

# Thermostable Newcastle Disease Vaccines

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## *Abstract*

Robust vaccines are required to protect village chickens against Newcastle disease. Thermostable vaccines derived from avirulent Australian strains of Newcastle disease virus (strains V4 and I-2) have proved successful for this purpose. These vaccines have been developed through ACIAR projects and have been adopted by other international aid agencies. It is now possible to offer integrated workshops in which administrators and field workers are trained in the use of thermostable vaccines, poultry-specific extension activities and gender aspects of poultry production. At the same time, laboratory workers are taught the skills required to produce and test thermostable vaccine on a small scale, and to assure the quality of the product. Vaccine seed material is supplied without cost. Recent events in Australia have led to some concern among potential users of the vaccine. Virulent strains of Newcastle disease virus have apparently risen from the avirulent strains that have been circulating in Australia for at least 30 years. Some have suggested that the virulent strains of Newcastle disease virus first recognised in 1926 were derived by a series of mutations from pre-existing avirulent viruses. The virulence of strains of Newcastle disease virus is currently judged by the sequence of amino acids at the cleavage site of the viral fusion (F) protein. Work in several laboratories has indicated that the sequences in V4 and I-2 are similar to those in other mild vaccine strains. One mesogenic vaccine strain used in developing countries has the same sequences as velogenic viruses. It is also argued that the Australian 'virulent' viruses do not produce a disease with high mortality and would be insignificant pathogens compared with Newcastle disease viruses that circulate in Africa and Asia. Thermostable Newcastle disease vaccines, locally produced and widely distributed, would allow village chickens to contribute fully to alleviating poverty and improving nutrition in rural areas. Suitable systems of extension and cost recovery would make the enterprise sustainable.

MOST people attending this meeting would agree that village chickens are a very important resource for populations in rural and peri-urban areas in developing countries. Most would agree that village flocks are not managed to produce at their full potential. In most cases, the major constraint to enhanced productivity is the viral disease known as Newcastle disease, which devastates poultry populations in developing countries. Commercial vaccines and attention to biosecurity are important steps in the control of Newcastle disease in commercial chickens. Only over the past 10 or 15 years have we been able to contemplate the control of Newcastle disease in village chickens. The key has been the production of vaccines that are cheaper, less complex

and more robust than commercial Newcastle disease vaccines.

The problems have been many. The flocks are small, scattered and multi-aged. The owners of the chickens (often women) lack economic or political influence, and veterinary and extension services are seldom responsive to their needs. Commercial vaccines are not suitable for use in resource-poor villages. Individual vials contain at least 1000 doses (economies of scale), the vaccines are heat-labile and require continuous refrigerated storage and if imported they are a drain on foreign exchange.

The author believes that these problems can be overcome, and bases this assertion on his experience with control of Newcastle disease since 1984. In many places, it is not yet feasible to construct cold chains to link vaccine producers to village flocks, so thermostable vaccines are required. Not all the chickens can be caught readily for vaccination, so

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the vaccines must be able to spread between chickens, and to be given on food if necessary. Local production of vaccine will reduce the demand on foreign exchange. Vaccination packages are required that include vaccine production, training at all levels and effective and specific extension programs.

The present paper will address the virological aspects of these vaccination packages. It will trace the development of thermostable vaccines and indicate the several countries where these vaccines have been successfully tested and the few where they are in general use. The difficult transition from successful trial to implementation needs to be explored. The paper will also touch on two safety issues that have recently arisen. One concerns the recent occurrence of clinical Newcastle disease in Australia, probably the result of evolution of Australian viruses. The other is the view of the author that village vaccines need not be made in specific-pathogen-free eggs.

### **Newcastle Disease Viruses in Australia**

Newcastle disease was first recognised and the disease named in 1926. Some have argued for an earlier origin. However, the virus spread very widely and reached Australia in the early 1930s. Classical veterinary control measures, with diagnosis, slaughter and movement control, sufficed to eradicate the disease. The causal virus was isolated and maintained. It is still available within laboratories.

There were no further occurrences of clinical Newcastle disease and it caused some surprise when Newcastle disease virus was isolated from a healthy domestic chicken in Queensland in 1966 (Simmons 1967). The virus, designated V4, proved to be unusual. It was not pathogenic for chickens and failed to kill chick embryos. It was soon shown that avirulent viruses of this type were widespread in Australia.

V4 virus was relatively thermostable and it was demonstrated in the author's laboratory and in Malaysia that it would respond rapidly to selection for enhanced thermostability. V4 was soon being used as a vaccine, in its native form in commercial chickens and in its heat-resistant form in village chickens. The heat-resistant variant is now the basis of vaccines for commercial chickens. Early stocks of the V4 vaccine were held for possible emergency use in Australia and they were sold commercially in Asia and Africa. The heat-resistant V4 (NDV4-HR) has been used in recent control operations in Australia.

The author had warned the commercial poultry industry in Australia that an incremental increase in virulence of endemic strains of Newcastle disease virus was probably a greater risk than the entry of virulent exotic strains. An examination of 45

contemporary isolates of Australian viruses failed to demonstrate any pathogenicity for chickens. One of the viruses in this collection was the strain designated I-2 (Spradbrow et al. 1995).

In 1999, there were reports of the association of Australian strains of Newcastle disease virus with late respiratory disease in broiler chickens. Shortly after there were outbreaks of clinical Newcastle disease which triggered initially a test and slaughter response and later a salvage and vaccinate response. Viruses with biochemical markers for virulence (see later) were isolated. Details of these outbreaks have yet to appear in the scientific literature. It is difficult to establish what the Newcastle disease specific mortality was, or how the virus affects laboratory chickens when it spreads by contact.

Reports to *Office International des Epizooties* (OIE), placed on the Internet by ProMED, are available. They indicate that, at least for some outbreaks, morbidity was extremely low and there was no mortality. However, by the new OIE standards, the virus was virulent.

### **Thermostable Newcastle Disease Vaccines in Developing Countries**

Almost since its foundation in 1984, ACIAR has had an interest in village chickens. Dr John Copland became convinced in the early 1980s that the control of Newcastle disease was an essential start to the improvement of productivity in village chickens. He brought together the author's group from the University of Queensland and Professor Latif Ibrahim's group at the Universiti Pertanian Malaysia to consider the problem. It was decided that a possible approach would be to develop a thermostable vaccine from the Australian V4 strain and to exploit its ability to spread between chickens. Because village chickens were seldom housed in Malaysia at that time, it was decided to produce a vaccine that could be given on food if necessary.

The author has reviewed the ensuing literature on thermostable Newcastle disease vaccines, which is now extensive (Spradbrow 1993-94). An update of this literature was placed on the website of the FAO electronic conference *The Scope and Effect of Family Poultry Research and Development* (Spradbrow 1999). The permanent site of this information is <http://www.fao.org/waicent/faoinfo/agricult/aga/agap/lps/fampo/fampo.htm>.

Only a summary need be given here. The thermostable vaccines, either NDV4-HR or I-2 have been tested successfully in many countries in Asia and Africa. Eye drop vaccination is most effective but some suitable food carriers have been identified. These can be used where it is impossible to catch

chickens, or where there is a strong local preference for food vaccination for other reasons. A few countries have proceeded to local production and distribution of thermostable vaccines. Others would like to follow this path and seek guidance.

Laboratory protection trials with thermostable vaccine have been almost universally successful. Countries commencing to produce vaccine can probably dispense with these. Thermostable vaccines, if properly applied, are protective. The procedure that will require experimentation is the field delivery of vaccine. The response to vaccination can be judged adequately by the serological response of the vaccinated chickens. There is no call for further challenge trials. Countries wishing to use food vaccine will have to validate suitable food carriers.

Many of the research requirements have been completed. Those that remain are very practical—how to optimise vaccine yields, how to determine potency rapidly, what are suitable quality assurance standards, what are the best diluents for maintaining thermostability? Future assistance packages will have large training components and small research components.

The initial ACIAR training workshop established a format that, with modifications, has been successful in other countries and for other agencies. One workshop is held for administrators and extension workers. This covers Newcastle disease, Newcastle disease vaccines, village chickens, extension methods, gender aspects of poultry production and ethnoveterinary medicine. A parallel workshop for laboratory workers teaches the skills needed to grow Newcastle disease vaccine in embryonated eggs, to harvest vaccine, to titrate Newcastle disease virus and to measure antibodies to Newcastle disease virus. Fortunately, the egg work and the serology are relatively simple so these activities can be undertaken in many laboratories in developing countries. To date workshops have been held in Pretoria (for ACIAR), Dar es Salaam (for ACIAR), Accra (for GRM International and World Bank), Yangon (for FAO), Phnom Penh (for FAO) and Thimphu (for AusAID). An extended workshop is being conducted in Brisbane in 2000 as part of a fellowship scheme for IAEA. Workshop manuals were prepared and have been revised for subsequent workshops (Alders and Spradbrow 1999; Spradbrow et al. 1998).

As part of the laboratory course, master seed material of I-2 vaccine is made available without cost. At some workshops, working seed and even vaccine is then produced. The intention is that laboratories will be able to make thermostable vaccine in some form in embryonated eggs. Often these will be commercial hatching eggs, already in use for making other vaccines. Some laboratories in developing countries have facilities for freeze-drying

vaccines. Others will produce 'wet' (that is liquid) vaccines, which will need to be distributed within a few weeks.

Distribution networks for vaccine will need to be established. These are discussed elsewhere in this volume. Extension activities that specifically target village poultry will be required. Vaccinators will need to be trained at farm or at village level. For vaccination projects to be sustainable, systems of cost recovery must be developed.

### **Avirulence, Virulence, Reversion to Virulence**

All strains of Newcastle disease are serologically similar when examined with polyvalent antiserum. The use of monoclonal antibodies does allow fine distinctions to be made. However, strains of Newcastle disease virus vary enormously in virulence, which is a measure of the degree of pathogenicity. Virulent strains of Newcastle disease virus will kill most of the chickens that they encounter. Strains of low virulence may produce disease in some chickens but no deaths.

Three useful terms were introduced to indicate the virulence of Newcastle disease viruses.

VELOGENIC viruses were highly virulent and might have tropism for respiratory and nervous tissues — NEUROTROPIC VELOGENIC — or for visceral organs — VISCEROTROPIC VELOGENIC.

MESOGENIC viruses were moderately virulent.

LENTOGENIC viruses were of low virulence.

Some added a fourth category, AVIRULENT, to cover virus strains like V4 and Ulster which do very little damage.

OIE unfortunately has ceased to recognise these terms.

The writer believes that the virulence of strains of Newcastle disease virus should always refer to their effect on chickens. The commonsense approach is that virulent viruses cause clinical disease and mortality. Alexander (1996) has pointed out the complications that arise when mild strains or even vaccine strains of Newcastle disease virus are isolated from clinical disease that is exacerbated by co-infecting microorganisms or environmental factors. Laboratory tests were required that might indicate the potential virulence of a strain of virus.

Three tests came into accepted use. The mean death time is measured in embryonated eggs from a specific-pathogen-free source inoculated by the allantoic cavity route with a minimal lethal dose of virus. Velogenic viruses killed in under 60 hours and mesogenics in 60 to 90 hours. Avirulent viruses fail to kill. Another test measures the reaction of one-day-old specific-pathogen-free chicks to intracerebral

injection of virus. The result is converted to an index (ICPI or intracerebral pathogenicity index) that varies from zero to a maximum of 2.0 for velogenic viruses. A similar index (IVPI or intravenous pathogenicity index) with the values 0 to 3.0 is calculated after intravenous inoculation of specific-pathogen-free chickens at 6 weeks of age.

Recently, some of the molecular determinants of virulence in Newcastle disease virus have been determined. Most attention has been devoted to one of the surface proteins of the virus particle, a protein known as the fusion (F) protein that is concerned with the entry of virus into cells. Viral genes code for an inactive form of this protein, referred to as F<sub>0</sub>. The virus particle will not be infective until this inactive protein is cleaved into two portions F<sub>1</sub> and F<sub>2</sub> that combined have fusing ability. Cleavage is achieved by host nucleases that act on a specific cleavage site. For some strains of Newcastle disease virus, a particular protease is required. Infectious virus is produced only when suitable cells are infected. In the chicken, these viruses are restricted to the cells of the enteric and respiratory tracts and little or no disease results. In embryos inoculated in the laboratory, the virus is restricted to the layer of cells lining the allantoic cavity. The embryo itself is not infected and survives. Other strains of Newcastle disease virus have an F<sub>0</sub> that is readily cleaved by many proteases. These viruses can colonise a wide variety of cells and spread throughout the host, killing chickens and embryos.

Thus, the chemistry of the fusion site of the F<sub>0</sub> protein is probably important in determining virulence of Newcastle disease viruses. The sequencing of viral genes is now a relatively simple procedure, and from the nucleotide sequence of the gene, the amino acid sequence of the protein that is that gene's product can be deduced.

Biochemists use a simple alphabetical code to indicate a particular amino acid. Those of relevance now are:

**R arginine;**

**K lysine.**

These are in heavy type because they seem to be part of a virulence motif when they occur against the cleavage site at the end of the F<sub>2</sub> portion of the F<sub>0</sub> protein. They are two of the three basic amino acids:

Q glutamine;

G glycine;

E glutamic acid;

L leucine.

These are written here in normal type, as they seem to have no effect on virulence.

**F phenylalanine.**

This is also written here in heavy type. It is a virulence indicator when it occurs immediately after

the cleavage site, as the first amino acid of the F<sub>1</sub> protein. It is not a basic amino acid. Avirulent viruses have leucine at this site.

Collins et al. (1993) studied this area of protein in 26 Newcastle disease viruses. There is now substantial unpublished work on many recent Australian isolates.

The fusion site is at an R (arginine) residue at the 116<sup>th</sup> amino acid on the F<sub>0</sub> protein. This is present in all strains of the virus.

Most virulent viruses have the following sequence:

112 **R** or **K-R-Q-K** or **R-R** 116 117 **F**

There are four basic amino acids at residues 112, 113, 115 and 116, and phenylalanine at 117 after the cleavage site.

Avirulent viruses are different. Collins et al. (1993) gave this general scheme

112 **G** or **E-K** or **R-Q-G** or **E-R** 116 117 **L**

There is evidence from Ireland (Collins et al. 1993) for the mutation of an avirulent virus to a virulent pathotype. This involved the replacement of amino acids at 3 residues (112, 115 and 117). The authors commented that this '... would not be a simple process or frequent occurrence ...'. Studies with monoclonal antibodies indicated that the virulent and avirulent viruses were similar and probably a common gene pool.

The Australian V4 strain has the low virulence sequence

112 **G-K-Q-G-R** 116 117 **L**

Strain I-2 (D.J. Alexander, pers. comm.) and strain Ulster share this configuration.

The strain known as Blacktown and associated with disease in the recent Australian outbreaks sequenced as follows, as reported by Anon (1999) in a newsletter distributed to industry.

112 **R-R-Q-R-R** 116 117 **F**

Collins et al. (1993) included some vaccine strains of Newcastle disease in their study.

The vaccine strains B1, La Sota and F had identical sequences

112 **G-R-Q-G-R** 116 117 **L**

Komarov, a vaccine still used in some countries, has the structure of a virulent virus

112 **R-R-Q-K-R** 116 117 **F**

Table 1 gives an indication of the lessons that have been drawn from these studies.

Obviously, there are factors other than the arrangement of six amino acids at a particular site that influence the virulence of strains of Newcastle disease virus. However, the technology to make these determinations has become routine but the official decision to rely on amino acid sequences may be too simplistic. An OIE Committee resolved and OIE adopted in 1999 an amended standard. Newcastle

disease was defined as an *infection* by a virus that had either

‘an intracerebral pathogenicity index of 0.7 or greater, or at least 3 basic amino acids in the position of residues 113 to 116 and phenylalanine at 117.’

**Table 1.** Amino acid sequences at the F protein cleavage site.

Residue number	Pathotype	
	High virulence	Low virulence
112	<b>R</b> or <b>K</b> (basic)	G or E (not basic)
113	<b>R</b> or <b>K</b> (basic)	<b>R</b> or <b>K</b> (basic)
114	Q (not basic)	Q (not basic)
115	<b>R</b> or <b>K</b> (basic)	G or E (not basic)
116	R (basic)	R (basic)
Cleavage site 117	F	L

There is no mention in the definition of clinical disease or death in chickens. Australian authorities also look for a basic amino acid at residue 112.

The definition assumes that all viruses of this configuration will be pathogenic. Veterinary laboratories in developing countries wishing to comply with these requirements would require a molecular biology laboratory or access to one-day-old chicks from specific-pathogen-free stock. This test also has limitations, apart from those of animal welfare. It has long been recognised (Mims 1960) that intracerebral inoculation is a crude way of introducing an inoculum to the blood stream. Only that part of the inoculum along the needle track is likely to remain in the brain. Kim and Spradbrow (1978) found moderate intracerebral pathogenicity indices (0.9 and 1.0) for strain V4, but the viral lesions were in liver and spleen, and not in the brain.

Perhaps the ability of Newcastle disease viruses to cause disease and death in the field should also be part of a consideration of virulence. Viruses with ribonucleic acid (RNA) genomes will readily produce mutants. Vaccines produced from seed lots to minimise the chance of genetic change. The strains of Newcastle disease virus that circulate in the field are not clonal (Hanson 1988). Hanson (1988) noted that isolates of Newcastle disease virus contained variants that remained hidden until their presence was revealed by applying suitable selection pressures. When pathogenicity indices or sequences or

thermal stability are measured, it is the predominant population in the mix that is being measured. Suitable selection pressures are required to reveal the variants whose presence may be required for viral persistence. The author prefers not to clone viral seed lots, to preserve any heterogeneity that may be important for immunity. Selection pressures can be controlled in the vaccine production laboratory. Once in the chicken, the vaccine virus will be subjected to the selection pressures that also mould the wild type viruses. It is difficult to envisage that the progeny of any lentogenic Newcastle disease vaccine virus will do serious harm. They are extremely unlikely to match the pathogenicity of the virulent strains already in circulation.

### Specific-pathogen-free eggs

Modern egg-based vaccines are made from eggs derived from specific-pathogen-free (SPF) flocks. The breeding chickens are in isolated buildings, often supplied with filtered air. They are derived from parents free from a long list of pathogens (the specified pathogens), and frequent testing assures the microbiological integrity of the flock. These flocks are very expensive to maintain, and consequently SPF eggs are very expensive. The SPF birds receive no vaccines. If vaccines are made from SPF eggs, in premises that meet standards of Good Manufacturing Practice, the vaccines should also be SPF. The status of the products is compromised if they are produced in other premises.

SPF eggs were introduced to exclude from vaccines, or from progeny chickens, certain pathogens that are transmitted through the egg. Avian leukosis virus and mycoplasmas were the agents causing most concern. Infectious agents have been transmitted with SPF egg-based vaccines. Often these were agents such as reticuloendotheliosis virus or chicken anaemia virus that were newly emerging and that were not included in the list of specific pathogens. Vaccines used in commercial poultry in developed countries should be produced in SPF eggs.

Some argue that vaccines for use in village chickens should also be made in SPF eggs. The costs would be prohibitive in many countries, and the chickens would not receive vaccine. In many developing countries, any locally made avian vaccines for commercial poultry are already produced in conventional eggs. The author knows of no instances where this has been harmful. For village chickens, any risks are far outweighed by benefits. Strains of Newcastle disease virus that cause extremely high mortality circulate in their environment.

Few potential pathogens will kill a high percentage of chickens, and these can be excluded from

vaccines: virulent Newcastle disease will; *Salmonella pullorum* and some pasteurellas will; and some strains of avian influenza will. Bacterial contamination can be detected by cultural techniques. Virulent strains of Newcastle disease will kill embryos, which will not then be harvested for vaccine production. The vaccine is given usually to older chickens, and it is given on mucosal surfaces and not injected. The contaminated commercial vaccines that have caused problems have usually been injected vaccines, given to young chicks.

The risks of harming village flocks with locally produced vaccines must be balanced against the fatal outcomes of contact with circulating strains of Newcastle disease virus. If we refrain from vaccination because SPF eggs are not available, chickens will continue to die unnecessarily, and an important source of protein will be lost to villagers.

### Postscript

A long time ago in Cambridge, the author met an old man who remembered the vaccine calf being brought around English villages. The calf would have been infected with what we now call vaccinia virus, the vaccine that protected against human smallpox. Not an ideal source of vaccine, but better than bovine exudate that had been transported by other means, and safer than the transfer of lymph from a human vaccine reaction. It was the best technique available at the time. Village chickens require access to Newcastle disease vaccine now, and flock owners deserve this service. We should strive for the best remedy that can be implemented, now.

### References

- Alders, R. and Spradbrow, P.B. 1999. Newcastle Disease in Village Chickens. A Field Manual. Version prepared for AusAID workshop in Bhutan, p. 47.
- Alexander, D.J. 1996. Paramyxoviridae (Newcastle disease and others). In: Jordan, F.T.W. and Pattison, M. ed. Poultry Diseases. Fourth edition. London, W.B. Saunders, 139–155.
- Anon. 1999. Newcastle disease again threatens NSW industry. In: Bolla, G. ed. In An Eggshell. No. 7. Gosford, Australia. NSW Agriculture, 1–14.
- Collins, M.S., Bashiruddin, J.B. and Alexander, D.J. 1993. Deduced amino acid sequences at the fusion protein cleavage site of Newcastle disease viruses showing variation in antigenicity and pathogenicity. Archives of Virology, 128: 363–370.
- Hanson, R.P. 1988. Heterogeneity within strains of Newcastle disease virus: Key to survival. In: Alexander, D.J. ed. Newcastle Disease. Boston. Kluwer Academic Publishers, 113–130.
- Kim, S.J. and Spradbrow, P.B. 1978. Properties of lentogenic Australian strains of Newcastle disease virus. Veterinary Microbiology, 3: 129–141.
- Mims, C.A. 1960. Intracerebral injections and the growth of viruses in the mouse brain. British Journal of Experimental Pathology, 41: 52–60.
- Simmons, G.C. 1967. The isolation of Newcastle disease virus in Queensland. Australian Veterinary Journal, 43: 29–31.
- Spradbrow, P.B. 1993–94. Newcastle disease in village chickens. Poultry Science Reviews, 5: 57–96.
- Spradbrow, P.B. 1999. Thermostable Newcastle disease vaccines for use in village chickens. In First INFPD/FAO Electronic Conference on Family Poultry. The Scope and Effect of Family Poultry Research and Development. Free communication No. 10. <http://www.fao.org/waicent/fao-info/agricult/aga/agap/lps/fampo/fampo.htm>
- Spradbrow, P.B., MacKenzie, M. and Grimes, S.E. 1995. Recent isolates of Newcastle disease virus in Australia. Veterinary Microbiology, 46: 21–28.
- Spradbrow, P.B., Bensink, Z. and Grimes, S. 1998. Small Scale Production and Testing of Newcastle Disease Vaccine. Laboratory Manual. Brisbane, Australia. University of Queensland, p. 31.