VIRULENT NEWCASTLE DISEASE IN AUSTRALIA
WHAT DID WE LEARN? WHAT CAN WE EXPECT?

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Summary

Australia experienced outbreaks of virulent Newcastle disease (ND) in chickens in New South Wales in 1998, 1999 and 2000. Genetic analysis of the outbreak viruses demonstrated that they were of Australian origin, evolving, in fact, from a low virulence virus known to be quite widespread in poultry, at least in NSW. The outbreaks of virulent disease were controlled using a "stamping out" program supported by targeted use of ND vaccines, and the disease has not recurred. However the so-called progenitor virus, i.e. the virus, from which the virulent virus arose, is still present in poultry. This allows the possibility, if conditions are favourable, for the re-emergence of virulent virus and disease. Options for control of re-emergent virulent ND are discussed.

I. INTRODUCTION

Australia experienced virulent outbreaks of Newcastle disease (ND) in and near Melbourne in 1930 and 1932 (Johnstone, 1933; Albiston and Gorrie, 1942) with stamping-out programs being used on both occasions to eradicate the disease. These outbreaks occurred soon after the initial description of the disease by Kraneveld (1926) and Doyle (1927) and were confirmed by laboratory testing (Doyle, 1933). The virus that caused these outbreaks is called the Albiston-Gorrie (AG) (or Australia-Victoria (AuV)) strain and is held in various ND virus repositories around the world. Australia's national poultry flock was considered to be free of NDV infection from 1933 until 1966 when Simmons (1967) reported the isolation of an non-virulent strain of the virus, designated V4, from chickens in the state of Queensland. Serological testing subsequent to this isolation showed that infection was widespread throughout the country, though there was no evidence of a history of ND-like disease in these serologically positive flocks (Anon 1966). An earlier serological survey in 1964 of 1425 sera collected from 17 hatcheries in four states of Australia had revealed no detectable antibody to the virus (French, 1964). This information, together with other investigations of flocks and hatcheries, led McIntosh (1964) to conclude that the Australia national poultry flock was free of infection with NDV at that time. However, there are some doubts about the validity of this claim given current perspectives of statistically based sampling. Nevertheless this information provides a convenient starting point (i.e. mid 1960's) for this paper.

Further isolations of the virus were made in subsequent years (Westbury and Geering, 1977) though all these viruses were demonstrated to be, or were considered to be, non-virulent, like V4 following non-parenteral infection of chickens (Hall et al., 1967; French et al., 1967; Kim et al., 1978; Westbury, 1979; Hooper et al., 1999). This established the myth in Australia that all Australian isolates during this period were V4. This was, in fact, not the case as both Turner and Kovesdy (1974) and Westbury (1979) demonstrated variation among these non-virulent virus strains in some of their biological characteristics.

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Isolates of NDV able to induce respiratory disease in experimentally infected chickens were detected in Australian poultry during the 1980's and 1990's (Hooper et al., 1999; Hansson et al., 1999) though not all viruses isolated during this time behaved this way (Hooper et al., 1999). These low virulence strains induced lesions in the respiratory tract qualitatively the same as those induced by three commercial La Sota vaccines (Hansson et al., 1999).

The ND situation in Australia up to 1998 can thus be summarised as follows:
- Australian poultry free of infection with NDV as judged by a serological survey in 1964.
- Non-virulent virus (strain V4) detected in chickens in 1966 and infection with NDV found to be widespread, but no ND-like disease.
- Emerging diversity among Australian isolates from 1967 to the early 1980's detected, as judged by nucleotide sequencing of the genes encoding the HN and F proteins, and testing of some biological characteristics, though the virus continued to be non-virulent.
- Detection from mid 1980's, or thereabouts, of isolates able to induce respiratory disease in chickens equivalent to that induced by typical lentogenic strains of the virus e.g. LaSota.
- Increased concern in the poultry industry during the 1990's about the impact strains able to induce respiratory disease were having on chicken farming, particularly their role in the so-called "late respiratory disease". This is a complex respiratory disease of older broilers seen in some parts of the country during summer months.
- Continued concern that the presence of endemic strains of the virus would severely compromise attempts to control incursions of virulent ND.

II. OUTBREAKS OF VIRULENT NEWCASTLE DISEASE

Outbreaks of virulent ND occurred in the Sydney, Mangrove Mountain and Tamworth areas of NSW between 1998 and 2000. An official ND control and eradication scheme was implemented to control and eradicate the disease. This involved the compulsory slaughter of chickens on affected premises, the depopulation of chickens on many farms through orderly abattoir processing, controls on the movement of poultry and poultry products, the disinfection of premises, and, finally, the use of ND vaccines. The control program caused severe financial loss and hardship to the NSW poultry industry, and, as a result, there was much critical comment and discussion about the effectiveness of the disease control strategy used, and the implementation of the strategy. This paper will not add to that commentary but rather concentrate on the origin of the virulent viruses responsible for the outbreaks.

Planning for emergency outbreaks of virulent Newcastle disease (ND) in Australia has been done over many years on the supposition that such an outbreak would be caused by an exotic strain derived from a foreign source. It was therefore a complete surprise to virologists when genetic analysis of the virus responsible for the 1998 outbreak of virulent ND, and subsequent outbreaks, indicated that it was an Australian derived virus (Gould et al., 2001a). Indeed, phylogenetic analysis indicated that the virulent viruses causing the outbreaks were very closely related to a low virulence ND virus strain called Peat's Ridge, and, most likely, derived from it. The term *progenitor strain* is sometimes used to describe this virus strain. The Peat's Ridge virus was, in turn, closely related to two other viruses (NSW 12/86 and Qld 1/87) isolated in Australia in 1986 and 1987 (Gould et al., 2001a). The strains have a family lineage or tree that can be defined by genetic analysis of at least two genes of the ND virus called the HN and F genes. The NSW 12/86, Qld 1/87, Peat's Ridge and the outbreak virulent viruses belong to the HN/9 lineage based on HN gene sequence analysis, and are similarly grouped on the basis of their F gene relationship.
Sequence analysis of a specific section of the F gene also provides a means of predicting whether a ND virus is virulent or not virulent. This is because the F protein of ND virus must have a specific amino acid (the building blocks of proteins) sequence at the so-called cleavage site of the F protein for the virus to be virulent. If it does not have this amino acid sequence (the amino acid sequence can be predicted from the gene sequence) then the virus is not able to induce virulent disease in chickens. Thus sequence analysis of the F gene is a powerful tool because it provides information about relationships among ND virus strains as well as the ability to predict the capacity of a virus strain to cause disease. Analysis of a large number of Australian ND virus strains has allowed Gould et al. (2001a), Gould et al. (2001b) and Stevens et al. (2001) to cluster Australian ND virus strains into a number of lineages, such as HN/45, HN/14, HN/9, or HN/7, etc., and to determine that within at least some of these lineages that there is phenotypic (e.g. pathotype) variation. Thus virulent and non-virulent viruses, and virus strains that are in the transitional stages between non-virulent and virulent have been demonstrated within the HN/9 lineage. Virulent virus has also been detected as a sub-population within viruses in the HN/7 lineage (AR Gould unpublished data). Indeed genetic analysis demonstrates that all so-called ND virus strains are, in reality, mixtures of closely related viruses, within which there will be a predominant phenotype, but also a collection of sub-ordinate phenotypes. The idea of mixtures of viruses within a particular virus strain is embraced within the general concept of viral quasi-species (Eigen 1993), a concept that provides a more expansive and dynamic view of viral populations (particularly viruses that have RNA as their genetic material). The quasi-species idea implies that any ND virus strain actually represent a heterogenous population of viruses, with the major phenotype within the population being determined by evolutionary selection pressures within the environment within which the virus is growing eg: a chicken. How does this occur?

III. VIRUS REPLICATION

This paper was “spell-checked” by a computer program before it was submitted for publication in an attempt to ensure that random errors in keyboarding and inadvertent errors in grammatical style were reduced to a minimum. Viruses, particularly those that use DNA to encode their genetic inheritance, also proof read during replication in an attempt to ensure that the progeny of a replication cycle or cycles, are identical or near identical to the original virus. Viruses that use RNA as their genetic material, such as ND virus, have no such proof reading capacity and so when they multiply, random errors occur in the sequence of the replicated RNA of progeny viruses. This ensures genetic diversity by creating swarms of ND viruses that vary randomly and slightly from the original virus. Some of these random changes will confer no survival advantage or disadvantage, some will be inimical to the virus, while others may confer a survival advantage on the progeny viruses, under given sets of circumstances. Thus the virus population arising following replication of the virus will consist mainly of the virus sub-populations within the swarm that are best adapted to that environment, as in any evolutionary process. Thus the phenotype, or biological characteristics, of a ND virus strain can be altered by selection pressures that confer advantage to variants within the population swarm. That selection pressure allows advantaged members of the swarm to out-compete the more disadvantaged members, and consequently become the predominant phenotype. Conversely if the selection pressures remain static then the structure of the population remains essentially unchanged. These twin phenomena of viral genetic diversity created by lack of proof reading, and evolutionary selection for fitness to survive, underpin the concept of viral quasi-species. Can the quasi-species concept be used to better explain and understand what happened with ND virus in Australia?
IV. NEWCASTLE DISEASE VIRUS IN AUSTRALIA – THE QUASI-SPECIES CONCEPT IN ACTION?

The situation with ND virus in Australia up to 1988 as described in the introduction to this paper, and the subsequent outbreaks of virulent ND caused by Australian derived virus, suggest a slow evolution of virulence characteristics in our ND viruses. This began in the 1960’s with the detection of non-virulent viruses, followed by the appearance of ND viruses able to induce mild respiratory disease in the 1980’s and eventually the detection of virulent virus in 1998. Molecular epidemiological studies have demonstrated that the changes in the virus genome necessary to drive this evolution have slowly accumulated within the genome over this period and that this has occurred within defined lineages of Australian viruses eg: the HN/9 and the HN/7 lineages or groups. The changes occurred in endemic strains that naturally circulated in the Australian poultry population, not as a consequence of the sudden appearance of exotic (foreign) strains of the virus. Molecular studies also point to the fact that these changes have not arisen because some Australian ND viruses lineages are inherently “unstable” and prone to mutation. Rather the changes have occurred at random and slowly accumulated, as quasi-species theory would suggest, as it is chance that determines whether a change in the genome impacts on the phenotype (virulence) of the virus, and then whether this confers some selective advantage. The situation of the molecular evolution of ND virus within Australia is the best recorded example anywhere in the world, in my opinion, of the emergence of virulence within a native population of ND viruses.

The quasi-species concept also requires selection for fitness, implying that something changed in the poultry world to assist the emergence of disease causing ability within our ND viruses. Those concerned with poultry health have conjectured about what may have driven or influenced this process. This conjecture includes:

- the role of international breeds of meat and egg-laying chickens that have replaced local breeds in the national marketplace during this time
- ongoing intensification of the integrated poultry industry, though many poorly managed multi-aged egg-laying farms remain on the fringe of large cities, particularly Sydney
- the appearance of virulent Marek's disease virus in Australia. Proponents of this idea suggest that the immunosuppressive impact of such strains in some way encouraged to emergence of pathogenic strains of ND virus.
- natural infection with endemic strains of NDV continued to induce immunity in chickens, a process that may have encouraged the selection of strains better able to survive in the presence of such immunity

There may be other possibilities.

Can the quasi-species concept also help explain the small numbers of chickens that developed clinical signs of ND on some farms infected with virulent virus. This puzzled many involved in the outbreak, to the extent that there were concerns expressed by some people about the real significance of the virulent virus as a disease causing agent. This issue is quite complex, largely because we have little to no information about the prior exposure of chickens on some of these farms to ND virus. Without the information it is difficult to assess what the real susceptibility of these flocks was to challenge with virulent virus. Nevertheless on a significant number of farms in was possible to detect a mixture of virus isolates within the same flock. Thus Peat’s Ridge virus, virulent virus and strains with a transitional
genotype between low virulence and full virulence were detected on the same farm. The pattern of detection was that the virulent virus was detected in the flock following the earlier detection of Peat's Ridge virus in that flock i.e. in a sequential study of the viruses circulating on the farm. Thus it is conceivable that on farms where there were a mixture of viruses that some birds, and perhaps most, were exposed to virus strains that caused mild disease, but induced immunity to subsequent infection with virulent virus. Other birds, however, might be exposed only to virulent virus, and so develop virulent disease. This might be particularly so if the transitional virus strains, and the virulent viruses, evolved during the multiple replication cycles of the virus that occurred within the one flock following its initial infection with the Peat's Ridge virus. Does this represent quasi-species in action?

V. THE FUTURE

Viruses from which virulent ND virus evolved or emerged are still present in the Australian poultry industry – they were detected a number of times during the first six months of 2001. The theoretical possibility of outbreaks of virulent ND, predicted following the first detection of ND viruses that had accumulated genomic changes in critical segments of the viral genome such that full virulence could easily develop, became reality in 1998. Whether the separate outbreaks of the disease in Sydney, Mangrove Mountain and Tamworth resulted from the spread of a virulent virus between these sites, or resulted from the emergence of virulent virus in each region or farm, or from a combination of such events, is not known. What is known is that the progenitor virus was present in each of the outbreak areas, and therefore there was the potential for virulent virus to evolve in each region. This is, perhaps, irrelevant since it is perfectly feasible that virulent virus could emerge again, given sufficient time and favourable conditions (whatever they may be). The presence of virulent virus within Australian ND viruses has now been detected in two Australian ND virus lineages (HN/9 and HN/7), although only viruses within HN/9 have actually caused virulent disease outbreaks at this time. Clearly there remains a risk of further outbreaks and this risk is increased the more prevalent viruses that require fewer genomic changes for virulence to emerge become in the Australian poultry industry. This leads to the almost inevitable conclusion that it is necessary, in some way, to control these types of viruses (essentially low virulence viruses). Vaccination seems to be the only practical option.

VI. WHAT DID WE LEARN? WHAT CAN WE EXPECT?

The poultry industry and community experienced the largest emergency animal disease control effort in the history of Australian animal health. All involved with disease control in the poultry industry learnt from the experience and it is likely that a future control program would be different. Indeed this experience is being used to refine and further develop plans for national emergency animal disease control programs, in particular the role that vaccination can have and should have in emergency animal disease control. Issues such as the biosecurity of farming enterprises have also become paramount.

The outbreak also provided a unique insight into the biology of ND virus and, in particular, the real threat of the emergence of virulent ND viruses from populations of naturally circulating endemic low virulence ND viruses. The Australian poultry scene has, perhaps, been a giant field laboratory in which the virus has had the opportunity to develop and evolve over many years. Newcastle disease virologists now include emergence as a means by which outbreaks of virulent ND can arise (Alexander, 2000). This requires animal health professionals to think about the need for – and the necessity of – disease control strategies when there is a background of the presence of potentially virulent virus in a
livestock population. The situation with virulent avian influenza in turkeys in Italy (Banks et al., 2001) provides considerable support to this need.

What can we expect if the present situation with regard to ND viruses remains the same? More outbreaks of virulent ND, in an indeterminate timeframe.

REFERENCES


