

Sero Investigation of Newcastle Disease Virus in Pigeons at Chittagong Metropolitan Area, Bangladesh

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Abstract

Newcastle disease is one of the major killer diseases in chicken and pigeon. Pigeon is considered as a fancy bird by the rearer because of its beauty. The squab meat is very nutritious for debilitating human being as it is easily digestible. The objective of this study is to evaluate the clinical signs of Newcastle Disease (ND) in infected pigeon lofts at Chittagong Metropolitan Area and to determine serological status against NDV of these lofts. From 10 pigeon lofts, 100 blood samples were collected and examined with Hemagglutination Inhibition (HI) test. The samples were divided into ten different groups by sample collected from pigeon lofts. Descriptive statistical analysis was performed based on age, breed, housing and their association with system affected by chi-square test through STATA statistical package. Geometric mean antibody titer (GMT) for NDV in serum of pigeon was found to be $\log_2^{3.09}$ although the pigeons were not vaccinated against NDV. Some of the pigeons exhibited nervous and diarrheic symptoms. From the investigation, we can conclude that most pigeons have antibody against NDV in the bottom line of protection. This antibody level is not sufficient to give protection in the birds infected with a velogenic strain of the virus. The owners are advised to administer NDV vaccine routinely and monitor antibody at regular intervals.

Keywords: Pigeons, Newcastle disease, HI, Chittagong Metropolitan area.

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Introduction

Newcastle Disease (ND) ranked among the most infectious diseases of great importance by WHO for Animal Health [1]. It has been identified that it can infect 200 species of birds [2]. The pathogenicity ranges from subclinical infections to highly virulent depending on the susceptibility of the host and the pathotype of the virus [3]. In 1984, the neurotropic ND occurring in pigeons was first recorded in Europe continent, and infection has been reported being transmitted from one type of bird to another without prior adaptation [5, 6]. The avian paramyxovirus-1 (APMV-1) or NDV which is the most pathogenic serotype [7], belongs to the order Mononegavirales. The genus is Avulavirus of paramyxoviridae family and subfamily paramyxovirinae [8]. The variant of APMV-1, Pigeon Paramyxovirus serotype-1 (PPMV-1), causes ND in pigeons and is characterized by the unique monoclonal antibody. NDV genome is negative sense, single-stranded RNA molecule containing 15,186 nucleotides [9]. The pigeon variety of NDV is an enigma, as the virus can produce high mortality in pigeons, yet it can react as a lentogenic virus in susceptible chickens. The only velogenic

strain causes disease in pigeons [10] and produces greenish feces, dull and inappetence, misshapen eggs with decline in production [11] and some egg shell defects [12]. PPMV-1 circulates in domesticated and wild pigeons, but strains of APMV-1 often cause little or no disease in pigeons, although there have been reports of neurological signs. Estimates of virulence for PPMV-1 from pigeons may not be accurate when assessed with routine pathotyping. Serological assays may be useful in some circumstances. Routinely HI test is carried out to monitor and evaluating of antibody response for NDV [13]. However, diagnosis is based on vaccination history, types of vaccine and existing disease condition. [14]; High antibody titer against NDV indicates the birds have been exposed to the virus, but it will not give any clue about circulating strain in that area [15]. Evaluation of exact distribution of ND is difficult due to lack of proper reporting, but the vNDV strains are prevalent in Asia, Africa along with North and South America to some extent [16]. Contacts between feral and racing pigeons in environments favored the spread of

pigeon-strain vNDV through newly introduced ones with subclinical infections or in carrier state [17].

Pigeons are fancy petbirds which are valuable to owners so vaccination should be employed in conjunction with good management practice, biosecurity and good hygiene [18] to control ND. However, due to lack of a specific vaccine for ND in pigeons in Bangladesh, high mortality in pigeons by ND is overviewed. Considering the potential risk of contamination of poultry species by pigeons carrying NDV, it is important to study the pathogenesis of the disease in pigeons. The survey was conducted with the goal of antibody level determination against NDV at different pigeon lofts via area based study to formulate suitable vaccination calendar in pigeons along with coordinated control strategy.

Materials and methods

A preset questionnaire was designed to collect information such as feeding, management, vaccination and signs/symptoms from sampled pigeon. The data thus recorded were stored in Excel sheet. Samples were collected from 10 pigeon lofts suspicious to infect with ND, throughout the Chittagong Metropolitan Area, Chittagong, Bangladesh. Totally 100 sera samples were collected from 10 understudy lofts: Loft I(15 samples), Loft II(5 samples), Loft III(10 samples), Loft IV(10 samples), Loft V(10 samples), Loft VI(25 samples), Loft VII(5 samples), Loft VIII(10 samples), Loft IX(5 samples) and Loft X(5 samples). The informed consent was obtained from pigeon owners. All the pigeons were between the ages of 8 to 24 months. The serum samples were tested using standard HI method for antibodies detection against NDV in the laboratory of Microbiology and Veterinary Public Health Department of the Chittagong Veterinary and Animal Sciences University, Chittagong. The antigen, Avinew® vaccine (Advance Animal Science Co. Ltd. Dhaka, Bangladesh) containing VG/GA strain of ND was used. 5 ml of chicken blood was collected with a disposable syringe containing 1 ml of 4% sodium citrate as an anticoagulant. Blood was centrifuged at the speed of 1500 rpm for 15 minutes. Buffy coat along with plasma was decanted keeping only RBC. 1% of chicken RBC suspension in Normal Saline (NS) was prepared after washing three times with NS. HI, the test was done according to the method mentioned in OIE manual (2002).

In brief, two-fold serial dilution of test serum up to 10th well was made then 4 HA unit of NDV was added upto 11th well and kept at room temperature for 30 minutes. In each well, 1% chicken RBCs

suspension was added. By this way, 12th well was kept for RBC control and 11th well was kept for virus control containing 4 HA unit. The samples showing buttoning of RBCs at the center were considered positive. The end point is a maximum dilution of each sample causing hemagglutination inhibition. The reciprocal of the serum dilution was used to determine HI titer of each sample.

Statistical analysis

The geometric mean titer was estimated, and results were analyzed statistically by applying ANOVA and t-test for the calculation of means/ statistical significance between housing system with system affected in NDV infection in pigeons [Table 2].

Results

From 100 serum samples, 34 were positive in HI test for antibodies against NDV; this gives a seroprevalence of 34%. The antibody titer levels at 4 and above were considered positive. Here, HI titer level ranges from 2²-2⁵. The geometric mean titer was found to be 3.09. The average antibody titer in 10 pigeon lofts (I-X) were 3.3, 2.8, 3.1, 2.9, 3.2, 2.8, 2.4, 3.8, 3.6 and 3.0 respectively (Figure 1).

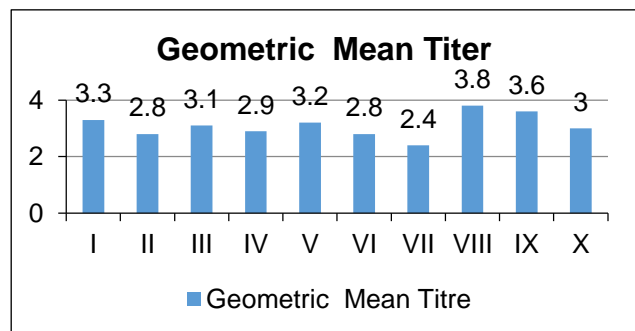


Figure 1: Geometric mean titer of different loft in Pigeon

From farm statistics, we observed that ND infections in breeds of pigeons such as Jalali(26%), Giribaz(29%), Siraji(11%), Fantail(12%) were found higher compared to other(18%). It was found that age-group of 12-24 months were mainly affected and showed the signs of ND. We found that caged pigeons were more susceptible to infections than free ranging and coop housed pigeons (Figure 2,3,4). Insignificant relation ($p>0.05$) was found between the system affected by ND and housing system of pigeons (Table 2). In each farm proportion of pigeons having antibody titer against NDV greater than 8 HI titers were calculated. It was observed that in farm number I, VIII and X 80% of the pigeons have more than 8 HI titer. In farm number 2 and 7 the

least 40% bird has antibody titer more than HI titer (Table 1).

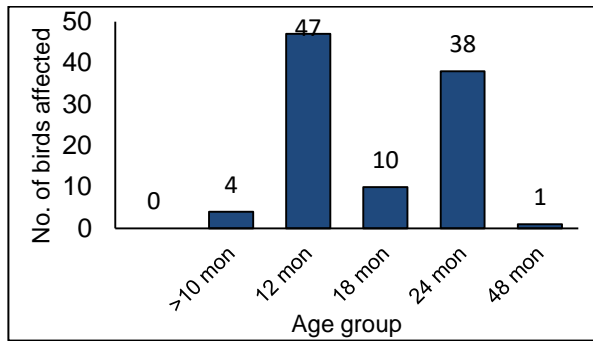


Figure 2: Occurrence of ND in relation to age

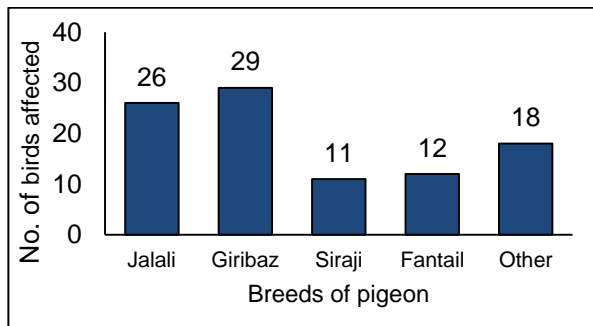


Figure 3: Occurrence of ND in relation to breed

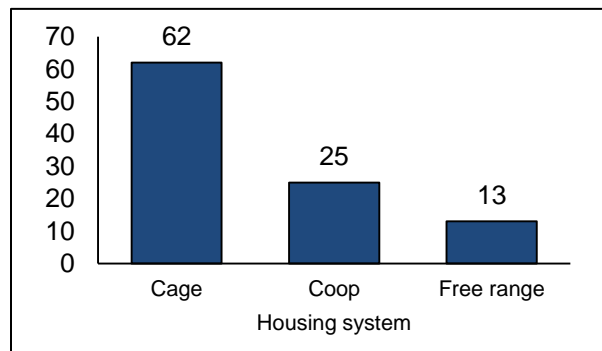


Figure 4: Occurrence of ND in relation to housing system

Table 1: Proportion of farm pigeons having antibody titer greater than 8 HI titer

Loft #	Cut off (No)	Cut off (yes)	Total
I	2(13.33%)	13(86.67%)	15
II	3 (60%)	2 (40%)	5
III	3 (30%)	7(70%)	10
IV	5 (50%)	5 (50%)	10
V	3(30%)	7(70%)	10
VI	11(44%)	14(56%)	25
VII	3 (60%)	2(40%)	5
VIII	2 (20%)	8(80%)	10
IX	7 (40%)	3(60%)	10
X	1 (20%)	4(80%)	5

Discussion

In the present study, antibodies levels in serum of Newcastle Disease (ND) affected pigeons were analyzed to evaluate the serological status in pigeons against ND. Of the total serum samples (n=100) collected from 10 pigeon lofts, only 34% were seropositive for Newcastle virus using Hemagglutination inhibition (HI) test. 66% of birds were negative and had HI titer 2-3. 34% of birds had $2-4 \leq \text{HI titer} \leq 2-5$. Most of the owners did not vaccinate their pigeons against ND though they were aware of the disease. However, many said they had faced ND as the major threat. History of a vaccination program is important in the interpretation of results. Similarly, PPMV-1 was isolated from 67.8% of tested pigeons [19]. Management practices may be responsible for the difference in titer.

From statistical analysis, it was found that age-group of 12-24 months pigeons are more susceptible. Some authors had estimated that morbidity is approximately 10% in adult pigeons, with minimal mortality in the absence of co-infections. Association of Housing system with system affected calculated by chi-square test showed that there was an insignificant relationship ($p > 0.05$) in antibody prevalence between groups and within groups.

Clinical signs observed were greenish white diarrhea, torticollis, incoordination and death. Similar signs were reported [20]. The dead pigeons brought to the hospital were subjected to postmortem examination. Primary lesions observed on necropsy were hemorrhage on proventriculus and button ulcers in the intestine. It is reported that ND as one of the major cause of impaired production [21] and increased mortality [22]. It was reported to cause 70% mortality in experimentally infected pigeons. However, transmission of vNDV strains from poultry to pigeons [23] and vice-versa may occur.

Several serological and molecular tools are applied to diagnose the infection. Hemagglutination inhibition (HI) is commonly used in diagnostic laboratories to determine antibody titers against NDV. Some level of cross-reactivity may be pragmatic among the assorted avian paramyxovirus serotypes in HI test. In this study, we observed antibody titer level ranging from 2-5 although pigeons were not vaccinated. This antibody may be cross reacting antibody with another paramyxovirus which was carried by a pigeon. Thus, most pigeons have antibody against NDV in the bottom line of protection, but it is not sufficient to give protection when there is an outbreak of NDV especially in the case of very virulent NDV. Many

Table 2: Association between rearing system and clinical signs

Housing system	System affected						Total	χ^2	df	p
	A	B	C	D	E	F				
1	25 (3.4%)	17 (13.6)	13 (11.8)	1 (1.24)	3 (1.86)	3 (3.10)	62	11.8	10	0.295
2	7 (6.37%)	3 (2.86)	2 (2.47)	0 (0.26)	0 (0.39)	0 (0.65)	13			
3	17 (12.2%)	2 (5.50)	4 (4.75)	0 (0.50)	0 (0.75)	2 (1.25)	25			
Total	49	22	19	2	3	5	100			

Note: 1= Cage, 2= Coop, 3=Free range, A= No signs, B= Digestive, C=Nervous +Digestive, D=Oral, E= only Nervous, F= Respiratory;

df=Degree of freedom; p=Probability

owners believed that ND vaccine of chicken is not safe to use in pigeons. Some research trials of ND vaccines of chicken in pigeons proved safe to use and twice vaccination at 4 weeks interval could produce protection against ND. Some field data suggested that only birds with HI titer above 16 after twice vaccination will survive a vNDV challenge [24]. Therefore, it is vital to monitor the antibody titer before and after vaccination. The owners are to be advised to administered NDV vaccine routinely and monitor antibody at a regular interval.

The above findings showed that there is dire need to carry out regular vaccination in pigeons against ND. The same recommendation has also been put forward by several others [25-27]. Lately, the oral vaccine like oiled rice coated with I-2 ND strain in chickens [28] has been developed. A similar practice in pigeons and other semi-domesticated birds is required. This was a small area-based study that covered only five different areas in Chittagong district, but a bigger study covering more areas throughout the country and a more thorough questionnaire about management would be necessary to get a more exact picture of the prevalence and other possible problems.

Conclusion

Newcastle disease is prevailing among pigeons in Bangladesh. Signs and symptoms of the disease were greenish or white diarrhea, respiratory distress, and torticollis. Sudden deaths, with few or no preceding clinical signs, were also seen frequently. Prevalence of low level of HI titers in the pigeons may be due to the early phase of infection whereas a high prevalence due to post-outbreak. A large percentage of antibodies against avian PMV-1 and their relatively high titers revealed the presence of virus in the pigeon population. If PPMV-1 strains circulate in chickens, it could evolve hazardous condition in the poultry industry.

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Conflict of Interest

No conflict of interests regarding the publication of this paper.

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