International Journal of Agriculture and Biosciences 2023 12(4): 222-227.
https://doi.org/10.47278/journal.ijab/2023.068

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Seroconversion study of thermostable NDI-2 vaccine at the free-ranging village chickens in two localities of South-central Niger

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ABSTRACT

Background and Aim: The search for a heat-tolerant and less expensive vaccine should be the central point for controlling Newcastle disease in village poultry farming in endemic countries. The objective of the present study was to quantify and monitor the level of antibodies present in the sera of village chickens after vaccination with the thermostable NDI-2 vaccine produced by the Central veterinary laboratory of Niamey. Materials and Methods: The trial involved 88 local chickens, less than one-year-old, raised in two localities in the Maradi region of Niger. Sera samples were first collected from the selected chicken population and - subjected to a competitive enzyme-linked immunosorbent assay to determine their serological status and to eliminate Newcastle disease virus seropositive individuals. The seronegative chickens were then vaccinated with eye drops and monitored for 16 weeks. During this period, serum samples were collected and tested with the indirect enzyme-linked immunosorbent assay to determine antibody titers. A comparative analysis of the antibody titers means was performed using the t-Student test at the 0.05 cut-off. Results: The test results showed that herd immunity (88%) was achieved at the fourth week after vaccination. Mean antibody titers were statistically significant at - 0.05 level of significance between weeks 2 and 4 and between weeks 12 and 16. Conclusion: The NDI-2 vaccine produced by the Central veterinary laboratory of Niamey can be used to protect village chickens against Newcastle disease.

Keywords: Newcastle disease, Seroconversion, Thermostable NDI-2 vaccine, Vaccination, Village chickens.

Article History
Article # 23-365
Received: 20-May-2023
Revised: 11-Jul-2023
Accepted: 28-Jul-2023

INTRODUCTION

Newcastle disease is one of the most devastating diseases in the poultry industry worldwide (Bello et al., 2018; Lebdah et al., 2022). It is a highly contagious viral disease capable of causing 100% mortality in a non-protected poultry population (Dzogbema et al., 2021; Fellahi and Boudouma, 2021). In family poultry farming, the disease causes significant economic losses and negatively impacts the household dietary diversification and its animal food consumption (Khatun et al., 2018). Annual outbreaks of Newcastle disease according to estimations -killed between 70-80% of unvaccinated chickens in villages (Nega et al., 2012).

In Niger, poultry production is mainly dominated by the traditional system. About 80% - of Niger Republic - population is practicing poultry farming and 98% are from the traditional sector. Traditional poultry farming plays an important role in the household economy, in food security, and in alleviating poverty in rural areas (MAG/EL, 2020). Despite this potential role, the sector faces several constraints among wich health constraints related to Newcastle disease (Moustapha et al., 2022).

To avoid the disease outbreak, vaccination is the only effective preventive means that can be used (Fentie et al., 2014; Dimitrov et al., 2017). Athought -the conventional vaccines have been successfully used over time (Getabalew et al., 2019), their efficacity is becomming more questionable now a day (Ahmed et al., 2022).

But these Vaccines made with strains whose genotype wrongly matched - that of wild field strains do not provide complete organ protection for the vaccinated host (Manar et al., 2020). In addition, these vaccines are not suitable for sustainable use in traditional chicken production systems

Cite this Article as: Moustapha A, Talaki E, Akourki A, Gagara H, Oumarou M and Yansambou MS, 2023. Seroconversion study of thermostable NDI-2 vaccine at the free-ranging village chickens in two localities of south-central niger. International Journal of Agriculture and Biosciences 2023 12(4): 222-227. https://doi.org/10.47278/journal.ijab/2023.068

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due to their cost, high doses and thermolability (Nega et al., 2012). The development of an inexpensive thermostable Newcastle disease vaccines represents the alternative means of controlling Newcastle disease in endemic countries (Alders, 2014). Another advantage of this type of vaccine is its ability to be used in remote areas in countries where cold chain facilities are limited (Hu et al., 2022). Vaccination trials of village chickens with vaccines based on I-2 strains selected by the Australian Center for International Agricultural Research (ACIAR) have been successfully conducted in several African and Asian countries (Adwar and Lukešová 2008; Nega et al., 2012; Acharya et al., 2019). In addition to their efficiencies, these vaccines also offer the potential to be administered through many routes such as eye drops, in drinking water, or in the diet (Thekisoe et al., 2004; Napit et al., 2023).

Niger's veterinary authorities opted for local production of vaccines based on thermotolerant strains of the Newcastle disease virus in order to make the vaccine more accessible to poultry farmers to improve vaccination coverage against Newcastle disease. No efficient evaluation study has been conducted for the vaccine in the perspective utilization of this type of vaccine. Thus, the present study is part of the evaluation of the efficacy of the thermostable NDI-2 vaccine produced by the Central veterinary laboratory (LABOCEL) of Niamey and more specifically, it aims to determine the antibody titers induced by the vaccine in traditionally bred chickens and to study the kinetics of post-vaccination antibodies.

MATERIALS & METHODS

Ethical Approval

Due to the absence of an Animal Care Committee available at the University of Lomé at the time of this research, the research was conducted under the supervision of the research team leader following the guidelines of the Canadian Council on Animal Care (2009).

Study Area

The study was conducted in the Maradi region (South-central Niger), in the localities of Takassaba and Dan Maimouna located in the communes of Mayahi and Kanembakaché respectively, in the department of Mayahi (Fig. 1). The annual temperature in the study area ranges from 21.54 to 35.38°C and the annual relative humidity is 27 to 57.6% (INS, 2020). The climate is Sahelian with a single rainy season from June to September. The average rainfall is 450.78 mm per year (INS, 2018). The population is predominantly rural and is mainly embarked in agriculture (88.3%), trade (6.9%), and breeding (2.7%) (INS, 2016). The study map clearly shows the study area (Fig. 1).

Vaccine Administered

The vaccine administered is a live, thermostable vaccine produced by the Central veterinary laboratory of Niamey (LABOCEL). It is a freeze-dried vaccine in the form of a dry white pellet, packaged in 100 doses per vial. It is prepared from the modified Australian avirulent NDV4-HR strain, called "I-2". This vaccine strain belongs to genotype I of class II (Asl Najjari et al., 2017). It is provided by the Pan African Veterinary Vaccine Center (PANVAC) to laboratories in African countries to support local production projects of thermotolerant vaccines, accessible to small village producers to vaccinate chickens against Newcastle disease.

Vaccine Transport, Preparation and Administration

Vials of 100-dose I-2 vaccine and 100-mL vials of diluent were transported on the field under ice in a cooler. Each 100-dose vial of I-2 vaccine was prepared in a shade, dust-free environment with 5 mL of diluent provided by the vaccine manufacturer. The mixture was then administered in drops corresponding to the vaccine dose into one of the eyes of chickens in a reclined, restrained position.

Experimental Protocol

The experiment was conducted on a young village local chickens breed of less than one-year-old at the beginning of the operation. The experimental flock consisted of 88 chickens, among which 18 roosters and 70 hens, belonging to thirthy (30) traditional poultry farmers located in the -localities of Takassaba and Dan Maimouna. The number of chickens monitored was limited by the availability of poultry farmers. The animals were kept in a free-range breeding system and were identified with tags numbered to facilitate their serological monitoring.

An initial serum sample (or screening sample) was taken from the chickens prior to vaccination at the beginning of the experiment, to determine their serological status in order to eliminate Newcastle disease virus seropositive individuals. Seronegative chickens were then vaccinated with the I-2 vaccine produced by the Central veterinary laboratory of Niamey by dropping the vaccine throughocular route in one of the chicken's eyes. A series of serum samples were also collected from these chickens after vaccination and according to a collection schedule. Five collections were performed at different time intervals including two weeks, four weeks, eight weeks, twelve weeks, and finally sixteen weeks to monitor the dynamics of post-vaccination antibodies.

Sampling Method

Two localities from the communes of Kanembakaché and Mayahi were selected in the department of Mayahi to carry out the present study, based on the socio-economic and nutritional importance of poultry in the households of these localities and the consequences of poultry diseases reported to the local veterinary service. Households carrying poultry farming were selected on voluntary basis and the acceptance of serological monitoring of animals over a sixteen-week period.

Sample and Data Collection

Serum samples were collected from August 26 to December 18, 2022, during a 16-week period. To facilitate the collection, all chickens monitored were previously identified by placing rings numbered on their leg. Samples of 1.5 to 2 ml of blood were collected from the wing vein of the chickens in tubes without anticoagulants. Serum samples were separated from clotted blood samples by centrifugating at 1500 rpm for 15 minutes. Serum samples were then decanted, aliquoted into microtubes, and frozen at -20°C at the Central veterinary laboratory of Niamey, Niger, until testing.

A survey questionnaire with open-ended and semi-open-ended questions was administered to poultry farmers face-to-face to collect information on poultry numbers owned by the farmers, mortalities recorded, and causes of mortality before and after chickens vaccination against Newcastle disease in the two study localities.

Methods of Analysis and Interpretation of Results

First, the competitive enzyme-linked immunosorbent assay (cELISA) was used as a screening test to determine the serological status of chickens (Ahamidou et al., 2023) prior to vaccination in order to eliminate Newcastle disease virus seropositive individuals. In a second step, the indirect enzyme-linked immunosorbent assay was used to detect post-vaccination antibodies in seronegative chickens, to quantify the level of specific antibodies present in their sera, and to follow up on vaccination.

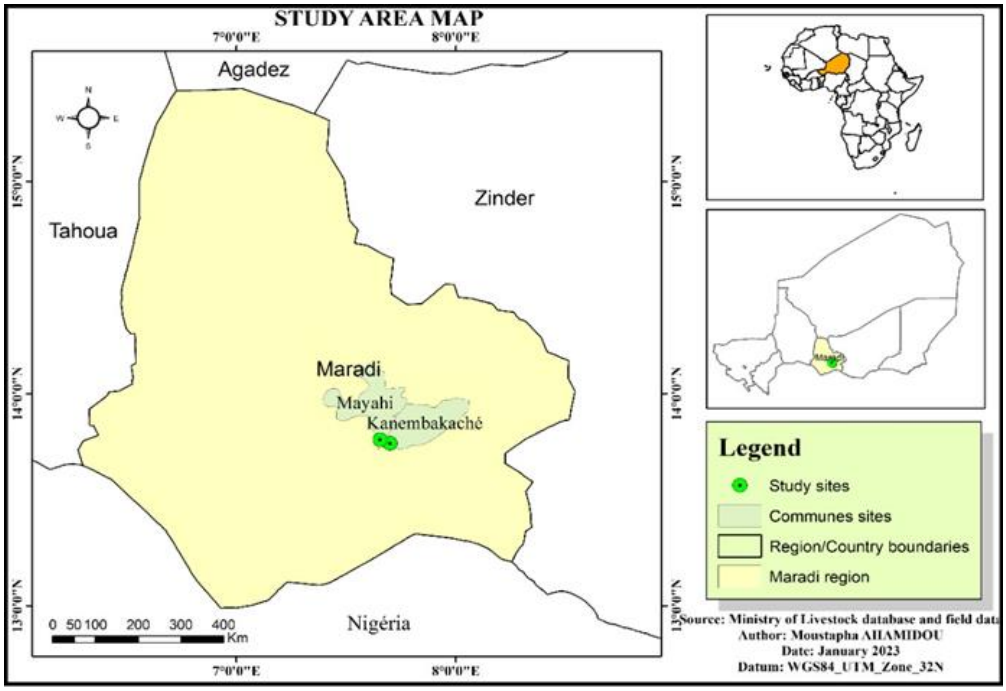

Fig. 1: Study area

The competition ELISA (Reference NDVC-2P LOT K34) and indirect ELISA (Reference NDVS-CV-5P LOT J36) diagnostic kits used were obtained from the Innovative Diagnostics IDVet laboratory in Marseille, France. The procedures used in the laboratory were conducted according to the manufacturer's instructions. Plates were read with SkanIt Software v.3.1 at an optical density (OD) of 450 nm.

The competitive ELISA is validated when the mean optical density value of the negative control is greater than 0.6 (OD_{CN} > 0.6) and the percent inhibition (PI%) of the positive control is greater than 40% (PI_{CP} > 40%). For each sample tested, the percentage inhibition value was calculated (PI %) according to the following formula: PI% = (OD_{CN} -OD_{sample} /OD_{CN})*100. Samples with a PI% value above 40% are considered positive, those with a value between 30 and 40% are considered doubtful and finally, those with a value below 30% are considered negative.

Regarding the indirect ELISA, it is validated when the average optical density value of the positive controls (OD_{CP}) is greater than 0.250 (OD_{CP} > 0.250) and the ratio between the average of the positive controls (OD_{CP}) and the average of the negative controls (OD_{CN}) is greater than 3 (OD_{CP}/OD_{CN} >3). For each sample tested, the S/P ratio and antibody titer were calculated. The S/P ratio was calculated using the following formula: S/P = OD_{sample} - OD_{CN}/OD_{CP}-OD_{CN}. The antibody titer was calculated with the formula: $\log10^{(titer)} = 1.00x\log10^{(S/P)} + 3.520$ hence titer = $10^{\log_{10}(titer)}$. The results obtained were interpreted according to Table 1.

Statistical Analysis

The data obtained were analyzed using STATA 13.0 software. Descriptive statistics were performed to describe the data by calculating frequencies, means, standard deviations, minimums, and maximums. Student's t-test was used to compare the mean antibody titers present in the serum of the chickens according to the period of seroconversion. The objective of this comparative analysis is to see if there is a significant difference between the mean antibody titers at 0.05 probability level according to the periods of vaccine seroconversion in order to get an idea of the antibody dynamics.

RESULTS

Table 2 tabulates the distribution of Newcastle disease prevalence in village chickens by locality before vaccination. Out of 88 chickens tested, 23 were positive for Newcastle disease virus, with an overall prevalence of 26.13%. The p-value of the Newcastle disease prevalences between the two localities was 0.467. The difference in the proportions of prevalences between the two localities was not significant at p<0.05. Therefore, the locality factor did not affect the prevalence of Newcastle disease.

More than half of the vaccinated chickens, that is 76% of the total number of chickens, were protected by the I-2 vaccine against ND the second week after vaccination. However, effective immunity was achieved at the fourth week. Indeed, it was in the fourth week that 88% of the vaccinated chickens had an antibody titer above the threshold for protection (Titer > 993) against Newcastle disease (Table 3).

In response to the antigen, the average number of antibodies detected in the serum of chicken by indirect ELISA was approximately 2410 ±199 in the second week after vaccination. At 16 weeks, -chicken could have an average antibody titer of 6157±411 in its serum. In addition, the mean antibody titer was 3128±201, 3247±205, and 3853±261 at 4 weeks, 8 weeks, and 12 weeks, respectively (Table 4). The minimum antibody titers were 203 at week 2 and the maximum was 11006 at week 16.

Table 1: Reference values for determining the immune status of chickens against Newcastle disease

S/P value	Antibody titer ELISA	Immune status of ND
S/P ≤ 0.3	Titer ≤ 993	Negative
S/P >0.3	Titer > 993	Positive

Table 2: Distribution of Newcastle disease in village chickens by locality before vaccination

Localities	Chickens tested	Negative chickens	Positive chickens		P-value
			No.	%	
Takassaba	44	34	10	22.73	0.467
Dan Maimouna	44	31	13	29.54	
Total	88	65	23	26.13	

Table 3: Group immunity by vaccine seroconversion periods

Period (weeks)	Number of chickens tested	Number of positive chickens (Titer > 993)	Number of negative chickens (Titer ≤ 993)	Herd immunity Percentage (%)
Two	58	44	14	76
Four	58	51	7	88
Eight	58	54	4	93
Twelve	58	55	3	95
Sixteen	58	57	1	98

Table 4: Average post-vaccination antibody titers according to the periods

Variable	Mean	SE	[95% Conf. Interval]	
			Inf	Sup
Titer weeks2	2410.3	199.1	2011.6	2809.1
Titer weeks4	3127.8	201.3	2724.7	3530.9
Titer weeks8	3247.1	205.1	2836.4	3657.8
Titer weeks12	3853.2	261.3	3330.1	4376.4
Titer weeks16	6156.6	411.9	5331.8	6981.4

Table 5: Results of the Student's t-test for comparison of mean antibody titers

Titers	Diff (Mean)	t	P-value
Titer S2 vs Titer S4	-717.5	-2.5	0.0126*
Titer S4 vs Titer S8	-119.2	-0.4	0.679
Titer S8 vs Titer S12	-606.1	-1.8	0.0708
Titer S12 vs Titer S16	-2303.4	-4.7	P< 0.0001*

*Significant difference

From the 2nd to the 4th week, the increase of detected antibodies was significant whereas it was not significant between the 4th and the 8th week and between the 8th and the 12th week. After the 12th week, the increase in detected antibodies had become significant again in the 16th week (Table 5).

Moreover, at the end of the seroconversion study, the number of chickens in the households surveyed tripled from 106 to 381 chickens of all ages at sixteen weeks after vaccination. At the same time, mortality dropped from 248 cases before vaccination to 34 cases after vaccination, with a mortality reduction of 61.86%. The main causes of mortality reported after vaccination were chick trampling, predators, climatic hazards, and fowl pox.

DISCUSSION

The overall seroprevalence of Newcastle disease in village chickens in the present study was 26.13%. The present results are comparable to previous studies of Hassan et al. (2013) and Khatun et al. (2022) where reported a prevalence of 28.72% and 28.58% in Nigeria and Bangladesh, respectively.

The immune response induced by Newcastle disease vaccination is classically assessed by the haemagglutination-inhibiting antibody titer. However, this titer depends on many factors, including the vaccine used and its route of inoculation, the vaccination program followed, and environmental and individual factors (Rauw et al., 2009). In the present study, the proportion of chickens having antibody titer above the threshold of protection (Titer > 993) against Newcastle disease at four weeks after vaccination was 88%. The present results are in agreement with an Ethiopian study where Nega et al. (2012) reported a proportion of 90.4% of the overall chicken population with a protective titer above the threshold for protection against Newcastle disease at three weeks after primary vaccination with the locally produced I-2 vaccine.

The antibody titer in the present study was lower in some chickens. These results may be explained by reduced immunocompetence due to immunosuppression from concurrent infection in extensively raised chickens (Abera et al., 2017).

The immune coverage rate of the herd in the present study was 88% at week 4. It progressively increased to 98% at week 16. This higher level of immune coverage in the herd may be explained by the absence of an epidemic outbreak after vaccination. However, the gradual increase in antibody titers in free-ranging chickens may be due to continuous vaccine-induced antibody synthesis or potentiation of serum conversion following a natural infection challenge. Van Boven et al. (2008) reported that herd immunity can only be achieved in a Newcastle disease-vaccinated bird population if a high proportion of birds (>85%) has a high antibody titer (log2 hemagglutination inhibition titer ≥3) after vaccination.

The present results are in agreement with those reported by Kapczynski et al. (2013) who stipulated that detectable antibodies in the blood of chickens immunized with a live vaccine reach their maximums in 21-28 days after vaccination. However, in the present study, a second peak of antibodies was reached between the 12th and 16th-week post-vaccination.

At twelve weeks post-vaccination, the antibody titers mean in the present study were found to be 3853 ± 261. These titers mean are lower, compared to the reported antibody titers mean of 8813 ± 2022 of Dzogbema et al. (2021) study in Benin which used killed vaccines against Newcastle disease in village chickens three months after primary vaccination.

The present reports showed that the antibody titers induced by the I-2 vaccine produced by the Central veterinary laboratory of Niamey were sufficient to protect chickens against Newcastle disease at sixteen weeks after vaccination. These reports are comparable to previous studies. Results from a Nepalese study showed that the level of immunity was sufficient up to 90 days after vaccination with locally produced live attenuated I-2 vaccine to protect chickens against Newcastle disease in the field (Acharya et al., 2019). In a German study, Oberlander et al. (2020) also reported that vaccination of chickens with live attenuated ND Clone 30 vaccines under field conditions resulted in sufficient seroconversion at twelve weeks post-vaccination to protect the animals from Newcastle disease.

In a comparative study, Mahmood and Sabir (2021) documented that vaccination of chickens with an

adjuvanted bivalent inactivated vaccine for the I-2 strain of Newcastle disease (ND) and avian influenza (AI) was more effective than a commercially available bivalent inactivated vaccine.

Conclusion

In the present study, the antibody titers detected in the sera of the village chickens were significant between weeks 2 and 4 and between weeks 12 and 16 after vaccination with the NDI-2 vaccine produced by the Central veterinary laboratory of Niamey, Niger. In addition to the good herd immunity presented by this vaccine from the fourth-week post-vaccination, it is able to induce an antibody titer above the threshold for protection against ND at sixteen weeks after vaccination. To ensure good immune coverage against the ND virus in village chickens, it is recommended that vaccination should be repeated at least twice after the primary vaccination within one year. However, further studies should be considered to determine the exact duration of vaccine-induced immunity.

Conflict of Interests

The author declare that they have no conflict of interest.

Acknowledgment

This study was funded by the Regional Center of Excellence in Avian Science of the University of Lomé in Togo (CERSA/UL). The authors wish to express their warm gratitude to World Bank Impact, which is the main sponsor of CERSA, to the General Director of Veterinary Services of Niger, and to the General Director of the Central veterinary laboratory of Niamey (LABOCEL) for their technical support and the facilities in carrying out this work.

Authors' Contribution

Ahamidou Moustapha designed the protocol, collected and analyzed the samples, and draft the manuscript. Essodina Talaki and Adamou Akourki validated the protocol, supervised the data collection, and revised the manuscript. Haladou Gagara participated in the analysis of the samples. Mariama Oumarou participated in the vaccination and the collection of samples. Mahamadou Seyni Yansambou participated in the critical checking of the manuscript.

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