The Definition of Newcastle Disease - Report of the Scientific Committee on Animal Health and Animal Welfare Adopted 24 March 1998

1. Request for an Opinion

The Office International des Epizooties [OIE] has proposed a new definition for Newcastle Disease. The Scientific Committee has been requested to comment on the suitability and acceptability of the new definition proposed by the O.I.E. and to make recommendations for changes in the proposed definition or in Community legislation.

The Committee was particularly asked to consider the possibility of providing alternative *in vitro* tests for virulence to those using living animals.

2. Current Definitions

The definition of Newcastle disease [ND] currently in use in all member states of the European Union is defined in Directive 92/66/EEC as follows;

"an infection of poultry caused by an avian strain of the paramyxovirus 1 with an intracerebral pathogenicity index [ICPI] in day-old chicks greater than 0.7".

In the ICPI test, described in Council Directive 92/66/EEC, virus is inoculated into the brains of 10 one-day-old chicks and the index is the mean score per bird per 24 hour observation over 8 days when each bird is scored 0 if normal, 1 if sick and 2 if dead.

In contrast the current OIE definition of ND is as follows;

" *ND is a disease of birds caused by strains of avian paramyxovirus type 1, significantly more virulent than lentogenic strains...."* International Animal Health Code - Mammals, Birds & Bees 6th Edition. (Paris, Office International des Epizooties), 1992.

3. New definition proposed by OIE

The new definition proposed by the OIE is as follows;

- *Newcastle disease is a disease of poultry caused by a virus of avian paramyxovirus serotype 1 (APMV-1) which has an intracerebral pathogenicity index (ICPI) in day-old chicks of 1.2 or greater.*
- *Virulent virus can also be confirmed by the presence of multiple basic amino acids at the at the C-terminus of the F2 protein and F (phenylalanine) at residue 117, the N-terminus of the F1 protein, failure to demonstrate this amino acid sequence would require characterisation by ICPI test.*

4. Need for a definition

It seems likely that the vast majority of birds are susceptible to infection with ND viruses of both high and low virulence for chickens, although the disease seen with any given virus may vary enormously from one species to another. Other animals may be infected with ND virus. In humans such infections are usually seen as mild to severe conjunctivitis.

The clinical signs seen in birds infected with ND virus 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. Virulent viruses have been divided into those in which the predominant signs produced in infected chickens are intestinal lesions [termed viscerotropic] and those which induce mainly neurological signs [neurotropic]. However, in

practice such 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.

ND viruses show a considerable range of virulence for susceptible hosts such as chickens. Generally, variation consists of clusters around the two extremes in tests used to assess virulence, but, for a variety of reasons, some viruses may show intermediate virulence [mesogenic].

The enormous variation in virulence and clinical signs mean it is necessary to define carefully what constitutes ND for the purposes of trade, control measures and policies.

The current OIE definition is insufficiently specific and thus allows considerable interpretation. In many Asian and African countries mesogenic viruses are used as live vaccines [with ICPI values in the region of 1.5] that could cause disease and high mortality in susceptible poultry.

The current EU definition is much more specific. It is important to note that this definition does not make any reference to clinical disease and that a definition of poultry is required. Poultry have been defined in Directive 90/539/EEC, modified by Directive 92/65/EEC as:

"fowl, turkeys, guinea fowl, ducks, geese, quails, pigeons, pheasants, partridges and ratites reared or kept in captivity for breeding, the production of meat or eggs for consumption, or for re-stocking supplies of game."

5. Molecular Basis for Pathogenicity

During replication ND virus particles are produced with a precursor glycoprotein, F0, which has to be cleaved to F1 and F2 for the virus particles to be infectious. This post translation cleavage is mediated by host cell proteases. Trypsin is capable of cleaving F0 for all NDV strains.

It would appear that the F0 molecules of viruses virulent for chickens can be cleaved by a host protease or proteases found in a wide range of cells and tissues thus spreading throughout the host, damaging vital organs, but F0 molecules in viruses of low virulence are restricted in their sensitivity to host proteases resulting in restriction of these viruses to growth only in certain host cell types.

Most ND viruses that are pathogenic for chickens have the amino acid sequence ^{112}R/K-R-Q-K/R-R ^{116} at the C-terminus of the F2 protein and F (phenylalanine) at residue 117, the N-terminus of the F1 protein; whereas the viruses of low virulence have sequences in the same region of ^{112}G/E-K/R-Q-G/E-R ^{116} and L (leucine) at residue 117. Some of the pigeon variant viruses [PPMV-1] examined have the sequence ^{112}G-R-Q-K-R-F ^{117}; but give high ICPI values. Thus there appears to be the requirement of at least a pair of basic amino acids at residues 116 and 115 plus a phenylalanine at residue 117 and a basic amino acid [R] at 113 if the virus was to show virulence for chickens.

6. Consideration of new OIE definition

The new OIE definition is in two parts [see section 3 above]. The first part is comparable to the current EU definition [section 2] but differs in two respects. Firstly "is a disease" is used in place of "is an infection" and secondly the ICPI value is 1.2 instead of 0.7.

The use of the word "disease" can be questioned. Some species of birds may be infected with ND viruses virulent for poultry but show no clinical signs. Nevertheless these birds may excrete virus and represent a threat to susceptible poultry and other birds. Similarly normally susceptible birds which have antibodies to ND virus may be infected and excrete virus without exhibiting clinical signs. Therefore the use of the phrase "is a disease" is misleading.

In considering the higher ICPI value, it was noted that Article 5 of Directive 92/66 gives a derogation allowing lesser control measures for vaccine viruses with ICPIs in the range 0.7-1.2. During the late 1980s live vaccines, based on the

La Sota strain which had ICPI values >0.7 were being used in the EU, but these have largely disappeared [although two viruses, demonstrably related to La Sota with ICPI values >0.7<1.2 were isolated in Germany between 1991-96] following the restriction on vaccines with ICPI >0.4 imposed in the EU [Commission decision 93/152 of 8. February 1993 laying down the criteria for vaccines to be used against Newcastle disease in the context of routine vaccination programmes]. Some viruses of the so-called pigeon variant PPMV-1 (or NDV) have shown ICPI values <1.2, but these values have increased on passage through chickens and these viruses are genetically similar to virulent ND viruses. The Committee considers that there was no justification for increasing the EU definition from ICPI 0.7 to ICPI 1.2 and that the derogation in Directive 66/92 should be deleted.

The Committee also considers that the use of the undefined term "poultry" in the OIE definition is unsafe as it is interpreted to include or exclude different birds by different groups. It is suggested that the current EU definition of poultry be used [see section 4] but instead of including a list of types of birds in the definition, it should be extended to all birds reared or kept in captivity for breeding, the production of meat or eggs for consumption, the production of other commercial products or for re-stocking supplies of game. It was pointed out that this would include endangered species kept in captivity for breeding purposes, but derogations to the control measures imposed in such cases could overcome this problem.

7. *In vitro* tests for virulence

A range of *in vitro* tests relating to virological properties have been used in the past to estimate virulence for chickens. Many of those employing conventional virological techniques merely measured phenotypic differences between a limited number of virulent and avirulent tested and could not be applied universally to estimate virulence. The ability of viruses to produce plaques or other cytopathic effects in cell cultures is a useful tool for detecting the presence of virulent virus, but those employing this technique in their laboratories report it to be unreliable. Similarly, monoclonal antibodies, either individually or in panels, may be employed to identify viruses and give a good indication of their likely virulence, but these really complement rather than replace *in vivo* tests for assessing virulence.

All the evidence available at present indicates that the presence of multiple basic amino acids at the C-terminus of the F2 protein and phenylalanine at the N-terminus of the F1 protein is directly related to the virulence of the virus for chickens. The Committee considered this represents the only valid *in vitro* method for determining virulence of ND viruses at present. The amino acid motifs associated with virulent viruses to date are: ^{112}R-R-Q-R-R-F ^{117}, ^{112}G-R-Q-K-R-F ^{117}, ^{112}R-R-Q-K-R-F ^{117}, ^{112}K-R-Q-K-R-F ^{117}, ^{112}R-R-K-K-R-F ^{117}. While the evidence points to an absolute correlation between virulence for chickens and the presence of phenylalanine at position 117, it is felt that since it is most probable that the requirement is for an amino acid motif that is recognised by a ubiquitous host protease, it is necessary to define more than a single amino acid. If the F protein cleavage site sequence is to be used for detecting the presence of virulent virus the minimum requirement should be "at least 3 basic [lysine or arginine] amino acids between amino acid residues 113 to 116 and phenylalanine at residue 117". Further, the detection of a virulent amino acid motif at the F0 cleavage site should only be used as confirmation of the presence of virulent virus and failure to detect the motif or detection of a sequence usually associated with viruses of low virulence for chickens does not confirm the absence of virulent virus. This is in accord with the thinking behind the new OIE definition.

Several techniques have been used to detect the motif at the F0 cleavage site. These have included, RT-PCR, RT-PCR and sequencing, antipeptide antibodies and recombinant phage antibodies. At present none of these can guarantee detection of all virulent ND viruses. It is felt that detailed definition of the techniques to be used is not necessary, since these will only to be used for confirmatory purposes. It is up to the National Laboratory to agree with the authorities in each country what tests could be used and to what standard. Probably the quickest *in vitro* test for detecting virulent virus reported to date is RT-PCR directly on tissues [Kant et al 1997]. At present such RT-PCR techniques are not as sensitive as virus isolation in eggs, but it is foreseeable that they may eventually be sufficiently refined to identify the presence of virus even when it is undetectable by isolation in eggs. At present it is necessary to run virus isolation in parallel and this was felt to be likely to continue to be a requirement for confirmation, epidemiological purposes and for future reference.

8. References

- Council Directive 92/66/EEC; Official Journal, L 260 of 05.09.1992 p. 1
- Council Directive 90/539/EEC; Official Journal, L 303 of 31.10.1990 p. 6
- Commission Decision 93/152/EEC; Official Journal L 59 of 12.03.1993 p.35
- International Animal Health Code - Mammals, Birds & Bees 6th Edition. (Paris, Office International des Epizooties), 1992.
- Kant et al., 1997; Avian Pathology 26 837-849

9. Recommendations

The Scientific Committee on Animal Health and Animal Welfare makes the following recommendations:-

I **.** For the purposes of Newcastle Disease notification, it is imperative to define "poultry". The following definition is recommended.

- Poultry are all birds which are reared or kept in captivity for breeding, the production of meat or eggs for consumption, the production of other commercial products or for restocking supplies of game.

The Committee recognises that this definition would include endangered species kept in captivity for breeding purposes and suggests some derogation to the slaughter policy should be applied to such birds.

II. The Committee recommended the definition of Newcastle disease should be as follows:

- "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.

III. The Committee recommends that the derogation for vaccine viruses specified in paragraph 3 Article 5 of Directive 92/66/EEC should be removed.

Acknowledgements

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The working group was chaired by Dr D.J. Alexander. The members of the group are listed below.

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