002) certainly considered this situation may have been important for the epizootic occurring in Italy in 2000, stating: *"It therefore appears that these viruses may be circulating undiagnosed throughout Europe. A possible explanation could be that the clinical disease appears only in an immunologically naive population (Scandinavia, the UK, and for the reasons discussed earlier, in this case Italy), and that in the face of high mortality levels farmers and veterinarians submit samples to diagnostic laboratories. In contrast, in countries in which a vaccination policy is applied, the infection may circulate easily without causing any clinical signs, thus making diagnosis more difficult.*" It would seem imperative that any future legislation or recommendations for control and eradication of ND in the EU also address this potential problem.

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Table 1. *Reported PPMV-1 infections of pigeons*[a] *in EU countries 2000-2009 confirmed by virus isolation and characterisation*

Year	Country (number of isolates[b])	Total isolates
2000	Belgium (10), Denmark (10), Germany (50), Ireland (2), Italy (16), Portugal (2), Slovenia (3), Sweden (5), UK (30)	138
2001	Belgium (7), France (8), Germany (22), Italy (31), Portugal (1), Slovenia(1), UK (24)	94
2002	Belgium (7), France (4), Germany (40), Ireland (3), Italy (16), Poland (3), Slovakia (1), UK (27)	81
2003	Austria (3), Belgium (5), Czech Republic (1), France (1), Germany (36), Ireland (5), Italy (11), Portugal (1), Sweden (8), UK (39)	110
2004	Austria (2), Belgium (6), France (6), Germany (20), Greece(1), Ireland (2), Italy (12), Slovenia (1), Sweden (2), UK (25)	77
2005	Belgium (6), Czech Republic (1), France (7), Germany (53), Ireland (1), Italy (39), Poland (2), Romania (1), Slovakia (23), Slovenia (3), Spain (1), UK (17)	154
2006	Austria (2), Belgium (9), Germany (13), Italy (38), Latvia (4), Slovakia (14), UK (1)	81
2007	Austria (7), Belgium (12), Bulgaria (1), Cyprus (2), Czech Republic (3), Denmark (1), Estonia (1), France (4), Germany (19), Italy (17), Slovenia (4), UK (39)	110
2008	Austria (5), Belgium (6), Cyprus (1), Denmark (2), Finland (21), France (2), Germany (16), Ireland (1), Italy (2), Netherlands (9), Portugal (1), Slovenia (1), UK (54)	121
2009	Austria (3), Belgium (9), Finland (5), France (1), Germany (29), Hungary (1), Italy (8), Latvia (3), Netherlands (289), Portugal (14), Slovakia (1), Sweden (2), UK (33)	398
	Total number of isolates	1364

[a]Most countries do not distinguish between racing pigeons etc and wild pigeons, some may include other species of *Columbidae.* All viruses were confirmed as PPMV-1 by mAb binding (group P) or genotyping in sub-lineage 4b (VIIb) or both.

[b]It should be noted that listed in this table are the number of isolates reported by the National reference Laboratory for each country and not the number of outbreaks.

Data from Aldous *et al*., (2007); Alexander & Manvell, (2002-2006); Alexander *et al*., (2008); Brown (2008); Brown & Bashford (2008); Brown & Henry, (2009, 2010) and C.M. Fuller & R.J. Manvell personal communications (2011).

Table 2. *Newcastle disease (APMV-1) viruses virulent for chickens isolated from or detected in wild birds (excluding pigeons) by EU country 2000-2009.*

Year	Country	Number of viruses and host	ICPI[a]	Cleavage site[b]	mAb group[c]	Genetic group
2000	Denmark	2 x pheasants[d,e]	1.6, 1.7	RRQRR*F	C1	5b (VIIb)
	Italy	6 x collared doves[f]	0.7-1.3	RRKKR*F	P	PPMV-1
2001	Denmark	1 x cormorant	1.4	RRQRR*F	C1	5b (VIIb)
	Italy	34 x collared doves	0.6-1.4	RRKKR*F	P	PPMV-1
				RRQKR*F	P	PPMV-1
2002	Italy	16 x collared doves	0.6-1.2		P	PPMV-1
2003	Italy	9 x collared doves	0.7-1.3	RRQKR*F	P	PPMV-1
	Spain	1 x turtle dove[h]	0.2		P	PPMV-1
2004	Cyprus	1 x partridge[i]	1.2	KRKKR*F		4a
	Italy	18 x collared doves	0.4-1.4	RRQKR*F	P	4b PPMV-1
2005	Cyprus	1 x wild bird faeces	1.5	KRKKR*F		4a
	Italy	14 x collared doves	0.7-1.4	RRQKR*F	P	4b PPMV-1
		1 x robin[j]	1.2	RRQKR*F	P	4b PPMV-1
	Romania	1 x diver[k]	1.8			?
	Slovakia	1 x turtle dove	nd	RRQKR*F		4b PPMV-1
	Slovenia	1 x collared dove	nd	RRQKR*F	P	PPMV-1
2006	Czech Rep.	1 x collared dove	0.35	RRQKR*F		PPMV-1
	France	2 x collared dove	0.9-1.3	RRQKR*F		4a
	Germany	1 x 'wild waterfowl'	1.3	RRQKR*F	P	PPMV-1
	Italy	13 x collared doves	0.7-1.3	RRQKR*F	P	4b PPMV-1
	Slovakia	22 x turtle doves	nd	RRQKR*F	nd	4b PPMV-1
		6 x turtle doves	nd	RRKKR*F	nd	4b PPMV-1
2007	Belgium	1 x goshawk[l]	nd	RRQKR*F	P	PPMV-1
	Italy	9 x collared doves	0.7-1.4	RRQKR*F		4b PPMV-1
	Romania	1 x starling[m]	1.7			5d (VIId)
	Spain	3 x doves		RRQKR*F	P	PPMV-1
		1 x pigeon[n]		RRQKR*F	C1	
		1 x turtle dove		RRQKR*F	C1	
2008	Slovakia	1 x turtle dove		RRQKR*F		4b PPMV-1
	Spain	1 x dove		RRQKR*F	P	4b PPMV-1
		1x turtle dove		RRQKR*F	P	4b PPMV-1
		1 x turtle dove		RRQKR*F	C1	
2009	Italy	5 x collared dove	0.7-1.1	RRQKR*F		4b PPMV-1
	Portugal	1 x collared dove		RRQKR*F		4d (VId)
	Spain	3 x turtle dove		RRQKR*F		

[a]Intracerebral pathogenicity index (Alexander, 2008)

[b]Deduced amino acids at positions 112 to 117 of the F0 precursor protein.

[c]Antigenic group determined using mouse monoclonal antibodies (Alexander *et al.*, 1997).

[d]Reared birds that had been released into the wild.

[e]*Phasianus colchicus*; [f]*Streptopelia decaocto*; [g]*Phalacrocorax carbo sinensis*; [h]*Streptopelia turtur*; [i]*Perdix* sp; [j]*Erithacus rubecula*; [k]*Gaviidae* sp; [l]*Accipter gentilis*; [m]*Sturnus vulgaris*; [n]*Columba livia*

Data from Aldous *et al*., (2007); Alexander & Manvell, (2002-2006); Alexander *et al*., (2008); Brown (2008); Brown & Bashford (2008); Brown & Henry, (2009, 2010) and C.M. Fuller & R.J. Manvell personal communications (2011).

Table 3. *Isolation of PPMV-1 viruses from poultry in EU countries 2000-2009*

Year	Country	Number of viruses and host	ICPI[a]	Cleavage site[b]	mAb group[c]	Genetic group
2000	Germany	1 x ornamental poultry			P	4b (VIb)
2001	Germany	1 x ornamental chickens		RRQKR*F	P	4b (VIb)
	Sweden	1 x broiler breeders	1.2	RRQKR*F	P	4b (VIb)
	Germany	1 x ornamental chickens		RRQKR*F	P	4b (VIb)
2003	Germany	1 x ornamental chickens	0.88	RRQKR*F	P	4b (VIb)
	Sweden	1 x backyard turkey	1.25	RRQKR*F	P	4b (VIb)
2005	France	1 x meat pigeon	1.0	RRQKR*F	P	4b (VIb)
		1 x pheasant	1.6		P	4b (VIb)
2006	Belgium	1 x chickens		RRQKR*F	P	4b (VIb)
	France	1 x meat pigeon	nd	RRQKR*F		4b (VIb)
	UK	1 x partridges	1.0	RRQKR*F		4b (VIb)
2007	Estonia	3 x chickens	1.2-1.6	KRQKR*F		4b (VIb)

[a]Intracerebral pathogenicity index (Alexander, 2008)

[b]Deduced amino acids at positions 112 to 117 of the F0 precursor protein.

[c]Antigenic group determined using mouse monoclonal antibodies (Alexander *et al.*, 1997).

Data from Aldous *et al.*, (2007); Alexander & Manvell, (2002-2006); Alexander *et al.*, (2008); Brown (2008); Brown & Bashford (2008); Brown & Henry, (2009, 2010) and C.M. Fuller & R.J. Manvell personal communications (2011).

Table 4. *Other virulent ND viruses isolated[a] from poultry in EU countries 2000-2009*

Year	Country	Number of viruses and host	ICPI[b]	Cleavage Site[c]	mAb Group[d]	Genetic group
2000	Italy	2 x breeders 17 x broilers 86 x rural chickens 8 x turkeys 5 x pheasants 3 x guinea fowl 1 x ostrich 1 x quail	all>1.5	all RRQRR*F	all C1	all 5b (VIIb)
2001	Italy	1 x rural chicken	1.9	RRQRR*F	C1	5b (VIIb)
2002	Denmark	3 x layers	1.7-1.8	RRQRR*F	C1	5b (VIIb)
2003	Italy	2 x rural chickens	1.6-1.8	RRQKR*F	?	?[e]
2004	Finland	1 x meat turkeys	1.6	RRQRR*F	C1	5b (VIIb)
	Germany	3 x turkeys	1.82	RRQRR*F	C1	5b (VIIb)
	Greece	1 x broilers	1.7	RRQRR*F		5d (VIId)
	Sweden	1 x layers	1.45	RRQRR*F		5b (VIIb)
2005	Denmark	1 x broiler breeders	1.79	RRQRR*F		5b (VIIb)
	France	1 x pheasant [no virus]		RRQRR*F		5b (VIIb)
	Greece	6 x broilers	1.6-1.9	RRQKR*F		5d (VIId)
	Romania	96 x chickens	1.3-1.9			5d (VIId)
		1 x partridge	1.75			
	Sweden	2 x layers	1.3-1.9	RRQRR*F		5b (VIIb)
	UK	3 x pheasants	1.3-1.6	RRQRR*F		5b (VIIb)
2006	Bulgaria	4 x chickens	nd	RRQKR*F		5d (VIId)
		1x turkeys	nd	RRQKR*F		5d (VIId)
	Hungary	4 x chickens	1.8-1.9	RRQKR*F		5d (VIId)
	Sweden	1 x layers		RRQRR*F		5b (VIIb)
2007	Bulgaria	1 x chickens	nd	RRQKR*F		5d (VIId)
		1x turkeys	nd	RRQKR*F		5d (VIId)
	Greece	1 x chicken	1.49	RRQKR*F		5d (VIId)
	Portugal	4 x chickens		RRQKR*F		
	Romania	46 x broilers		RRQKR*F		5d (VIId)
		20 x layers	all ~1.8	RRQKR*F		5d (VIId)
		1 x layers		RRQRR*F		5b (VIIb)
2008	Bulgaria	34 x chickens				5d (VIId)
	Portugal	1 x chickens		RRQKR*F		
	Romania	5 x chickens	1.6-1.8			5d (VIId)
	Sweden	1 x layer hens		RRQRR*F		5b (VIIb)
2009	Bulgaria	2 x layer hens		RRQKR*F		5d (VIId)
	Romania	1 x layers		RRQKR*F		5d (VIId)
	Spain	1 x pheasants		RRQRR*F		5b (VIIb)
	Sweden	1 x broiler breeders		RRQRR*F		5b (VIIb)

[a]It should be noted that listed in this table are the number of isolates reported by the National reference Laboratory for each country and not the number of outbreaks.

[b]Intracerebral pathogenicity index (Alexander, 2008)

[c]Deduced amino acids at positions 112 to 117 of the F0 precursor protein.

[d]Antigenic group determined using mouse monoclonal antibodies (Alexander *et al.*, 1997). largely replaced by genotyping after 2004.

[e]This virus has not been characterised genetically, it does not react with mAb 161 (C. Terregino personal communication), but could still be a PPMV-1 virus as some viruses placed in genetic group 4b(VIb) do not show typical mAb binding.

Data from Aldous *et al*., (2007); Alexander & Manvell, (2002-2006); Alexander *et al*., (2008); Brown (2008); Brown & Bashford (2008); Brown & Henry, (2009, 2010) and C.M. Fuller & R.J. Manvell personal communications (2011).