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Sero-prevalence and Associated Risk Factors of Avian Influenza Virus Infection in Backyard Chicken at Sylhet Region, Bangladesh

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ABSTRACT

Avian Influenza (AI) infection persists in the northeastern part of Bangladesh, Sylhet region. The presence of AI as natural infection in backyard chicken was recently carried out by one of our pilot studies using rapid antigen detection kit from cloacal swab sample. This study aimed at assessing sero-prevalence (antibody positivity) and associated risk factors of avian influenza (AI) infection in three locations of Sylhet region by observational epidemiological and questionnaire-based approach. A total of 282 individual back-yard chicken serum raised under free ranging or semi-intensive housing system; pooled to 156 household sample was subjected to serological examinations (ELISA and Hemagglutination inhibition test). Among the Univariate odds ratio of the risk factors and prevalence: feeding rice bran, poultry house location, mud house, bamboo house, Upazilas location and contact with wildlife variables result was significant. To be precise, our data shows a significant association of AI risk for poultry houses in yard rearing system (1.48-11.61% of 95% CI of OR). Also, seroprevalence was higher in backyard chicken encountered with wild birds (1.34-8.21 of 95% CI of OR). Surprisingly, mud Poultry house and feeding rice bran reduced AI seropositivity as a contributing risk factor (1.48-10.68% and 1.48-15.15% of 95% CI of OR, respectively) in backyard chicken. Based on our findings, an overall higher prevalence of 54.84 and 25.83% was observed from samples of Dakshin Surma and South Sunamganj upazila, respectively whereas the lowest prevalence of 25.83% was observed in Kanaighat upazila. Findings from this study merit that bird-level AI infection persists in the study location and associated risk factors or protective factors needs to be further assessed.

Key words: Avian Influenza, Sero-prevalence, Risk factor, ELISA.

INTRODUCTION

Poultry rearing has been an integral part of human livelihood since they are domesticated and reproduced under human care. Approximately 80% of rural households in developing countries is engaged in poultry rearing and of which chicken contributes 92% of total poultry population (FAO 2020; Du et al. 2023). According to the recent reports of Department of Livestock services Bangladesh, chicken population is around 275.81 million and backyard chicken contributes the livelihood and social needs of rural families (Islam et al. 2015; DLS 2020; Kencana et al. 2023).

The majority of rural households have indigenous chickens that are raised under backyard /semi-intensive or free-range farming system. Such traditional family-based rearing of backyard chicken became an important source of income generation, safe protein consumption and uplifting of socio-economic livelihood. However, a major constraint to chicken rearing is avian viruses which adversely affect the poultry health and productivity. It has been estimated that avian virus accounts for around 28% of infection in backyard chicken rose under traditional farming system (Owoade et al. 2006). Of all avian viruses, avian influenza outbreaks resulted massive culling of poultry and severe economic losses to farmer in Bangladesh (Biswas et al. 2008). Unfortunately, in Bangladesh avian influenza problem is often overlooked due to mild/subclinical (LPAI form) infection occurs after viremia (Biswas et al. 2008; Islam et al. 2023).

Avian influenza is a highly infectious notifiable disease of poultry and wild birds and regarded as a risk group-2 pathogen by OIE (OIE 2009). The causative agent of avian influenza belongs to Orthomyxoviridae family and

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is specified to the genus Influenza A (Chen et al. 2006; Garten et al. 2009). Based on the surface protein Haemagglutinin (H) and Neuraminidase (N) subtypes (H5 & H7) two different Pathogenic form of Avian influenza are circulating; namely: highly Pathogenic Avian Influenza (HPAI) that causes systemic infection and low pathogenic Avian Influenza (LPAI) causes respiratory infection (OIE 2009; Tong et al. 2013; Islam et al. 2023; Suardana et al. 2023).

The virus is prevailing in developing countries like Egypt, Indonesia, India and northern zone of Bangladesh (Biswas et al. 2008). AI (Bird flu) also poses threat to public health in Bangladesh after first reported human case (Brooks et al.2009). But the major threat remains infection to poultry which are intensified in recent years after recurrence episode of Avian Influenza (HPAI) in Bangladesh (Ahmed et al. 2010; Carnegie et al. 2023).

The risk factors that favoring HPAI and LPAI outbreaks are pre-dominated by live bird marketing (LBM), biosecurity concern, geographical locations and frequent exposure to wild birds could result a silent sero-positivity and asymptomatic Avian influenza (Hassan et al. 2020). Hence, it is expected that Avian Influenza infection could persist in the northeastern part of Sylhet region due to availability of risk factors. To our understanding, there is presence of natural infection in backyard chicken as a pilot study recently carried out by rapid antigen detection kit using cloacal swab sample (unpublished data; information retrieved by personal communication) in three different upazilas of Sylhet region namely: Dakshin Surma, South Sunamjong and Kanaighat. Apparently, avian influenza is prevalent in Sylhet but the epidemiological parameters and their association with AI infection is poorly understood.

Besides, epidemiological information regarding infection burden and associated risk factors in Bangladesh is mainly based on passive reporting (Ahmed et al. 2010). So, an in-depth sero-survey could be a handy tool to exploit the sero-epidemiology and associated risk factor of infectious disease like Avian Influenza. Development of such sero-surveillance strategies using modern epidemiological approach for AI will embark opportunity to explore host and risk factor analysis in different topographical areas of Bangladesh (Ahmed et al. 2011).

MATERIALS AND METHODS

Ethics Statement

The Animal Use and Ethics Committee of Sylhet Agricultural University approved this research in accordance with the guidelines (Memo no: SAU/Ethical committee/AUP/20/05). All invasive sample (Blood) collection was handled and maintained by the guidelines and regulations established by the Bangladesh Veterinary Council for handling biological materials.

Study Area

Three different upazilas (Sub-district) of the Sylhet region- namely South Sunamganj upazila (location: 24°49' and 25°10' north latitudes and in between 91°14' and 91°27' east longitudes), Dakshin-Surma (location: 24°43' and 24°54' north latitudes & in between 91°47' and 91°58' east longitudes), and Kanaighat (location: 24°53' and 25°06' north latitudes & in between 92°01' and 92°26' east longitudes) were chosen for study to be carried out. Attention to selected areas was based on the hypothesis of availability of unvaccinated backyard chicken, not knowing the previous epidemiological record and the convenience of sampling (Fig. 1).

Study Design, Target Population, Sampling Method and Sample Size Estimation

A cross-sectional observational study was performed from July 2020 to June 2021 to determine the avian influenza burden in backyard chicken raised under free ranging or semi-intensive housing system from study area. A complete list of households having at least four (4) backyard chickens will be generated belonging to 2 villages in each of selected study area. Systematic random sampling technique with an interval N/n (i.e., the population size divided by the sample size) will be conducted from individual back-yard chicken emphasized on serological examination. The approximate sample size required to estimate sero-prevalence will be based on the use of software WINPEPI (WINPEPI: Describe: K. Sample size…: Estimating a proportion: Systematic random sample) designed by The University of Edinburgh by depicting the formula of Thrusfield (2005) at 50% expected prevalence with 5% absolute precision and 95% confidence interval (CI).

$n = \{z^2 P_{exp.} (1\text{-}P_{exp})\}/d^2$,

Where: n = required sample size,

$P_{exp.}$ = expected prevalence,

d = desired absolute precision,

z = multiplier from the Normal distribution (1.96 at 95% CI)

Therefore, by substituting the values of variables in WINPEPI software and Pocket calculator the sample size will be 385, which is used as representative bird to know AI Prevalence.

Sample Collection

To estimate individual bird level sero-prevalence, blood from individual chicken will be aseptically withdrawn and let it clot at 56°C for 45min. serum was separated from clotted blood cells by centrifugation for 5min at 2000rpm and used to determine antibody titer by ELISA (Enzyme-linked Immuno-sorbent Assays) and HI (Hemagglutination Inhibition) test as referred by OIE terrestrial manual 2018. Rest of the unused serum was stored at -20°C.

Serological Examination

ELISA Test

To confirm bird- level antibody presence, a highly sensitive ELISA test was conducted. ELISA test was performed by using a commercial BioChek ELISA kit (Product Code: CK121; OIE registration number 20080203) to measure the amount of antibody to AI in the serum of chicken according to the protocol provided by the manufacturer. The sample and control optical density (OD) values were measured using an ELISA reader (Multiskan FC, Thermo scientific) at 405 nm. Samples with an S/P ratio of 0.5 or greater contain anti-AI antibodies and are considered positive. The presence of antibodies against AI in the absence of vaccination indicates the bird has been

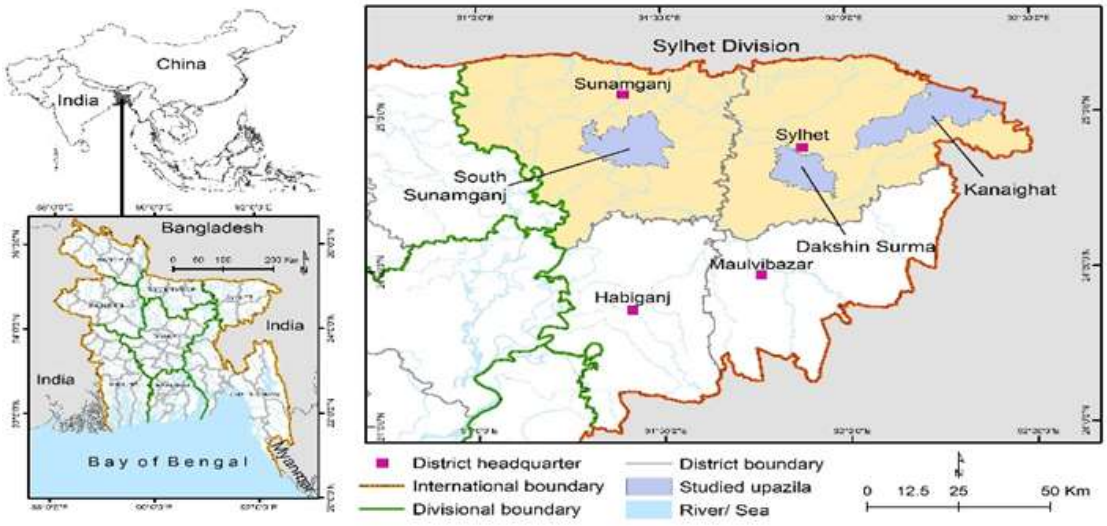

Fig. 1: Geographical location of the sampling area (created by ArcGIS 2.0).

infected with the virus; not necessarily at the time of sample collection, it could be before that. A household was classified as positive if one or more chickens in the flock tested positive (OIE 2018).

HI Test

Samples that showed positive antibody response to ELISA test were further confirmed by using HI test. The inclusion criterion for positivity is considered as titer of inhibition at a serum dilution of 1/16 ($\log 2^4$ when expressed as the reciprocal) (OIE 2018; Nooruddin et al. 2006; Islam et al. 2019). Micro plate HA (Hemagglutination) test to determine 4HA units was carried out by two-fold serial dilution of the 25µL of viral suspension from first well to 12th well of 96 well microtiter plate with 25µL phosphate buffer solution in each well. 25µL of 0.5%cRBC were dispensed in each well of 96 well "V" bottomed plate and incubated at room temperature for 20min. The reciprocal of the highest dilution of antigen in which positive pattern of agglutination was observed considered as HA Unit.

Final HI titer of the sera samples was assessed by using constant 4HA unit antigen and decreasing serum method (β procedure). A 25µL of test serum was dispensed in 1st well and serial dilution from 2nd to 12th well was carried out. 25µL of prepared 4HA unit (1:128 dilution) was dispensed in each well except 12th well (control) and allowed to incubate at room temperature for 20min to facilitate antigen antibody reaction. A final 25µL volume of 0.5%cRBC (v/v) was added in each well upto 12th well by tilting to mix with virus and serum sample and incubated 30min to setup reaction. Samples showing peculiar central button shaped settling of RBCs were recorded as positive and maximum dilution of each sample causing hemagglutination inhibition was considered as the endpoint, which was used to estimate the HI titer. A serum antibody titer of log 2^4 without history of vaccination in backyard chicken was considered as positive.

Data Management and Analysis

Percentage function was used for measuring prevalence and Chi-square test (χ^2) function were used for measuring association between the risk factors as well as prevalence at 95% confidence interval and $P<0.05$ set for significance. Descriptive statistics of the qualitative and quantitative explanatory variables was performed to explore the distribution in relation to the dependent variable. Univariable logistic analysis applied to test relationships between AI infection and categorical (ordered/unordered)/and dichotomized variables. Explanatory variables with expected $P \leq 0.05$ in univariable analyses was used in the multivariable logistic regression analysis. All data was entered and managed in CSV (comma-separated values) format using R-studio (version 4.0.2).

RESULTS

Prevalence of AIV

To get a comprehensive idea of bird level seropositivity and risk factors associated with geographical location, a higher prevalence of 54.84% and 60% was observed from samples of Dakshin Surma and South Sunamganj upazilas, respectively while the lowest prevalence of 25.83% was observed in Kanaighat upazila. Fluctuation of prevalence was due to the design effect (DE) was an intra-cluster association with sample type and sample number. We also observed high AIV association in aged birds (11-36 months).

Univariate Odds Ratio of the Risk Factors

The Univariate logistic regression analysis showed 5 possible risk factors associated with seropositivity based on backyard chicken's blood level binding antibody status ($P<0.05$) (Table 1).

Feeding rice bran, Poultry house location, Mud house, Bamboo house, upazila and contact with wildlife is significant in univariate analysis and included in multivariate modeling.

Multivariable Logistic Regression Analysis of Explanatory Variables that are Significantly Associated with AI Prevalence

We excluded upazila variables as confounders for other risk factors. Bamboo house was statistically insignificant in multiple logistic regression and excluded from final model.

Table 1: Univariate analysis of risk factors of AI

Variables	Unit	AI positive	Total	Prevalence	OR	95% CI of OR	P- value
Age	0-6 month	19	79	24.05	1(ref)		
	7-10 month	18	44	40.91	2.19	0.99-4.86	0.0641
	11-36 month	14	33	42.42	2.33	0.98-5.54	
Sex	Male	13	31	41.94	1.65	0.73-3.70	0.2272
	female	38	125	30.40	1(ref)		
Upazila	South Sunamganj	31	120	25.83	1(ref)		
	Dakshin Surma	17	31	54.84	3.49	1.55-8.01	0.0048*
	Kanaighat	3	5	60.00	4.31	0.68-33.85	
Duck rearing	Yes	44	141	46.67	1(ref)		
	No	7	15	31.21	1.93	0.64-5.70	0.2364
Flock size	3-8 birds	41	112	36.61	1.96	0.90-4.57	0.0895
	More than 8 birds	10	44	22.73	1(ref)		
Rear with pet animals	Yes	11	36	30.56	1(ref)		
	No	40	120	33.33	1.14	0.52-2.62	0.7544
Contact with wild life	Yes	41	93	44.09	4.18	1.96-9.62	0.0001*
	No	10	63	15.87	1(ref)		
Poultry house location	Within living house	34	124	27.42	1(ref)		
	Yard	17	32	53.13	3.00	1.35-6.74	0.0070*
Scavenging area	Both	42	138	30.43	1(ref)		
	Household area	9	18	50.00	2.29	0.84-6.26	0.1055
Litter disposal	Throw along roadside	8	42	19.05	1(ref)		
	Spread on field	27	76	35.53	2.34	0.98-6.08	0.0593
	Throw in bush	16	38	42.11	3.10	1.16-8.79	
Ventilation	No ventilation	31	98	31.63	1(ref)		
	Open air	5	13	38.46	1.35	0.38-4.39	0.8825
	Wall house opening	15	45	33.33	1.08	0.50-2.28	
Bamboo poultry house	Yes	37	134	27.61	1(ref)		
	No	14	22	63.64	4.59	1.81-12.34	0.0012*
Mud poultry house	Yes	5	47	10.64	1(ref)		
	No	46	109	42.20	6.13	2.44-18.80	0.0000*
Concrete poultry house	Yes	8	21	38.09	1.32	0.49-3.36	0.5746
	No	43	135	31.85	1(ref)		
Feed rice bran	Yes	28	115	24.38	1(ref)		
	No	23	41	56.10	3.97	1.89-8.51	0.0003*
Feed whole rice	Yes	48	151	31.79	1(ref)		
	No	3	5	60.00	3.22	0.52-25.04	0.2034
Feed commercial food	Yes	1	6	16.67	1(ref)		
	No	50	150	33.33	2.5	0.39-48.58	
Feed scraps	Yes	17	42	40.48	1.00	0.49-2.02	1
	No	34	114	29.82	1(ref)		

Table 2: Multivariate regression analysis of risk factors associated with AI sero-positivity

Variables	Unit	Odds ratio	95% CI of OR	P-value
Upazila	South Sunamganj	1(ref)		
	Dakshin Surma	6.30	2.13-20.00	0.0000*
	Kanaighat	5.76	0.77-52.58	
Contact with wildlife	No	1(ref)		
	Yes	2.67	1.06-6.96	0.0457*
Feed rice bran	Yes	1(ref)		
	No	5.36	1.92-16.11	0.0003*
Mud poultry house	Yes	1(ref)		
	No	4.02	1.29-14.48	0.0030*
Poultry house location	Within living house	1(ref)		
	Yard	4.08	1.48-11.61	0.0001*

Multivariable logistic regression analysis showed highly significant (P<0.05) sero-positivity to risk factors association was associated with contact with wildlife (OR 2.67; 95% CI: 1.06-6.96) no supplementation with feeding rice bran (OR 5.36; 95% CI: 1.92-16.11), mud poultry house (OR 4.02; 95% CI: 1.29-14.48 and poultry house location (OR 4.08;95% CI: 1.48-11.68). No protective factor was identified and house cleaning frequency (data not shown), bamboo poultry house had least association with AI seropositivity (Table 2).

DISCUSSION

Persistence of avian influenza infection in the birds implies significant zoonotic importance. Stakeholders, Farmers and Poultry handler is highly prone to the infection due to direct and frequent exposure to chickens (Capua and Catolli 2013; Turner et al. 2017; Zhang et al. 2019; Sajjad et al. 2021). However, factors like location, season, bird species, vaccination, hygiene, poultry floor type, rearing system ventilation, stress, feeding system can potentiate the

occurrence and outbreak of AI infection in poultry (Le et al. 2013; Chang et al. 2014). The inclusion criteria for seropositivity in this study was referenced from OIE manual 2018; $\log 2^4$ for HI test and s/p ratio of greater than 0.50 in AIV specific ELISA test.

Our study was designed to investigate the sero-prevalence of avian influenza in the household chickens of three selected upazila's of Sylhet. We observed a significant ($P<0.05$) variation based on location (Table 1) and higher prevalence in serum samples obtained from Kanaighat upazila. From Kanaighat upazila we have received 12 samples, hence the data related to overall prevalence contrast owing to small and uneven sample size from this area.

A similar study endorsed in five districts of Pakistan (Fatima et al. 2017) showed location-based difference in sero-prevalence and aligned with other studies (Osman et al. 2015). We also explored whether backyard chickens reared within living house were significantly ($P<0.05$) less prevalent to AI infection than those reared in yard system. This was further corroborated by our findings of no mud poultry house and bamboo house as drivers ($P<0.05$) of AI in serum. Possibly, infections were further harnessed by the damp and sticky surroundings of house that intensifies sero-prevalence. The odd-ratio threshold had exceeded the above reference value in all significant variables we studied (Table 1, 2). While addressing contact with wildlife (specifically wild ducks) as a determinant of AI sero-positivity, we found a high odd ratio (1.34-8.21). This could be an important attributable risk factor for free-range birds as they have the access to scavenge in jungles and might encounter infection a similar manner of avian influenza (Mahmood and Sabir 2021; Ntakiyisumba et al. 2023).

In our studies, we did not find an association of whether a mixed farming with duck or reared with household pet could signify avian influenza infection. Neither of the concrete floor house, scavenging area or litter disposal system had minimal contribution of AI sero-prevalence. Our findings regarding duck-chicken rearing contrasted with previous findings were mixed farming aggravated AI infection (Rahman et al. 2012).

Also, ventilation and scavenging area had minimal effect as backyard chicken had less sero-prevalence to AI infection. Such air-borne transmission could be possible in controlled environmental settings (Bertran et al. 2017). This might be due to accessibility of fresh air and swiping out of pathogen in flowing waterbodies.

A major finding of this study is feeding rice bran to poultry had a strong effect on combating AI prevalence. Rice bran could be useful in treating Influenza like illness (Elsaid et al. 2021). This effect might be due to adding rice bran to poultry diet which can lead an increase of secretory Immunoglobulin (IgA) resulting clearing of mucosa associated HPAI and LPAI. We also propose rational vaccination strategy as a protective factor for backyard chicken if there is a chance of outbreak occurrence in specified area (Xu et al. 2023). Effective vaccination strategy for serendipitous nature of other paramyxovirus (e.g., Newcastle disease virus) was well achieved in several different studies (Rahman et al. 2015; Islam et al. 2019). However, genetic assortment of AIV might lead to emergence due to vaccine selection pressure.

Our study had limitations too. Since we designed the cross-sectional study over a short period of time, we might miss seasonal trends and serum sample was not equal amount from three upazilas. Also, positive samples were not further subtyped using H and N receptor due to limited resources and we did not investigate for clade association of HPAI and LPAI prevalence in studied area.

Conclusion

This research aimed at exploration of the current hypothesis that avian influenza (AI) is circulating in the study area. It is expected that, a clear in-depth view on present AI infection status and knowledge on available risk factors in north-western region of Bangladesh. In a broader sense this will allow us to understand when to vaccinate and what management challenges needed to be adopted to combat AI outbreaks in backyard chicken. As a consequence, findings from this research will help the farmers to minimize flock mortality by knowing possible entry and persistence of virus, betterment of backyard chicken health by tackling modifiable risk factors and selecting suitable intervention strategies (vaccination). Furthermore, outputs from this project will help to develop a nationwide host-prevalence and risk factor analysis strategy against AI by integrated one health approach (wild-domestic-human interface) in different topographical areas of world.

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