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Article

Development of flock immunity against Newcastle disease in native chicken in a locality

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Abstract: Preventive measures to combat with Newcastle disease virus (NDV) can be achieved by vaccination programs that are practiced in many countries of the world. Even after vaccination outbreaks of NDV occurred in the flock indicate that vaccination against NDV cannot provide effective immunity that may cause disease in partially vaccinated flock. Hence, the objectives of this study was to development of flock immunity in chicken against Newcastle disease in a local community. In this study a vaccination programme for the native flock in a locality (Dhamrai, Savar, Dhaka, Bangladesh) was conducted and effectiveness of the Ranikhet disease vaccine (RDV) was identified with detection of antibody titers that may protect the native flocks against the disease but mortality or the infection was present. The study showed that after routine vaccination with BCRDV (Baby Chicks Ranikhet Disease Vaccine) and RDV (Ranikhet Disease Vaccine) vaccine the native chicken showed high antibody titre in native chicken. Overall herd immunity of native chickens can be achieved if the maximum (>80%) chicken obtains high antibody titers (log_2 haemagglutination inhibition titer ≥3) after vaccinations. So, it can be concluded that successful vaccinations programme is the key point to control NDV with higher herd health immunity.

Keywords: Newcastle disease; HI; vaccine; herd immunity; control

1. Introduction

Newcastle disease virus (NDV) is a single stranded, negative sense, enveloped, non-segmented RNA virus under the family paramyxoviridae have some hemagglutinin and neuraminidase proteins (Miller *et al*., 2010). It is a highly contagious disease that causes respiratory, nervous, and digestive problems in wild and avian species (Seal *et al.*, 2000; Alexander, 2003). The severity of the disease is dependent upon the age and immunity status of the birds and on the virulence of the strains of the virus. NDV have three pathognomonic groups like velogenic, mesogenic and lentogenic strain in which velogenic strain causes serious effects of birds of Bangladesh (Samad, 2005). According to OIE 2012, NDV mainly caused by virulent strains (velogenic and mesogenic) where low virulence strains used as live vaccine. An outbreak of NDV was reported in semi-scavenging and backyard chickens of Bangladesh in 2001 (Barman, 2002) that affects at the average age between 33 days. NDV epidemics is most vulnerable and it causes significant morbidity and mortality of chicken and remarkable losses of poultry sector every year in Bangladesh (Ahamed, 2002; Biswas *et al*., 2005).

Vaccine for NDV of Bangladesh mainly produced by using lentogenic strain of Newcastle Disease Virus (NDV) named as 'Baby Chick Ranikhet Disease Vaccine' (BCRDV) and by using RDV-Mukteswar' strain of NDV named as 'Ranikhet Disease Vaccine' usually prepared by the Department of Livestock services (DLS), Bangladesh (www.dls.gov.bd). To protect native chickens from NDV a programme with the accordance of vaccine in combination with BCRDV and then also with RDV was used in the study. Administration strategy for vaccination of the native chickens in this study was by intramuscular injection at 30-60 days of age by RDV vaccine and another route was by aerosol or by drinking water with BCRDV vaccine at 3-7 day of age.

Protection and immunity against NDV after vaccinations was found to be good where other study claimed that infection and shedding of virus in NDV vaccinated native chickens may occurs without clinical signs. Immunity against NDV was found to be higher in individual flock than mass vaccinations with faster application of the live vaccines to each bird of a flock (Senne *et al*., 2004). Appropriate immune response to develop immunity prior to exposure to the challenge virus may largely depends on vaccines that are viable, administered correctly to healthy birds and time is allowed for vaccination in accurate time (Dortmans *et al*., 2012; Kapczynski and King, 2005). Day old chick of backyard poultry are safe from NDV due to their maternal antibodies that protect them at 10-12 days of life but immune response of young age may hamper due to higher level of maternal antibodies (Yosipovich *et al*., 2015). According to Department of Livestock Services (www.dls.gov.bd) through Livestock Research Institute (www.lri.gov.bd) the vaccination schedule of New Castle Disease was BCRD (100 dose) @ 1 drop/eye in 3-7 days of old and booster was done at 21 days of old. Whereas RDV vaccination was done @ 60 days and continued up to every 2 months interval @ 1 cc intramuscularly in thigh muscle of chicken.

Herd immunity is important for preventing the spread of infection in a flock following a primary virus introduction. Herd immunity to ND in broilers can be achieved following vaccination if at least 85% of the flock has haemagglutination inhibition log2 titres for Newcastle disease virus (HI-NDV) is ≥3 (tested against 8 HAU) (van Boven *et al*., 2008). However, maternal antibodies protect chicks during the first 10–14 days of life but high levels of maternal antibodies may interfere with the immune response following vaccination at a young age (Eidson *et al*., 1982; Gharaibeh and Mahmoud, 2013; Yosipovich *et al*., 2015).

Control of NDV with vaccinations is largely practiced by both commercial poultry producers and also on a small scale by backyard poultry rearer. Herd health immunity largely depends on successful vaccinations strategies mention in the above study. Our main objectives of this study was to study the immunity against NDV for native chickens with a series of vaccinations that can be identified using serology. So development of a control strategies for NDV with vaccine schedule to elucidate the immune response for native herd to combat the outbreaks to a new extend is our main key points.

2. Materials and Methods

2.1. Study area and period

The study was conducted at Dhamrai and Savar upazilla in Dhaka district of Bangladesh from 2019 to 2020 for the detection of herd health immunity of NDV of native chickens after successful vaccination campaign with mass vaccination in native chicken and intensive farming system of the area regarding native chickens of the farmers.

2.2. Experimental trial with vaccines

Vaccination with RDV and BCRDV of native chickens were performed after successful deworming (Table 1) @ 14 days before vaccination. A clinical trial was carried out with maintaining a treatment group of approximately 100 with 50 flocks per group. This study was undertaken to gain experience for implementing surveillance and vaccinations programs to endure field research and to improve capacity build up at the farm level of Bangladesh. Native chicken of approximately 0 days-3 months old were selected for the trial. Two groups were selected for this trial where first group referred as treatment group and second group as control group. Available vaccines of NDV of Bangladesh based on the seed strain of lentogenic, F-strain (BCRDV) and mesogenic, Mukteswar strain (RDV) were used for the treatment group of the study. Vaccinations were carried out by field staff and birds were injected with one dose vaccine in intraocular (BCRDV) and intramuscular route (RDV). All the programs of vaccinations were supervised by the registered veterinarian of the selected upazilla. Native chickens of treatment group were vaccinated three times at 3 days, 21 days, and 60 days of age. Secondary programmes of vaccinations were done within 1 km around the treatment flock as to reduce the risk of infection and also to reduce the introduction of virus in the study flock. Although it is not possible at all because biosecurity measures is not so high of this flock. Hence pre and post vaccination blood serum was collected above 60 days of age of birds.

Table 1. Age of vaccines given and sampling from the animals.

Type of birds	Vaccine given/day	Sampling age
Native chicken	3, 21 and 60 days	0, 37, 76 days
Control 100 chicken	21, 60 days	37, 76 days of age

2.3. Collection of samples

Blood samples were collected in 2020 from selected native chicken by venipuncture using sterile needle and then serum was separated from each individual blood sample and transferred to the Animal Health Research Laboratory of Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh and stored at -80^{0}c until use.

2.4. Experiment with serological test

Hemagglutinition Inhibition (HI) test was performed according to standard protocol (OIE, 2012) to detect immunity against NDV of native chicken. A serial two fold dilution as 1:2, 1:3, 1:4, 1:5 and 1:6 was performed in V-bottomed microtiter plates and agglutination was observed to detect the HA unit. Then 1% chicken RBC was prepared using anticoagulant (EDTA) (tube or 4% sodium citrate (one part to four parts blood) or Alsever's solution (equal volume) in the syringe into which the blood was drawn. HI was performed by adding ND antigen with 4HA unit and chicken RBC previously prepared and observed the inhibition at micro titer plate with two fold dilution. Complete inhibition with highest dilution was calculated with logarithmic value. Four $\log_2$ HI titre was considered protective threshold against NDV and below this titer considered as no protection.

2.5. Statistical analysis data

The data of this experiments was performed using student's t- test. Significance in survival rates of chicken were analyzed and *P* value of <0.05 is being considered as statistically significant.

3. Results

In the treatment groups the average titer after vaccinations with BCRDV and RDV was calculated after serological test of the samples. In vaccinated group samples were obtained from 1, 19, 37 and 76 days age of native chickens where antibody titre was found to be 5.00±0.7071, 6.00±0.5477, 6.20±0.4472, and 9.20± 0.7071 respectively (Table 2). In case of control group the average titer was calculated after serological test of the samples. In unvaccinated group samples were obtained from 1, 19, 37 and 76 days age of native chickens where antibody titer was found to be 6.30±0.7071, 5.00±0.7071, 4.20±0.4472, and 4.00± 0.4472 respectively (Table 2). Among the two groups, higher antibody titer was detected 9.20+0.7071 in treatment group after vaccinations at 60 days of age that assume as positive response. As a result, this can protect more than 85% of native chickens. On the other hands, in the control group where unknown vaccine was used along with same maternal antibody. Higher antibody was shown at first day of age that protect the flock at about 50%. As the days passed it reduced to 4.00 + 0.4472 at 76 days. Herd immunity to NDV is obtained whenever the virus is unable to cause a prolonged chain of infections; that is, if no epidemic can unfold after a primary virus introduction. In our context, 'herd immunity' is achieved if the fraction of birds that have a high antibody titre after vaccination exceeds the critical fraction of birds with a high antibody titre.In other words, titre may be as low as 58% or as high as 100%.

Table 2. Detection of HI titer after vaccinations with NDV.

Vaccine	Flock size	HI titer before vaccinations (days)	Age of vaccinations (days)			HI titre after vaccinations (days)				% protection
			1st days	Booster	RDV	1	19	37	76	
BCRDV and RDV	A (n=50)	12.75±3.85	3	21	60	5.00±0.7071	6.00±0.5477	6.20±0.4472	9.20± 0.7071	7 (70)
Control	B (n=50)	13.22±3.75				6.30±0.7071	5.00±0.7071	4.20±0.4472	4.00± 0.4472	0 (00)

4. Discussion

The aim of this study was to study the effectiveness of vaccination with LRI produced vaccine against NDV under field conditions by measuring antibody titers in the HI test and detecting outbreaks of NDV virus infection. The vaccine coverage of a flock was estimated from the titre values. Sentinel birds that were not

vaccinated against NDV were used to record major NDV outbreaks in the flocks included in the trial and were also necessary for correct interpretation of the antibody titres. Without surveillance system it is impossible to be sure whether the measured titers result from vaccination, infection with NDV viruses, or a combination of the two. The average titers after two vaccinations of native chickens in the treatment group was 6.00 which found to be similar with the findings of Kafi *et al*., 2003 and the coverage, here defined as the percentage of samples with a titre of 24 or above, was more than 95%. This trial demonstrates that, with LRI produced homologous vaccines, high antibody titres and a high coverage could be reached, at least in native chickens flocks. Philippa *et al.,* 2005 used a different cut-off value for vaccine effectiveness. They used a titre of >40, a value that is considered to be protective and to reduce virus replication in humans, in a situation in which challenge experiments were not ethical. After three vaccinations the coverage obtained using this cut-off value was approximately 90%. It would be helpful to have more data on the relationship between titre and protection against virus transmission, as has been described before. An important aim of vaccination is to reduce the transmission of virus within and between flocks to obtain herd immunity (Diekmann and Heesterbeek, 2000). It has been suggested that native chickens may be less responsive to vaccination. The lower responses were explained by concurrent diseases or immunosuppressive infections at the time of or after vaccination, but the underlying mechanism of the lower response needs to be explored. It is necessary to study the transmission of the virus in native chickens using vaccination challenge experiments under controlled conditions. Based on preliminary sequencing, the virus isolated from this flock was similar to the occurred only in a flock of the treatment group, one could infer at first sight that vaccination in this group was less effective than in the control group. This cannot be concluded, however. First, it is obvious that the number of outbreaks is far too low to be of any statistical significance. Second, farms were not randomly allocated to the two groups, either treatment or control, because participation was entirely voluntary. Therefore, we cannot exclude the possibility that, among other factors, farm management differed substantially between the two groups. When monitoring HI antibodies induced by vaccination it is essential to ensure that the antibodies are the result of vaccination only and are not caused by infection with an ND subtype virus, or a combination of vaccination and infection. Moreover, direct contact between the sentinels and the vaccinated birds did not occur, as they were all housed in cages. The number of farms included in this study was much lower than calculated for the power analysis. One of the reasons was that farmers were reluctant to participate, and much time and effort was expended on visiting farms and informing the farmers of the trial. In addition to this, the treatment was not allocated randomly, as some farmers were willing to cooperate only when they could apply their own vaccination programme. The programme set up to inform farmers and try to convince them to join the trial experienced difficulties, especially in convincing farmers to implement vaccinations strategy using sentinel chickens. Farmers were worried about the risk of these sentinels being a source of infection to other chickens in the flock.

5. Conclusions

A clinical trial was carried out to monitor vaccine-induced HI titres, vaccination coverage and the number of birds with high titre. The trial was only small-scale, and this prevents the drawing of conclusions about the effectiveness of vaccination in NDV control in Bangladesh. Nevertheless, valuable experience was obtained in the operation of veterinary services, sample handling, quality assurance of laboratory testing, and the implementation of a surveillance strategy using sentinel birds that were not vaccinated against NDV. The results and experiences of this trial may help to develop and improve future surveillance and control strategies in Bangladesh and in other countries in the region.

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Conflict of interest

None to declare.

Authors' contribution

Md. Zakir Hasan: data collection, analysis, reviewing and editing; Sonia Akther: manuscript writing, supervision, reviewing and editing. All authors have read and approved the final manuscript.

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