

Adoption of I₂ Vaccine in Immunization of Village Chickens against Newcastle Disease Virus in Southern Tanzania: Immune Status of Farmer Vaccinated Birds

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Abstract

Newcastle disease (ND) is an economically important disease of poultry for which vaccination is applied as a preventive measure in many countries. In June 2009 we conducted a survey to establish the immune status of village chickens against ND virus in villages where vaccination programs, using thermotolerant ND I₂ vaccine were adopted by farmers in southern Tanzania. One hundred farmers from ten villages in three district councils were involved. Blood samples were collected from 499 chickens and sera harvested for Hemmagglutination Inhibition (HI) test. Results indicated that 73.3% of the sampled birds had protective levels of antibodies against ND virus. A significantly large proportional of vaccinated adult chickens attained protective immunity as compared to growers and chicks. We recommend advocating ND I₂ in other village chicken keeping communities of the country for ND control. Additionally farmers need to be educated on control measures for other important diseases of village chickens.

Keywords: Village chicken, I₂ vaccine, HI test, Tanzania

1. Introduction

In many developing countries, village chickens are the livestock most commonly owned by rural families. In Tanzania they represent over 94% of chickens, predominantly kept by village farmers under scavenging production system (Ministry of Agriculture and Food Security, MAFS, 2003). According to Roberts (1992), village chicken can break the vicious cycle of poverty, malnutrition and disease. Chicken meat is rich nutritionally providing protein, fats, minerals and vitamins. Indigenous chicken can be a good source of cheap nutrition for resource poor households, the sick, malnourished and children under the age of five.

In the country, as in other parts of Africa, Newcastle disease (ND), a highly contagious viral disease affecting wild and domestic avian species (Seal et al., 2000; Alexander, 2003), is the major constraint to village chicken development. The impact of the disease is most notable in domestic poultry due to their high susceptibility and the severe consequences of outbreaks of virulent strains on the poultry industries. The disease may represent a bigger drain on the world economy than any other animal viral disease (Alexander, 2003). In response to the threat presented by ND, several countries have put in place vaccination campaigns to prevent epizootics. It is known that vaccination of poultry provides an excellent means to lessen clinical signs of infection caused by virulent Newcastle disease virus (NDV) (Alexander, 2003; Senne et al., 2004; Kapczynski and King, 2005).

Vaccination programs for NDV include the use of either low-virulent live-virus vaccines or inactivated vaccines to induce protective immunity while producing minimal adverse effects in birds. Inactivated vaccines which were the first to be available commercially to the poultry industry are known to be safe but provide incomplete protection against ND (Hitchner, 1964). They therefore require multiple booster immunizations to achieve and maintain protection. On the other hand live-attenuated vaccines based on lentogenic strains such as La Sota are known to be highly immunogenic, but have a drawback of safety concerns (Ikegami et al., 2009; Bouloy et al., 2009), as their use can result into clinical disease. They are however widely used because of their high efficacy and availability (Borland and Allan, 1980). Although the use of vaccines has reduced the disease rates, it also may have contributed to the fact that current vaccines and vaccination campaigns are not maximally effective in preventing infection and transmission (Burrige et al., 1975; Alexander, 2003; Senne et al., 2004; Kapczynski and King, 2005).

The Newcastle disease thermotolerant vaccines are among live-attenuated vaccines that offer an appropriate technology for ND control in rural free-range poultry management systems where cold-chain facilities, logistics, management and husbandry factors need consideration (Alders and Spradbrow 2001). In Tanzania, the use of a locally produced thermotolerant ND I₂ vaccine is being promoted for ND control in village chickens to increase poultry production in rural households for poverty eradication programs. Since 1994, it has been used in almost all districts of southern zone (SZ) of Tanzania in efforts to reduce chicken losses. Initially immunization of the birds was effected by the District councils' livestock officers at no cost. The free vaccination was however withdrawn within some few years and farmers were to vaccinate their birds at their own cost including buying the vaccine from Veterinary Investigation centre, district livestock offices and private veterinary centres. Sustainability of the initiated program differed among villages as well as among farmers within the same village. Chicken mortalities however continued to feature in vaccinated flocks and farmers attributed it to ND, thereby starting condemning the vaccine. The present study was therefore conducted to assess the immunity induced by the vaccine in farmer vaccinated village chicken in selected villages of Southern Tanzania.

2. Materials and Methods

2.1 Ethics Statement

This study was approved by the Institutional Review Board at Livestock Research Centre Naliende, Mtwara, Tanzania. Permissions to carry out the studies in the Districts were sought from the respective District council authorities. Farmers provided verbal consents which were preferred over written consents for consistency as some respondents could not read and write. Blood collection from birds was effected by qualified, registered veterinarians using standard procedures.

2.2 Study Area

The present study was conducted in the southern zone regions of Tanzania namely; Lindi and Mtwara. Project villages were selected from three district councils i.e. Lindi rural, Mtwara Municipal and Mtwara rural.

2.3 Sample Size Calculation

The sample size for the number of birds to sample was calculated using the formula for random sampling developed by Martin *et al.* (1987) as follows:

$n = Z^2 PQ/L^2$; where n = required sample size, Z is the Z value for a given confidence level, P is a known or estimated prevalence, $Q = (1 - P)$, and L = allowable error of estimation. For the purpose of this study a confidence level was assumed at 95% with an allowable error of estimation of 5%. The average prevalence of protective NDV antibodies in vaccinated birds using thermotolerant I₂ vaccine was estimated at 70% based on previous studies (Foster et al., 1999; Alders and Spradbrow, 2001). Therefore, $n = (1.96^2 \times 0.70 \times 0.30) / 0.05^2 = 323$ birds. For a wider coverage this study sampled 499 birds.

2.4 Selection of Study Villages

Inclusion of any village in this study considered the following criteria: 1) relatively higher chicken population in the district, 2) presence of farmers regularly vaccinating their chickens against ND using thermotolerant I₂ vaccine and 3) willingness of farmers and leaders to cooperate with researchers and livestock experts/extension staff during and after the project. Extension staff from the District Agriculture and Livestock Development Officers' (DALDOs) offices facilitated the selection process.

2.5 Selection of Project Farmers

Meetings with chicken keepers were organized in selected villages with involvement of the village leaders and extension staff. Farmers were introduced to the study objectives and principles and village leaders assigned a task to take a lead in the selection of farmers to be involved in the study.

2.6 Collection of Blood Samples

At household level, blood samples were collected from randomly picked vaccinated chicken from a wing vein, allowed to clot for 4 h and centrifuged. The sera obtained were kept at -20°C until used for serology.

2.7 Serological Evaluation of Birds' Responses to Vaccination

A convenient and most commonly used serologic procedure for determining the response of the flocks to ND vaccine, the hemagglutination-inhibition (HI) test, was adopted. The assay was performed by a conventional microtiter method as described by King (1996) using four hemagglutinating units of NDV La Sota strain. The titers were reported as the log₁₀ of the inverse of the highest sample dilution showing complete HI. NDV HI titers greater than or equal to log₂ 5 units were considered to be protective against a field challenge (Phillips, 1973). Samples were analyzed individually in duplicate.

2.8 Data Analysis

Gathered data were entered into Microsoft Excel spreadsheet and analyzed in Medcalc[®] software. Descriptive statistics, frequencies in particular, were computed for the levels of immunity in sampled birds. Differences in proportions of birds that attained protective immunity by village and age group were compared using Chi square for statistical significance at $p=0.05$.

3. Results

3.1 Project Villages and Farmers

Ten villages were selected from the three district councils based on the set criteria. Naumbu, Imekuwa (Mtwara rural); Mtawanya, Nachenjele, Mkangala and Naliendele (Mtwara Municipal); and Mnimbila, Kilangala, Kilimahewa and Kiwalala (Lindi rural). A total of a hundred farmers, ten from each village were involved in the study.

3.2 Blood Samples

Blood samples were collected from a total of 499 indigenous birds, an average of five birds per household. Majority of the sampled birds (>90%) were of the "Mbegu Kuza" breed composing 60-70% of village chicken population in Southern Zone. The remaining proportion came from chickens of the "Barred Neck" breed. The samples were always collected early in the morning before the chickens were released as the farmers were leaving for farms.

3.3 Birds' Responses to Vaccination

Following hemagglutination-inhibition (HI) test, 73.3% of the sampled birds ($n=499$) were found to have protective levels of antibodies against NDV. The proportions of birds with protective antibodies against NDV per village ranged between 63% and 97.3% (table 1). Some proportions differed significantly while others didn't. Responses of the birds to vaccination by age group are shown in table 2. The proportion of birds who developed protective immunity was significantly higher in adults as compared to growers ($p=0.0417$) and chicks ($p=0.0372$). Differences in proportions between growers and chicks didn't show any statistical significance. The distribution of ND virus antibody titre values among the different age groups of village chickens is displayed in table 3.

4. Discussion

We are reporting results of evaluating the immune status against NDV in regularly vaccinated village chickens in southern Tanzania. We aimed at disproving the perceptions by some farmers that the vaccine doesn't provide immunity to their birds resulting into chicken losses attributable to the disease they vaccinate against.

Poultry diseases remain the greatest threat to rural poultry farmers and are responsible for very large economic losses to producers. Vaccination is one of the main approaches to the control of infectious diseases of poultry (Aini, 1990). For a long time this approach has been used in attempt to control ND where by health birds are vaccinated. An effective NDV vaccine program and related biosecurity are essential components for NDV control.

In the present study 73.3% of the sampled birds, vaccinated using thermotolerant ND I₂ vaccine, attained protective levels of antibodies against ND virus infection. This finding concurs some other reports which found protective antibodies attained in less than 100% of vaccinated birds. Foster et al. (1999) found that at least 70% of the chickens vaccinated using thermotolerant ND I₂ vaccine would be protected against challenge with virulent virus. Field records in Mozambique indicate that ND I₂ vaccine provides approximately 80% protection in the field in the face of an outbreak, when given every four months via eye-drop (Alders and Spradbrow, 2001). It has been pointed out that ND vaccines do not provide unconditional immunity against infection and transmission, and the individual responses to vaccination can be highly variable; some birds attaining protective levels of antibodies against infection and disease, and others not (van Boven et al., 2008). Aini (1990) identifies husbandry system and concurrent infections to be more important interfering factors during vaccination on

backyard farms. A comparison study by Illango et al. (2008) found that, following vaccination using thermotolerant ND I₂ vaccine, housed chickens attained 100% protection to Newcastle disease compared with 89% protection by un-housed chickens. Other determinants on the success of vaccination have been documented by Calnek (1983). These include the vaccine itself and the immune response of the host to the vaccine. According to the author the response is affected by age, the route of vaccination, general health of the host and the nature of the antigen. The effect of age has featured in the present study whereby a significantly large proportional of vaccinated adult chickens attained protective immunity against NDV as compared to growers and chicks. This may be attributable to repeated exposure and/or well developed immune system in adult chickens leading to an adequate response.

Conventionally, humoral responses have been used to measure protective immunity. This was adopted in the present study in which protective immunity against NDV was evaluated by measuring ND virus (NDV) antibody titers in serum. Some other researchers have measured the NDV antibody titres in tracheal mucosal swabs and washes (Zoth et al., 2008). The systemic and mucosal immune systems are considered to function more or less independently with systemic antibodies providing protection against ND and the local antibodies limiting multiplication of NDV at the site of entry. It is however known that antibodies alone do not necessarily provide protection and that cell-mediated immune responses provide a crucial counterpart to humoral immunity (Bautista-Lopez et al., 2000; Eichner 2003; Ovsyannikova et al., 2004). Humoral and cellular immune processes are known to be integrally linked with each other during the initial induction of immunity to a pathogen (Lee et al., 2008). The evaluation of cell mediated immune responses involves methods such as measurement of production of cytokines such as Interferon γ (IFN- γ) which is the signature cytokine for T helper 1 cells whose predominance is associated with cell-mediated responses, which are most beneficial to viral infection (Klimpel et al., 1990; Sharma, 1999). Cytotoxic T lymphocytes specific against NDV have been detected in the spleen of vaccinated birds though their contribution to protection is yet to be explained (Al-Garib et al., 2003). Protection associated with vaccine can also be evaluated through challenge experiments. Such experiments assess protection of birds against overt clinical disease following vaccination upon challenge with highly virulent virus. Several challenge experiments have been conducted on ND virus vaccines in chickens (Foster et al., 1999; Parede and Young, 1990; Kapczynski and King, 2005; Miller et al., 2007; Bwala et al., 2011). The studies have reported protection in vaccinated birds from developing clinical disease. Some studies indicate that the protection is limited to disease development but not infection and replication of the virulent strains of the virus (Parede and Young, 1990; Kapczynski and King, 2005; Miller et al., 2007; Bwala et al., 2011).

ND vaccination projects have reported early success, but later reported the emergence of other causes of poultry mortality as birds live longer (Yongolo et al., 1998). An observation in Mtwara, Tanzania (Mbyuzi, 2009; personal communication) has revealed that in ND-immunised birds mortality is still high in chicks and growers. This suggests the importance of other disease conditions which do not receive much attention. Farmers' limited knowledge on these diseases makes them attribute the mortalities to ND and eventually associate it with inefficiency of vaccine. This may be the case in the area where the present study was conducted in which birds were found to be immune and yet exist complains of significant chicken losses; farmers attributing them to ND. Alders et al. (2002) and Muhairwa et al. (2008) proposed that in order to reduce heavy chicken losses, the control of ND must go along with improved husbandry practices and control of avitaminosis A, parasites, bacterial infections and fowl pox (only where pox is a problem).

5. Conclusions

We have shown that the use of thermotolerant ND I₂ vaccine by farmers in the study area induces a significant production of IgG in village chicken under free range production systems. The vaccine is therefore potent for ND control and adaptable for rural farmer use. Since the farmers who adopted the use of the vaccine still complain of continued chicken mortalities of especially chicks and growers, some starting condemning the vaccine, they need to be educated on other important diseases and their control measures so as to significantly reduce losses of village chicken. This will make village chicken production a profitable enterprise that will improve rural communities' livelihoods.

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Table 1. The proportions of village chickens with protective levels of ND virus antibodies by village (HI assay results)

Village (Number of birds sampled)	Birds with protective levels of ND virus antibodies	
	Number	Percentage
Naumbu (74)	55	74.3
Imekuwa (24)	18	75
Mtawanya (33)	27	81.8
Nachenjele (37)	27	73
Mkangala (54)	45	83
Mnimbila (54)	34	63
Kilangala (51)	35	68.6
Kilimahewa (74)	57	77
Naliendele (20)	15	75
Kiwalala (78)	53	68
TOTAL (499)	366	73.3

Table 2. The proportions of village chickens with protective levels of ND virus antibodies by age group (HI assay results)

Age group (Number of birds sampled)	Birds with protective levels of ND virus antibodies	
	Number	Percentage
Chicks (74)	50	67.6
Growers (313)	224	71.6
Adults (112)	92	81.2
Total (499)	366	73.3

Table 3. The distribution of ND virus antibody titre values among different age groups of village chickens (HI assay results)

Age group	Number of samples	Antibody titers following HI test						
		1:2	1:4	1:8	1:16	1:32	1:64	1:128
Chicks	74	13	11	29	13	7	1	0
Growers	313	36	53	103	90	18	13	0
Adults	112	3	17	29	25	21	15	2