EVALUATION OF THERMOSTABLE VACCINES AGAINST NEWCASTLE DISEASE IN VILLAGE CHICKEN USED IN TROPICS AND SUBTROPICS

ADWAR T., LUKEŠOVÁ D.

Abstract

Newcastle Disease (ND) in domestic poultry is a focus for concern throughout much of the worlds In poor rural communities because of severe economic losses that have occurred from illness. In the commercial poultry sector there are quite a number of conventional vaccines available for the control of Newcastle Disease. This has drastically reduced the incidence of Newcastle Disease in the commercial poultry farms. It is important to note here that most, if not all, of these conventional vaccines are heat labile and hence cannot be used in the rural areas since the provision of cold-chain facilities is practically impossible, coupled with the behavior of the rural scavenging chicken. An oil-adjuvant inactivated vaccine has, for some time now been used to control Newcastle Disease in rural chickens, but the vaccination coverage has always been very low because of the needed skills in its application. It is also relatively expensive with most farmers not being able to purchase it. The objectives of this study are: to evaluate ND heat resistant vaccine and if could be used successfully in a rural community (tropic and subtropic) to protect free-range chickens against ND.

Key words: immunize, heat resistant vaccines, avian paramyxovirus type 1, rural community, rural chicken

INTRODUCTION

Newcastle disease (ND) is a major constraint to village poultry production throughout developing countries, frequently causing mortality rates of 75% to 100% in unvaccinated flocks (Spradbrow, 1992). The resource derivable from the chickens cannot be fully utilized unless the disease is controlled particularly in the village poultry flocks that are believed to keep the virus in circulation and act as reservoirs and carriers to themselves and the more susceptible exotic breeds in commercial farms (Gomwalk et al., 1985). According to Jordan and Pattison (1996), ND is caused by a group of closely related viruses that form the avian paramyxovirus type (PMV-1). Nine serogroups of avian paramyxoviruses have been recognized: [APMV-1 to APMV-9] and APMV-1 remains the most important pathogen for poultry while others are known to cause disease in poultry and other types of birds (Alexander, 2003).

The incubation period of ND after natural exposure has been reported to vary from 2 to 15 days. The time for appearance of the symptoms varies, depending on the infecting paramyxovirus, host species and its age and immune status, infection with other organisms, environmental conditions, the route of exposure, and the dose (Alexander, 2003). Transmission can occur by direct contact with feces and respiratory discharges or by contamination of the environment including food, water, equipment, and human clothing. Newcastle disease viruses can survive for long periods in the environment, especially in feces. Generally, virus is shed during the incubation period and for a short time during recovery. Some psittacine species can shed the virus intermittently for a year or more. Virus is present in all parts of the carcass of an infected bird (OIE, 2000 a). Strains of NDV have been grouped into five pathotypes on the basis of the clinical signs seen in infected chickens (Jordan & Pattison, 1996: OIE, 2000 b) – Table 1.

Chickens infected with virulent NDV may die without showing any signs of illness The chicken fluffs its feathers and appears to have its coat dragging on the ground Chickens are sleepy and do not eat . Slight difficulties in breathing during infection, Chickens show severe difficulty breathing with distress and gasping, swelling of the head and neck, marked decrease in egg production, shaking, twisted neck and paralysis of wings and legs will sometimes be seen in advanced stages of the disease, lethargy and weakness, muscle tremors, conjunctivitis and nasal discharge, sternal and lateral recumbency. (Alders and Spradbrow, 2001, Olivier, 2004). No gross lesion may be observed in many of the first birds dying in a commercial poultry operation. Per acute deaths are generally due to collapse or dysfunction of the reticuloendothelial system before discernible gross lesions have developed. There is no pathognomonic gross lesion for VVND, but, generally, sufficient lesions can be found to make a tentative diagnosis if enough birds are examined (McDaniel and Orsborn 1973).The lesions are often particularly prominent in the mucosa of the proventriculus, caeca and small intestine. The macroscopic lesions that often noticed on infected chickens are: proventricular hemorrhage, caeca tonsil hemorrhage, air-sacculitis, lung congestion, nephritis and tracheitis. Air-sacculitis may be present even after infection with relatively mild

strains, often observed in association with secondary bacterial infections. However, the characteristic signs and lesions associated with the virulent pathotypes will give rise to strong suspicions of the disease (Alexander, 2003).

In the laboratory, virus isolation is attempted by inoculating 9- to 11-day-old embryonating chicken eggs. Chorioallantoic fluid (CAF) is collected from all embryos dying after 24 hours post inoculation and tested for hemagglutination (HA) activity. If positive, the hemagglutination-inhibition (HI) test is used with known NDV-positive serum to confirm the presence of NDV in the CAF (Beard, 1989).

Recommendations for the control and eradication of Newcastle disease include strict quarantine, slaughter and disposal of all infected and exposed birds, and disinfection of the premises. The reintroduction of new birds should be delayed for 30 days. Pests such as insects and mice should be controlled, human traffic should be limited, and the introduction of new animals with unknown health status should be avoided. Vaccines are available, though they may interfere with testing. Effective disinfectants include the cresylics and phenolics (OIE ,2000a).

People can be infected with velogenic Newcastle disease and show signs of conjunctivitis which resolve quickly, with virus shed in the ocular discharges for 4–7 days. Infected individuals should avoid direct and indirect contact with avian species during this time. Laboratory workers and vaccination crews are most at risk, with poultry workers rarely being infected. No known infections have occurred from handling or consuming poultry products (OIE, 2000a).

VACCINATION

Vaccination has been reported as the only safeguard against endemic ND (Usman, 2002). Inactivated vaccines give very good immunity without vaccinal reactions and have been widely used, but are relatively expensive and require considerable attention to training when used by non-veterinary personnel. Live vaccines are easy to apply and relatively inexpensive, and give moderately good immunity. Vaccinal reactions to them vary according to the vaccine strain. Among the live vaccines, the heat resistant vaccines require less stringent transport requirements in the field, and they have also been widely used in villages. Recombinant vaccines have the advantage that they can be serologically detected dependently of the wild virus (Bell**,** 2001) (Table 2):

Tab. 1: Five path types in infected chicken

Pathotypes	**Description**
Viscerotropic velogenic	A highly pathogenic form in which hemorrhagic intestinal lesions are frequently seen.
Neurotropic velogenic	A form that presents with high mortality, usually following respiratory and nervous signs.
Mesogenic	A form that present with respiratory signs, occasional nervous signs, but lower mortality.
Lentogenic	A form that presents with mild or sub-clinical respiratory infection
Asymptomatic enteric	A form that usually consists of a sub-clinical enteric infection

Tab.2: Summary of the advantages and limitation of the different vaccine types (Bell, 2001)

Example	**Newcavac**	**La Sota**	**Clone 30**	**I-2**	**Komarov**	**HVT/F**
Immunogenicity	Very good	Moderate	Moderate	Moderate	Good	Moderate
Vaccinal reaction	None	Moderate	Slight	Very slight	Severe	None
Ease of application	Difficult	Easy	Easy	Easy	Easy	Easy
Transportability	Good	Poor	Poor	Very good	Poor	Moderate
Previous village use	Extensive	Some	No	Extensive	Yes	No
Spread ability	No	Yes	Yes	Yes	Yes	Yes
Cost	Moderate	Low	Low	Low	Low	High

The selection of a ND vaccine for use in rural chicken will depend on the local conditions in each country. Selection criteria will include – ease of use, cost, thermostability, immunogenicity, availability and transportability. In circumstances where the cold chain is weak or absent, the only reliable option will be the use of thermostable ND vaccines; i.e. the live vaccines NDV4-HR (Ideris et al., 1987) and I-2 (Bensink and

Spradbrow, 1999), or inactivated vaccines such as ITA-NEW and Newcavac. In most cases where farmers are to contribute wholly or partially to the cost of the vaccine, the price of the vaccine will be a major factor. The lower price of the vaccine, the greater the number of farmers who will be able to afford to pay for it and, consequently, the greater the vaccination coverage. Many strains of Newcastle disease virus other than velogenic strains are used in the production of live vaccines. Eight of these strains are listed in Table 3.

Tab. 3: Eight strains of Newcastle disease virus used in live vaccines (FAO, 2002)

Strain	Description
F	Lentogenic. Usually used in young chickens but suitable for use as a vaccine in chickens of all ages.
B1	Lentogenic. Slightly more virulent than F, used as a vaccine in chickens of all ages.
La Sota	Lentogenic. Often causes post vaccination respiratory signs, used as a booster vaccine in flocks vaccinated with F or B1.
V4	Avirulent. Used in chickens of all ages.
V4-HR	Avirulent. Heat Resistant V4, thermostable, used in chickens of all ages.
I-2	Avirulent. Thermostable, used in chickens of all ages.
Mukteswar	Mesogenic. An invasive strain, used as a booster vaccine. Can cause adverse reactions (respiratory distress, loss of weight or drop in egg production and even death) if used in partially immune chickens. Usually administered by injection.
Komarov	Mesogenic. Less pathogenic than Mukteswar, used as booster vaccine. Usually administered by injection.

Thermostable live Newcastle disease vaccines

A thermostable vaccine enables distributors and users to reduce the problems associated with inadequate cold chains in the field. It is essential that users understand that a thermostable vaccine must still be treated with some of the respect due to a biological product, that is the vaccine cannot expose to sunlight and frequent shifts in temperature and still expect it to remain active (Alders and Spradbrow, 2001a)

The NDV4-HR vaccine

The heat resistant V4 (NDV4-HR) vaccine against ND has yielded encouraging results in many countries in Africa (Alders and Spradbrow, 2001a) and Southeast Asia (Spradbrow, 1993–94). NDV4-HR vaccine is a living vaccine with the following characteristics – it is thermostable, retaining its activity for 12 weeks at a temperature of 28°C in freeze-dried form (Ideris *et al*., 1987) it can be administered via eye-drop (intraocular), nose-drop (intranasal), oral drench, or drinking water; mixed with certain feeds or by injection (Spradbrow, 1993–94; Anon, 1991). Its ease of administration makes it suitable for use by village farmers; the vaccine strain can be transmitted by contact from vaccinated to non-vaccinated birds (Alders *et al*.,1994; Spradbrow, 1993–94); it is a virulent strain and can be safely administered to chickens of any age from day-old chicks to adult birds (Spradbrow, 1993–94; Anon, 1991) its biological safety is superior to that of other living ND vaccine strains such as B1 or La Sota (Anon,1991).

The ND I-2 vaccine

The Australian Centre for International Agricultural Research (ACIAR) commissioned workers at the Virus Laboratory in the University of Queensland to produce a seed virus similar to NDV4-HR that could be made available without cost to laboratories in developing countries (Bensink and Spradbrow, 1999). Forty-five isolates of a virulent ND were examined for antigenicity, safety and ability to spread. The most promising of these isolates were checked for their thermostability and the more resistant isolates selected for enhanced heat resistance. The result was strain I-2, which was amplified in eggs from a disease-free flock to form a master seed. The seed was tested for safety and for freedom from bacterial contamination.

Strain I-2 has undergone laboratory tests in several countries and has proved to be protective against local virulent strains of the ND virus (Alders and Spradbrow, 2001b). In Vietnam, after extensive laboratory and village trials, it has been officially recognized as the ND vaccine for village chickens (Tu *et al*., 1998). In Tanzania, it has given protection for at least two months after vaccination (Wambura *et al*., 2000). Field records in Mozambique indicate that I-2 ND vaccine provides approximately 80 percent protection in the field in the face of an outbreak, when given every four months via eye-drop (Alders and Spradbrow, 2001a).

ND vaccine of acceptable standard can be produced from strain I-2 in central laboratories or even regional laboratories in developing countries. The vaccine can be produced in eggs which are not specifically pathogen-free, but which come from a flock that is regularly screened for key poultry diseases. It can be produced and stored in liquid form, and suitably diluted in a protective solution such as 1 percent gelatin (in which the vaccine will maintain its activity for at least twelve weeks at 22°C; (Bensink and Spradbrow, 1999) before use. The thermostable vaccine is then best administered via eye drop. The I-2 vaccine produced in Mozambique will retain its activity for eight weeks at 28°C when freeze-dried and stored in the dark.

Administration of thermostable ND vaccines

Standard dose – as with other live ND vaccines such as La Sota, a minimum of 106 EID50/bird is required to produce an adequate level of protection. EID50 (50 percent embryo infectious dose) is a laboratory measure of the content of living infectious virus in a vaccine. It has been demonstrated that birds that received a higher oral dose of the NDV4-HR vaccine generated a higher immune response when confined in cages with wire floors (Spradbrow *et al*., 1988). This means that even though the thermostable vaccine can survive at ambient temperatures, attempts to improve its conservation will result in a slightly higher vaccine titer at the time of vaccination and consequently a higher and longer-lasting immunity. This is particularly important when birds are not housed together at night. Administration route - these vaccines can be administered via eye-drop, drinking water, certain feeds and injection. Field trials in Mozambique indicated that almost all farmers preferred eye-drop administration even though it entails the capture of birds. In their opinion, eye-drop administration produces a greater survival rate, has a lower frequency of administration and is easy. It is important that the eye-dropper used be made of virus-friendly plastic and that it is calibrated to ensure that one drop contains one dose.

Calibration of the eye-dropper and administration of the eye-drop to the bird is done with the dropper in a vertical position to make sure that drops of a uniform size are produced. Age of bird - the same dose is given to birds of all ages, from day-old chicks to adults. Vaccination schedule - for eye-drop administration, the vaccine should be administered once, with revaccination every 3–4 months. Via drinking water, the vaccine should initially be given on two occasions, 2–3 weeks apart, with re-vaccination at least every three months.

Storage and transport conditions for thermostable ND vaccines

If users have access to normal cold chain facilities, these should be used, even when dealing with a thermostable vaccine. Freeze-dried vaccine stored at 4–8°C will retain high titer for a longer period than that stored at ambient temperature. At 4–8°C, the vaccine should maintain an adequate titer for at least one year. When taking the vaccine to the field, it should be placed in a cool box with ice or an ice pack. The vaccine should not be frozen (unless the instructions specifically indicate that it may be frozen). Freeze-dried vaccine packaged under vacuum rather than with nitrogen will lose the vacuum and gain moisture if the vial is frozen. The rubber cap on the vial contracts when frozen enabling moist air to enter the vial. When this occurs, the shelf life of the vaccine is reduced. These vaccines are thermostable, but attention to the conservation of the vaccine once removed from refrigeration will ensure optimal results: the vaccine should always be kept away from sunlight. When transporting the vaccine in the field, it should be wrapped in a damp cloth and carried in a covered open-weave basket, this allows evaporative cooling which helps to keep the vaccine cool and the cover prevents contact with sunlight, the date the vaccine leaves the cold chain should be recorded as it will remain effective for 2–3 months only, the vaccine should be stored in a cool, dark location, for example, near the base of a clay water pot.

Genetic sequencing of thermostable live ND vaccines

Genetic analysis indicates a relationship between the chemical structure of surface proteins of limited areas of the genome of strains of ND virus and the virulence of these strains. An area of apparent importance is the cleavage site of the fusion protein on the surface of the virus particle. Particular aminoacid patterns around the cleavage site in virulent strains have become known as the virulence sequence. V4 and I-2 and other vaccines such as La Sota and HB1 lack the virulence sequence (Alders and Spradbrow, 2001a).

Safety issues

The avirulent live ND vaccines such as I-2 and NDV4-HR are unusual in that it is not possible to administer an overdose. They are harmless to both bird and handler. Both the I-2 and NDV4-HR vaccines produce no evidence of clinical respiratory signs, weight loss, mortality in young chickens or egg production drop after vaccination (Bensink and Spradbrow, 1999; Heath *et al*.,1992). The safety performance of the original V4 (avirulent) vaccine is superior to both the HB1 (lentogenic) and La Sota (mesogenic) vaccine strains (Table 4)

Tab. 4: Comparative safety of Newcastle disease vaccine strains (Heath *et al*., 1992).

Signs in vaccinated birds	Vaccine strain		
	V4	HB1	La Sota
Sneeze test	Nil	Definite signs	Pronounced signs
Respiratory disease	Nil	Clinical respiratory signs	Clinical respiratory signs
Weight gain	No effect	Significant reduction	Highly significant suppression
Mortality in young chicken	Nil	Yes	Yes
Egg production drop	Nil	5–10%	>10%

CONCLUSION

The thermostable vaccine induce protective immunity among free-range chickens when correctly applied is accepted, its cheap and thus make it affordable to all farmers to use, its not require strict cold chain facilities and easy to administer by farmers. It can make a vital contribution to the improvement of household food security in many developing countries. The control of ND will contribute to improved village poultry production as well as in commercial poultry by prevent to keep the virus in circulation and act as reservoirs and carriers to themselves and the more susceptible exotic breeds in commercial farms. In some circumstances, it will provide the first contact between small-scale farmers and national veterinary services.

REFERENCES

ALDERS R.G., BAGNOL B. (2000): Communicating with farmers – a vital element in the control of Newcastle disease in village chickens. Proceedings of the XXI World's Poultry Congress, Montreal, 20–24 August 2000.

ALDERS R.G., INOUE S., KATONGO J.C. (1994): Prevalence and evaluation of Hitchner B1 and V4 vaccines for the control of Newcastle disease in village chickens in Zambia. Preventive Veterinary Medicine, 21: 125–132.

ALDERS R.G., SPRADBROW P.B. (2001a): Controlling Newcastle Disease in Village Chickens: a field manual. Canberra, Australian Centre for International Agricultural Research.

ALDERS R.G., SPRADBROW P.B. (2001b): SADC Planning Workshop on Newcastle Disease Control in Village Chickens. Proceedings of an International Workshop, Maputo, Mozambique, 6–9 March 2000. ACIAR Proceedings Nº 103.

ALEXANDER D.J. (2003): Newcastle disease and other avian Paramyxoviruses infections In: Saif Y. M. (ed.): Disease of poultry, 11th ed. Ames, Iowa: Iowa State Press: 63–87.

ANON (1991). Websters Newcastle Disease Vaccine for Village Chickens (1991). Information Dossier. Castle Hill, Australia.

BENSINK Z., SPRADBROW P. (1999): Newcastle disease virus strain I-2 a prospective thermostable vaccine for use in developing countries. Veterinary Microbiology, 68: 131–139.

BEARD C.W. (1989): Serologic Procedures. In: A Laboratory Manual for the Isolation and Identification of Avian Pathogens. In: Purchase H.G., Arp L.H., Domermuth C.H., Pearson J.E. (eds.). Kennett Square, PA: Amer. Assoc. Avian Pathologist, pp 192–200.

BELL J.G. (2001): A comparison of the different vaccine available for the control of Newcastle disease in village chickens. In: Alders R.G., Spradbrow P.B. (eds.): SADC Planning Workshop on Newcastle disease control in village chickens. Proceedings of an International Workshop, Maputo, Mozambique, 6-9 March, 2000. ACIAR Proceedings, No. 103: 56–60.

FAO (2002). Laboratory Manual for the Small-Scale Production and Testing of I-2 Newcastle Disease Vaccine RAP publication (2002/22); ISBN 974-7946-26-2.

FAO (1997). Guidelines for the inclusion of improved household poultry production. Diversification component of the Special Programme for Food Security. Rome.

GOMWALK N.E., ADESIYUN J.T., BISHU G., ADESIVUN A.A. (1985): A serological survey of Newcastle disease virus in domestic poultry around Zaria. Nigerian Veterinary Journal, 14 (1): 70.

HEATH B.C., LINDSEY M.J., MCMANUS K.P., CLAXTON P.D. (1992): Webster's Newcastle disease vaccine for village chickens. In: Spradbrow P.B. (ed.): Newcastle disease in village chickens. Canberra, ACIAR Proceedings No. 39-104109.

IDERIS A., IBRAHIM A.L., SPRADBROW P.B., HUNG SENG C. (1987): Development of Food Pellet Newcastle Disease Vaccine. In: Copland J.W. (ed.): Newcastle Disease in Poultry: A New Food Pellet Vaccine. Canberra, ACIAR, pp. 20–23.

JORDAN F.T.W., PATTISON M. (1996): Poultry disease. 4th ed., London, W.B. Saunders company Ltd.

MCDANIEL H. A., ORSBORN J.S. (1973): Diagnosis of velogenic viscerotropic Newcastle disease. D.J.A.V.M.A., 163 (9):1075–1079.

OIE (2000a). Newcastle Disease." Manual of Standards for Diagnostic Tests and Vaccines. Paris. Available at http://www.oie.int/ eng/maladies/fiches/a_A060.htm

OIE (2000b). Manual of standards for diagnostic tests and vaccines. 4th ed. Available at: http://www.oie.int/eng/normes/mmanual.a_00036.htm [Accessed on 27/07/2004].

OLIVIER A. (2004). Newcastle disease (2004). Available at: http://www.nda.agric.za/publications/newcastle disease.htm [Accessed on 01/02/2005].

SPRADBROW P.B. (1987): In Newcastle Disease in Poultry. A new food pellet vaccine. Copland J.W. (ed.), Canberra, ACIAR: Newcastle Disease – an Overview pp. 12–18.

SPRADBROW P. (1992): A review of the use of food carriers for the delivery of oral Newcastle disease vaccine. In: Spradbrow P.B. (ed.): Newcastle Disease in village.

SPRADBROW P. (1992b) A review of the use of food carriers for the delivery of oral Newcastle disease vaccine. In: Spradbrow P.B. (ed). Proceedings of an international workshop, Kuala Lumpur, 6–10 October 1991. Canberra, ACIAR proceedings, No. 39: 18–20.

SPRADBROW P.B. (1993–94): Newcastle disease in village chickens. Poultry Science Review, 5: 57–96. Chickens: organized by Australian Centre for

International Agricultural Research. Melbourne: Brown Prior Aderson: 18–20.

TU T.D., PHUC K.V., DINH N.T.K., QUOC D.N., SPRADBROW P.B. (1998): Vietnamese trials with a thermostable Newcastle disease vaccine (strain I-2) in experimental and village chicken. Preventive Veterinary Medicine, 34: 205–214.

USMAN M. (2002): Effects of vaccination of chickens against Newcastle disease with thermostable V_4 and Lasota vaccines using different grains and their brans as vehicle. Available at: http://www.cipav.org.co/lrrd/lrrd18/5/nwan18060.htm [Accessed on 18/05/2006].

WAMBURA P.N., KAPAGA A.M., HYERA J.M.K. (2000): Experimental trials with thermostable Newcastle disease virus (strain I-2) in commercial and village chickens in Tanzania. Preventive Veterinary Medicine, 43 (2): 75-83.

Received for publication on February 7, 2008
Accepted for publication on April 28, 2008

Corresponding author:

Adwar Taimur, D.V.M.
Institute of Tropics and Subtropics
Czech University of Life Sciences Prague
Kamýcká 129
165 21 Prague 6 – Czech Republic
e-mail: taimur_adwar@yahoo.com