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Newcastle disease sero and viro-prevalence in rural poultry in Chittagong, Bangladesh

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Abstract

Bangladesh experiences some of the highest malnutrition rates in the world, and efforts are being made to increase food security and overall health status. One of the largest constrains on increasing food security is endemic diseases among livestock and poultry populations. Newcastle Disease (ND) is one of these viral endemic diseases reducing food security. However, the sero- and viro-prevalence of ND has not been thoroughly studied in rural poultry in Bangladesh. Knowledge of farm management practices and their effect on ND sero and viro-prevalence is needed before interventions can occur, and efforts to improve the endemic state of ND cannot begin without a baseline study. This cross-sectional study randomly sampled 129 rural households with 245 chickens for the sero-prevalence and active infection rate of rural chickens in two selected upazilas (sub-districts) of the Chittagong district. ELISA was used for the detection of sero-prevalence, and cloacal samples were analyzed for ND presence using one-step RT-PCR. The aims of this study were to describe farmer demography, determine the ND sero-prevalence at the household and individual chicken level, estimate the proportionate ND prevalence at the individual chicken level, determine potential risk factors for ND sero-prevalence at the household level, and determine challenges farmers face with household chicken farming. The overall household level ND sero-prevalence based on ELISA was 31.8% (41/129) (95% CI: 23.9–40.6%), whereas the overall bird level ND sero-prevalence based on ELISA was 21.2% (52/245) (95% CI: 16.5–26.8%). ND prevalence based on RT-PCR was 12.5% (4/32) (95% CI: 3.5–29.0%). The odds ofND sero-positivity was significantly higher in farms belonging to Rangunia than in farms belonging to Anowara with an odds ratio (OR) of 7.8 (95% CI: 3.3–18.6%). The odds of ND sero-

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Author's contributions

Joseph P. Belgrad did field and lab work as well as MS writing. Md. Ashiqur Rahman, Md. Sadeque Abdullah, and Md. Harun Rashid supported the field work. Md. Abu Sayeed supported in data analysis. M. Sawkat Anwer helped in writing proposal and editing MS. Md. Ahasanul Hoque helped in writing proposal and MS and overall supervision of the field and laboratory works.

Ethics

No ethical issues or conflicts of interest occurred with the conduction of research and subsequent writing of this manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2018.09.015.

positivity was significantly lower in poultry house cleaning frequency of once or twice weekly compared with once daily cleaning (OR = 0.3; 95% CI: 0.1–0.8%). High cleaning frequency may produce excessive stress on poultry predisposing them to infection. Poultry rearing is different between Anowara and Rangunia. Anowara (coastal) scavenging areas become restricted because of regular tide flow allowing small fishes and other aquatic animals to be the dominant scavengers in Anowara. The incoming tide also removes viral reservoirs such as feces and dead birds that may otherwise be readily accessed by healthy chickens.

Keywords

Rural poultry; Chittagong; Bangladesh; Newcastle disease; Prevalence

1. Introduction

Nutritional status of children under five is a strong indicator of a country's economic condition and overall health status. Bangladesh experiences some of the highest malnutrition rates in the world with 45% of children under five suffering from malnutrition (Rayhan and Khan, 2006). Between 1975 and 1996, the national prevalence of anemia has remained constant. Among the rural population, 54% of children and 49% of pregnant women are anemic (Ahmed, 2000). More recent studies found that 51.9% of children aged 6–59 months (Khan et al., 2016) and 37% of pregnant women are anemic (Chowdhury et al., 2015). So, while the overall prevalence of anemia is decreasing, a greater effort must still be made to further improve Bangladesh's malnutrition endemic. Proper nourishment during a mother's pregnancy and the first two years of the child's life is important because of this period's impact on structural and cognitive development (Crosby et al., 2013). The consumption of insufficient and poor food is the primary cause of malnutrition for individuals living in rural areas. Malnutrition increases vulnerability to a wide range of physical, mental, and social health problems that persist throughout life (Nord, 2014). Individuals malnourished during early childhood went on to earn up to 20% less than their well-nourished peers (Save the Children et al., 2013), thus continuing the cycle of poverty and malnourishment.

In Bangladesh, the livelihood of people living in rural areas relies on agriculture, and chickens are the livestock most commonly owned by rural families. Increasing chicken production can contribute to food security as the provided eggs and meat can greatly combat malnourishment (Alders and Spradbrow, 2001). Eggs can be stored more easily than most other animal products and provide essential amino acids, calcium, phosphorus, magnesium, iron, zinc, and vitamins A and B. One egg provides 11.5% of daily protein requirements and 5% of daily energy requirements for adults (Branckaert et al., 2000).

While chicken and egg production can greatly contribute to a family's nutritional resources, poor farming practices can lead to endemic diseases such as Newcastle disease. Newcastle disease is a contagious disease of birds and is a large constraint to rural poultry production in developing countries (Awan et al., 1994). Vaccination is one of the most effective controls but is often unperformed, performed incorrectly, or performed irregularly (Abraham-Oyiguh et al., 2014). The disease has significant economic impact as the disease decreases body

mass and egg laying capabilities (Alexander, 1992), and has also been associated with mortality rates as high as 100% without clinical signs, even amongst vaccinated poultry due to improper or incomplete vaccination techniques (Chukwudi et al., 2012). ND presents in the following three broad categories: velogenic (highly virulent), mesogenic (intermediate virulence), and lentogenic (nonvirulent). Approximately nine strains of NDV have been distinguished through the pathogenicity test (Alexander, 2000) with most of infections being caused by velogenic strains rather than mesogenic or lentogenic strains (Hasan et al., 2010). Data exists on the prevalence of ND in rural communities in Africa (Abraham-Oyiguh et al., 2014; Lawal et al., 2016), and on chicken mortality rate due to ND in Bangladesh (Biswas et al., 2005, 2006). However, an understanding of the overall NDV prevalence and sero-prevalence within live populations is lacking.

The aims of this study were to describe farmer demography, determine the ND sero-prevalence at the household and individual chicken level, and estimate the proportionate ND prevalence at the individual chicken level. Additional aims were to determine potential risk factors for ND sero-prevalence at the household level, and challenges farmers face with household chicken farming.

2. Materials and methods

2.1. Description of the study site

Anowara (costal) and Rangunia (inland) upazilas (sub-districts) of the district of Chittagong, Bangladesh were selected for the present study which was nested within the ongoing intervention study on rural poultry supported by Leveraging Agriculture for Nutrition in South Asia (LANSA) project (a UK funded project). Anowara is situated at 22.2167 °North and 91.9111 °East, whereas Rangunia is situated at 22.4667 °North and 92.0833 °East (latitude.to, 2017). The spatial location of the study sites was identified in the digitized map (Fig. 1) using ArcGIS-ArcMap version 10.2 (ESRI, USA) software. The number of sampled households with number of sampled chickens from each upazila is shown using a bar diagram in the map (Fig. 1).

2.2. Study design, sample size, and sampling

A complete list of households having at least one chicken belonging to 3 villages in Anowara (Raipur, Sorenga, and Gohira) and 2 villages in Rangunia (Tinchoudia and Lalanagor) of the Chittagong district has been developed through the UK funded LANSA Project of CVASU, Bangladesh for an intervention study of a total of 150 households. To investigate the ND sero-prevalence, a total of 123 households were required for a cross-sectional study within the intervention study assuming 50% expected ND sero-prevalence as there is no published report, ± 5.0% precision, with a 99% confidence interval and 1 design effect (Dean et al., 2018). However, the current study recruited a total of 129 households from the intervention study. A random sample of 1–2 chickens were selected from each household based on the chickens brought forth by the villagers for testing. A total of 245 chickens were sampled. Sampling took place over a total of five days during the months of June and July of 2017. A specific place per village was used for sampling chickens.

2.3. Sample collection, preservation, and transportation

Whole blood samples (0.5–3 ml, in all cases < 1% of body weight) were drawn aseptically from the medial metatarsal, jugular, or ulnar veins and then immediately transferred to individual sterile Eppendorf tubes (1.5 ml) with unique identity numbers. Blood samples were then transferred to the laboratory of Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh using an insulated cool box within 5–6 h. Blood samples were subsequently allowed to clot at ambient temperature on the bench in the laboratory, kept refrigerated overnight at 4°C, and then centrifuged at 10,000 rpm for 10min to separate the serum. Serum samples were then transferred to cryovials (1.5 ml), preserved at –20 °C, and tested within 15 days.

Cloacal and oropharyngeal swab samples were taken from birds by inserting and swabbing sterile cotton tipped applicator sticks deeply into the vent or oropharynx of each individual chicken. Each of the cloacal and oropharyngeal swab samples was placed separately into a vial containing 1 ml of sterile Viral Transport Medium (VTM), prepared according to the recipe described by Healing and Organization (Healing, 2006), with unique identity numbers. The samples were then stored in an insulated container with ice packs until transfer within 5–6 h to a –80 °C freezer at the CVASU laboratory.

2.4. Epidemiological data collection

A pretested questionnaire took the following information regarding households sampled: chicken and duck population size; household type (mixed versus single farming) and location; bird ages, sex, breed, and vaccination status against NDV. Supplementary data were also collected on Farmers' socio-economic status such as education level and primary sources of income. Data on household management practices included source of chicken feed, proximity to commercial poultry farms, poultry housing type, cleaning practices, litter and dead chicken disposal, and primary challenges to poultry farming. The full questionnaire can be viewed in supplemental Table 1.

2.5. Newcastle disease serology: ELISA

Serology samples were tested for anti-NDV antibodies in chicken serum using the IDvet ID Screen® Newcastle Disease Indirect ELISA kit according to manufacturer instruction (Catalog No. NDVS-CV-5 P). The optical density (OD) of the final plate was recorded at 450 nm. The results were interpreted as per the manufacturer protocol as follows with an S/P value > 0.3 as positive.

2.6. NDV RNA extraction

Cloacal swab samples (N = 245) were selected for viral RNA extracts using the Thermo Fisher Scientific PureLink™ RNA Mini Kit (Catalog No.12183018 A). The protocol used was provided in the manufacturer's laboratory manual and titled "Purifying RNA from Liquid Samples/RNA Clean-Up." Samples were pooled in batches of eleven and twelve producing nineteen pools of eleven (19*11 = 209) and three pools of twelve (12*3 = 36). All pool extracts were tested using RT-PCR as described below. However, we took 32 individual samples from the three pools with the lowest Ct (Florescent cycle threshold) values (highest positivity) for individual RNA extraction followed by RT-PCR testing.

2.7. NDV RNA real time PCR

Published One-step Real Time Polymerase Chain Reaction (RT-PCR) was used to evaluate RNA pooled extracts from cloacal swab samples for the presence of NDV (APHA, 2015), UK Protocol. Molecular detection of NDV RNA was performed by using one-step RT-PCR directed at the fusion (F) gene described by Kim et al. (2013). Any sample that was reactive to the assay (threshold Ct value ≤40) was considered as reactive for NDV.

QuantiFast® Probe RT-PCR Kit (Qiagen; Catalog No. 204454) was used for the detection of NDV in the viral extracts in ABI Fast Real-Time PCR Machine (ABI 7500).

The components of the master mix along with thermal profiles and the sequences of the primers and probes have been given in the supplemental Tables 2 and 3.

2.8. Statistical evaluation

Field and laboratory data were entered into Microsoft Excel 2007 spread sheet. Data were cleaned for errors and inconsistencies, sorted, coded and checked for integrity in MS Excel 2007. Afterwards, data were exported to STATA-IC-13 *(StataCorp, 4905, Lakeway Drive, College Station, Texas 77845, USA)* for conducting epidemiological analysis.

2.9. Descriptive analysis

Descriptive statistics (frequency number, percentages, mean, median, minimums and maximums) were calculated to express farmers and farm demographic characteristics and management information. The ND sero-prevalence based on ELISA was estimated both at the household level and the individual bird-level (with or without considering households as cluster variables). As more than one bird per household was also sampled and birds within household were correlated, “Household” was therefore considered as a clustered variable to adjust the cluster effect in calculating bird-level ND sero-prevalence. We used the following example commands in the STATA software:

svyset, clear

svysetHouseholdID

svy: prop ELISA_ND

The ND prevalence based on RT-PCR was estimated at the individual bird level using both pooled cloacal swabs and individual cloacal swab samples. The results of ND sero-and viro-prevalence were expressed in percentages and 95% confidence intervals. The major constraints faced by the farmers were also expressed in frequency numbers and percentages.

2.10. Risk factors for analysis

Risk factor analysis for the ND sero-prevalence based on ELISA was performed at the household level. A household having at least one ND sero-positive sample to ELISA was defined as a case household when assessing risk.

2.10.1. Risk factors for univariable analysis—Initially, thirteen different factors were considered for the univariable risk factor analysis as follows: 1) study sub-district

(Anowara or Rangunia), 2) farm size, 3) whether the flock included ducks, 4) chicken age, 5) chicken house cleaning frequency, 6) chicken feeder cleaning frequency, 7) chicken water cleaning frequency, 8) dead bird disposal, 9) litter disposal method, 10) frequency of litter disposal, 11) whether rice bran was fed, 12) whether whole rice was fed, and 13) whether cooked rice was fed. The response variable was used as binary (ND sero-positive household (Yes/No)).

Univariable chi-square tests were performed to evaluate association between binary response variable of ND sero-positive household (Yes/ No) and the thirteen independent variables.

2.10.2. Multivariable analysis—Factors with a trend toward significance ($p = 0.1$ or less in chisquare testing) were initially considered for inclusion in the multivariable analysis. Backward stepwise logistic regression analysis was applied to fit the model. At first a full model was run and only variables with $p \leq 0.05$ in the likelihood ratio test were retained. Biologically plausible interactions among the main factors were also tested and retained in the final stage if significant ($p \leq 0.05$). To improve the fit of the models, the variables of "Poultry house cleaning frequency" Parity, "Litter disposal" and "Dead bird disposal" were re-categorized during this stage. The "Poultry house cleaning frequency" variable was divided into "once daily" and "once or twice weekly/ once per three or four weeks". The "Litter disposal" variable was divided into "Spread on fields/Throw in bushes/Pond/Lake/ Canal/Sea" and "Compost/Bury/ Throw in pit/Left in yard." The "Dead bird disposal" variable was divided into "Feed to other animals like fish/Throw in bushes/Road/ Pond/ Lake/Canal or Sea" and "Bury and throw in pit". Confounding was checked by re-adding, one by one, the variables removed in the stepwise backward procedure. A variable was considered a confounder if its removal made the regression coefficients of the remaining variables showed a relative change (≥15%) (Dohoo et al., 2003). We tested for collinearity between categorical factors using the two-tailed p value using the Fisher's exact test (Hoque et al., 2015). Two factors were considered collinear if the p value was ≤0.05. The sensitivity of the final model was then assessed for goodness-of-fit using the Hosmer-- Lemeshow test described by Dohoo et al. (2003) while the post estimation of predictive ability was determined using the receiver operating characteristics (ROC) curve (Dohoo et al., 2003). The outputs were presented for each adjusted predictor variable as an OR, p value, and 95% confidence interval.

3. Results

3.1. Farmer and farm management demographics

Statistical analysis of farmer socioeconomic status data taken from Table 1 shows that most household farmers were below the age of 36 years. The farmers were mostly female and illiterate. The majority of household chicken owners also had some other self-business such as a small shop or food stand or making household items from raw materials as their primary form of income rather than chicken farming. Table 2 displaying farm demographics and management shows most of the rural chickens were unvaccinated against NDV, and most of chickens scavenged for food on household premises. Poultry also mixed with neighboring

poultry. Housing ventilation was largely performed by wall openings, and whole rice was the major food source.

3.2. ND sero- and viro-prevalence

The overall household level ND sero-prevalence based on ELISA was 31.8% (41/129) (95% CI: 23.9–40.6%), whereas the overall bird level ND sero-prevalence based on ELISA was 21.2% (52/245) (95% CI: 16.5–26.8%) and 21.2% (52/245, household as cluster = 129) (95% CI: 16.0–27.6%).

All 22 cloacal swab pools (100%) were RT-PCR positive. Of the 32 individual cloacal swabs obtained from 3 positive pools with the lowest Ct values in RT-PCR testing 12.5% (4/32) (95% CI: 3.5–29.0%) were positive.

3.3. Household level risk factor analysis

Univariable analysis between farm demographics and management practices compared with ELISA results for NDV in Table 3 shows that the five areas that included statistical significance at $p \leq 0.1$ were subdistrict ($p < 0.001$), farm size ($p = 0.058$), poultry house cleaning frequency ($p = 0.02$), dead bird disposal ($p = 0.09$) and litter disposal methods ($p = 0.05$). Table 4 presents the outputs of the multivariable analysis of sub-district and poultry house cleaning frequency. Neither confounding or interaction was detected in the model. The odds of ND sero-positivity was significantly higher in farms belonging to Rangunia than in farms belonging to Anowara with an odds ratio of 7.8 ($p < 0.001$). The odds of ND sero-positivity was significantly lower in poultry house cleaning frequency of once or twice weekly compared with once daily cleaning frequency (OR = 0.3; $p = 0.012$).

3.4. Challenges of household chicken rearing

Table 5 summarizes the biggest challenges associated with chicken farming with the top threat being disease and mortality followed closely by predators.

4. Discussion

In the sub-districts of Anowara and Rangunia of Chittagong, Bangladesh, Newcastle Disease Virus sero-prevalence is influenced by a variety of associated factors ranging from geographic location to frequency of chicken house cleaning. ND sero-prevalence and its associated factors are discussed in further detail below.

The multivariable association model showed that the most statistically significant factors for ND sero-positivity was the sub-district of Rangunia with an odds ratio of 7.8, followed by poultry house cleaning frequency with once or twice weekly cleanings having an odds ratio of 0.3 compared with daily cleanings. Clearly, farm management practices play a large role in the sero-positivity of ND. Daily cleaning may increase the spread of NDV due to disturbing the litter of chickens that are actively infected. A study conducted by Anderson et al. (1966) showed that increased dust, ammonia, and CO_2 that would be increased through high cleaning frequency did not produce a statistically significant increase in NDV infection rate. However, the study did not consider NDV harbored within the feces or stress to the

birds caused by the cleaning, and thus frequent disturbance of feces with NDV may in fact increase NDV infection rate. Furthermore, everyday cleaning may prevent the chicken house from fully drying, especially during the wet season. The damp environment may also allow for the survival of NDV in the chicken houses (Boyd and Hanson, 1958). Stress is another major factor that should be considered when chicken houses are cleaned. Mohamed and Hanson (1980) showed that NDV was more invasive and attained higher titers in stressed birds. Daily disturbances through frequent cleaning may lead to an increased stress response in rural poultry potentially leading to higher NDV infection rates and sero-prevalence. Future studies should be conducted to further assess whether the increased sero-prevalence of NDV is due to frequent cleaning or stress.

The most statistically significant variable for NDV sero-positivity was chickens within the sub-district of Rangunia with an odds ratio of 7.8 compared with Anowara ($p < 0.001$). Poultry rearing is different between Anowara and Rangunia. Anowara (coastal) scavenging areas become restricted because of regular tide flow allowing small fishes and other aquatic animals to be the dominant scavengers in Anowara. The incoming tide also removes viral reservoirs such as feces and dead birds that may otherwise be readily accessed by healthy chickens.

Overall, the lack of individuals vaccinated against NDV indicates that most if not all sero-prevalence is due to endemic NDV infections which are exacerbated due to poor farm management practices. Through vaccination usage, the endemic state of NDV in Bangladesh could be reduced. As shown in Table 5 disease and mortality play the largest role in constraining chicken farming emphasizing the need for this research and further research in infectious diseases in rural Bangladesh. Further efforts made to decrease the endemic state of NDV could greatly increase the food security of rural farmers of the country. By increasing the food security, Bangladesh can make efforts to reduce its malnutrition rate and economic stability.

In continuing future research, more data could be collected on the active infection prevalence through more RT-PCR. The NDV RNA prevalence obtained from this study, while small in sample size, indicated a prevalence of 12.5%. If this were to be extrapolated to the entire population in the study, it would indicate that roughly half the sero-prevalence is due to past infections highlighting the endemic nature of NDV in the study regions. Further analysis comparing the costal subdistrict of Anowara and the inland sub-district of Rangunia could offer insight as to whether disposal of dead chickens in the sea would help mitigate the prevalence of NDV. More data could also be collected from around other districts in Bangladesh to offer a larger picture of the overall endemic state of NDV.

Sources of error for this research could be explained through possible contamination of samples as samples were collected quickly and might have been exposed to each other. Errors could also have occurred in viral extraction if RNAses destroyed any of the individual cloacal samples. The greatest limitations to the study were time and money. The entire collection period lasted only two months, and funds were only available to perform 100 viral extractions, hence the necessity of pooling the samples. All pools tested positive, so rather than report a prevalence rate of 100%, 32 individual samples were chosen from the three

pools with the lowest Ct values with the understanding that the shedding prevalence statistical power was greatly reduced due to the diminished sample size. Since those samples with the lowest Ct values (most positive pools) were chosen, the viro-prevalence may be overestimated. Another source of error may have been due to the small within farm samples size of 2 chickens per household. Though the farms sampled ranged from 2 to 20 in size, farmers were not willing to let us take samples from more than 2 chickens. Therefore, farms may have been misclassified as negative because of a low level of sampling. To account for the variation of sero-positivity between household farms, a 1 design effect was considered in sample size calculation. There were some cases of informational biases from the farmers when answering questions. One of the questions was frequency of hand washing before handling chickens with the follow up question asking if they used soap. Several times farmers indicated that they never washed their hands before handling chickens while also indicating that they used soap, an obvious impossibility that either shows that the questions was not understood completely, or a biased answer was given. Similarly, farmers may have indicated that they cleaned their poultry houses daily rather than weekly, bi-weekly, etc. to appear a more responsible caretaker. The age of those questioned also had a considerate range from less than 14 to more than 70 with varying degrees of education which may have impacted how the questions were answered or whether the questions were even understood at all. However, despite these potential errors, these data still offer valuable insight into the endemic nature of ND in Chittagong, Bangladesh due to the relatively large sample size and trained veterinary interviewers using the local language.

5. Conclusion

These data and analysis presented by this research offer valuable insight into the endemic state of NDV in Chittagong, Bangladesh. Rural household owners cannot readily afford to vaccinate their poultry against NDV. Therefore, other measures are necessary to reduce the prevalence of NDV. High cleaning frequency may produce excessive stress on poultry predisposing them to ND infection. Poultry rearing is different between Anowara and Rangunia. Anowara (coastal) scavenging areas become restricted because of regular tide flow allowing small fishes and other aquatic animals to be the dominant scavengers in Anowara. The incoming tide may also potentially remove viral reservoirs such as feces and dead birds that may otherwise be readily accessed by healthy chickens though further research to this effect is warranted. Results of the present study suggest that simple changes to farm management practices such as reducing cleaning frequency and proper waste removal may greatly decrease the overall prevalence and sero-prevalence of NDV. This study also identified areas in which further research could be conducted to further benefit the rural and scientific community.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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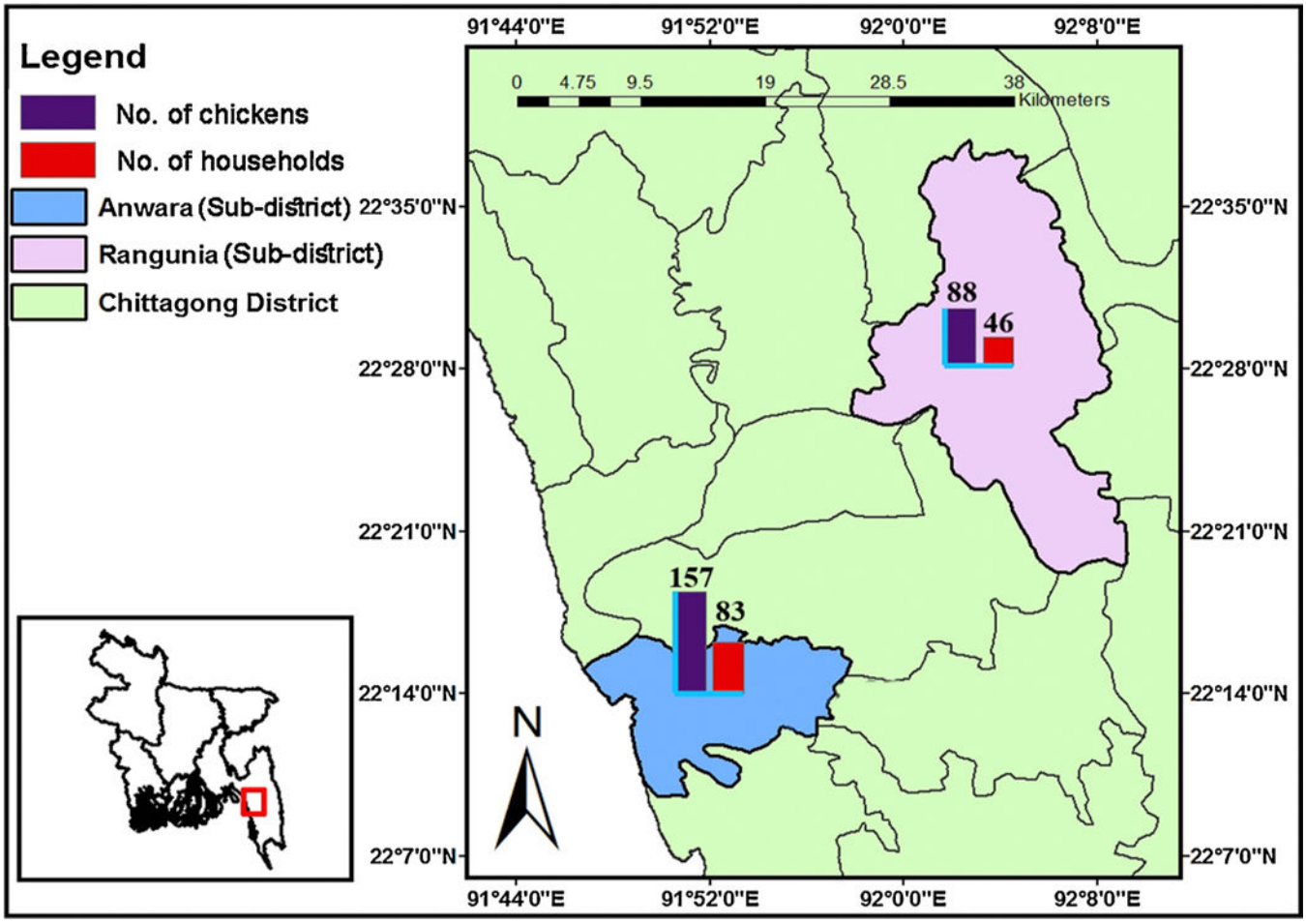

Fig. 1.
Geographical location of study areas with sampled households and chicken number.

Table 1

Household poultry farmers' demographic characteristics in Chittagong, Bangladesh June – July 2017. (N = 129).

Variable	Category	Frequency	Percentage	Mean	Median	Minimum-Maximum
Farmer age (Year)	≤15	4	3.1	14.8	15	14–15
	16–36	63	48.9	29.8	30	17–36
	37–45	31	24.0	41.5	40	37–45
	≥46	31	24.0	55.5	50	46–70
Sex	Male	27	20.9			
	Female	102	79.1			
Farmer Education	Illiterate	66	51.2			
	Primary	35	27.1			
	SSC/HSC/Graduate	28	21.7			
Duration of farming (Years)	≤2	5	3.9	2	2	2
	3–12	60	46.5	7.9	8	3–12
	13–20	36	27.9	17.9	20	13–20
	≥21	28	21.7	32.4	30	21–50
Farmers income source	Agriculture	23	17.8			
	Self-business	61	47.3			
	Service	45	34.9			

SSC: Secondary School Certificate; HSC: Higher Secondary Certificate.

Table 2

Frequency distribution of household poultry farm demography and management in Chittagong, Bangladesh June – July 2017. (N = 129).

Variable	Category	Frequency	Percentage	Mean	Median	Min-Max
Chicken farm size	≤2	10	7.7	1.7	2	2
	3–8	62	48.1	5.3	5	3–8
	9–11	29	22.5	10.1	10	9–11
	> 11	28	21.7	15.2	15	12–20
Duck farm size	0	15	11.6	0	0	0
	1–4	74	57.4	2.9	3	1–4
	≥5	40	31.0	7.2	6	5–15
Chicken age (months)	Below 5	14	10.9	0	0	0
	1 chicken over 5	38	29.5	1	1	1
	≥2 chicken over 5	77	59.7	5.1	3	2–20
Chicken with ND vaccine	Not vaccinated	114	83.4			
	No information	15	16.6			
Chicken deworming	No	109	84.5			
	Yes	5	3.9			
	No information	15	11.6			
Scavenging areas	Household premises	123	95.4			
	Paddy field	2	1.6			
	Both	4	3.1			
Mix with neighbors' chicken	Yes	128	99.2			
	No	1	0.8			
Distance from commercial farm (meter)	≤120	5	3.9	102	120	60–120
	121 to 492	63	48.8	332.7	300	150–492
	493 to 984	21	16.3	764.6	792	600–792
	≥984	40	31.0	984.3	984.3	984.3
Bamboo poultry house	Yes	12	9.3			
	No	117	90.7			
Mud poultry house	Yes	22	17.1			
	No	107	82.9			

Variable	Category	Frequency	Percentage	Mean	Median	Min-Max
Concrete poultry house	Yes	42	67.4			
	No	87	32.6			
Metal poultry	Yes	40	31.1			
	No	89	68.9			
Poultry house location	No house	2	1.6			
	Yard	52	40.3			
	Within living room	75	58.1			
Housing ventilation	No house	2	1.6			
	Wall openings	59	45.7			
	Open air	40	31.0			
	No ventilation	28	21.7			
Feed rice bran	Yes	62	48.1			
	No	67	51.9			
Feed whole rice	Yes	84	65.1			
	No	45	34.9			
Feed cooked rice	Yes	54	41.9			
	No	75	58.1			
Feed food scraps	Yes	2	1.6			
	No	127	98.4			
Feed commercial feed	Yes	3	2.3			
	No	126	97.7			

Table 3

Univariable association between household chicken farm demographic and farm management factors with the binary ELISA results of antibody of Newcastle disease at the household level in Chittagong, Bangladesh June–July 2017. (1=Yes; 0 = No) (N = 129 farms).

Variable	Category	ELISA n/N (%)	p-value (Chi-square test)
Farm size	min-4	16/33 (48.5)	0.058
	5–8	10/39 (25.6)	
	9–20	15/57 (26.3)	< 0.001
Sub-district	Anowara	14/83 (16.9)	
	Rangunia	27/46 (58.7)	
Having ducks	Yes	34/114 (29.8)	0.18
	No	7/15 (46.7)	
Chickens over 5 months of age	0	6/14 (42.9)	0.63
	Only one	12/38 (31.6)	
	≥2	23/77 (29.9)	
Poultry house cleaning frequency	Once daily	17/32 (53.1)	0.02
	Once or twice weekly	20/80 (25.0)	
	Once per three or four weeks	4/14 (28.6)	
Feeder cleaning frequency	Every time after use	36/109 (33.0)	0.6
	Once in a day	3/15 (20.0)	
	Once in a week	2/5 (40.0)	
Waterer cleaning frequency	Every time after use	9/28 (32.1)	0.8
	Once in a day	2/10 (20.0)	
	Once per three or four weeks	1/3 (33.3)	
Dead bird disposal	Bury and throw in pit	17/36 (47.2)	0.09
	Feed to other animals	1/3 (33.3)	
	Throw in pond/lake/canal or sea	18/76 (23.7)	
	Throw in bushes/road side	4/13 (30.8)	
Litter disposed	Spread on fields/throw in bushes	15/42 (35.7)	0.05
	Compost/ bury/throw in pit/left in yard	18/43 (41.9)	
	Throw in pond/lake/ canal/sea	8/44 (18.2)	
Litter removed	Once in a day	12/30 (40.0)	0.3

Variable	Category	ELISA n/N (%)	p-value (Chi-square test)
	Once in a week	18/53 (33.9)	
	Twice or more in a week	11/46 (23.9)	
Feed rice bran	Yes	16/62 (25.8)	0.16
	No	25/67 (37.3)	
Feed whole rice	Yes	29/84 (34.5)	0.4
Feed cooked rice	No	12/45 (26.7)	0.4
	Yes	15/54 (27.8)	
	No	26/75 (34.7)	

Table 4

Multivariable association between potential factors with the binary ELISA results of antibody against Newcastle disease at the household level in Chittagong, Bangladesh, June – July 2017. (1=Yes; 0=No) (N=129 farms) (Logistic Regression Model Outputs).

Factor	Category	OR	95% CI	P
Sub-district	Anowara	Ref		
	Rangunia	7.8	3.3–18.6	< 0.001
Poultry house cleaning frequency	Once daily	Ref		
	More (Once in a week/Twice or more in a week)	0.3	0.1–0.8	0.012

Table 5

Frequency distribution of major constrains faced by the farmers at household farms in Chittagong, Bangladesh June–July 2017.

Variable	Category	Frequency	Percentage
Challenge	Predators	40	31.0
	Disease and mortality	43	33.3
	Food/water availability	18	13.9
	Flood/tide water/heavy rainfall	20	15.5
	Toxins/poisoning	8	6.2