ORIGINAL ARTICLE

Seroprevalence and risk factors of avian reovirus in backyard chickens in different areas of Mymensingh district in Bangladesh

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ABSTRACT

Objectives: The present study estimated the seroprevalence of avian reovirus (ARV) infections in backyard chickens of the Mymensingh district in Bangladesh.

Materials and Methods: Considering several risk factors, a total of 460 serum samples were collected from backyard chickens from eight Upazilas of the Mymensingh district in Bangladesh. Blood samples were taken from the wing vein using 3-ml sterile syringes and kept at room temperature for clotting in a slanting position and then transported to the laboratory maintaining the cool chain. Subsequently, the prepared sera were harvested and stored at −20°C until used. Finally, an indirect enzyme-linked immunosorbent assay (ELISA) was performed to detect ARV-specific antibodies using a commercial ARV antibody detection ELISA test kit.

Results: The results revealed high prevalence rates of ARV antibodies, with a total seroprevalence of 69.78% (321/460). Area-wise, 74.55% (82/110) seroprevalence was recorded as the highest in Mymensingh Sadar, whereas 64% (32/50) was the lowest in Gauripur Upazila. With regard to sex, female chickens showed a significantly higher ($p < 0.05$) seroprevalence as 90.33% (271/300) compared to male chickens 31.25% (50/160). With regard to age groups, the seroprevalence of ARV infection was 59.33% (89/150) within 2–8 weeks, 82% (205/250) within 9–16 weeks, and 45% (27/60) within 17–20 weeks, respectively. Based on hygienic conditions, the highest seroprevalence of ARV was noted in backyard chickens housed in poor conditions 80% (120/150) than good conditions 50% (40/80). Backyard chickens reared in free-ranging conditions exhibited a significantly higher seroprevalence 73.33% (220/300) of ARV antibodies compared to rearing in separate houses 63.12% (101/160). The seroprevalence of ARV was higher in crossbreeds 71.67% (43/60), brought from market 76% (38/50), and unhealthy 78.57% (55/70) backyard chickens than non-descriptive indigenous 69.5% (278/400), home-reared 69.02% (283/410), and healthy chickens 68.21% (266/390).

Conclusion: The high prevalence of ARV antibodies revealed in the current study indicates an extensive exposure of ARV to backyard chickens in Bangladesh that may be transmitted naturally to other chickens, ultimately leading to ominous economic effects on the poultry sector.

Introduction

In low-income and developing countries, including Bangladesh, rural households raise backyard poultry as a source of quality foods and financial supports. Generally, rural women foster backyard poultry to provide additional economic assistance to their families [1]. About 77% of people in Bangladesh live in rural areas [2], and around 80% of rural families rear chickens, ducks, pigeons, and geese using a backyard production system [3]. Rearing backyard chickens has been regarded as a longstanding practice for many years among rural people in Bangladesh [4]. Nowadays, the poultry sector has become a particular segment in the production of animals by providing cheap and easily get-at-able sources of nutritious protein in terms of eggs and meat. As a result, the demand for poultry is being accelerated progressively in all classes of people

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in Bangladesh [5]. Based on the Household Income and Expenditure Survey 2016, the daily protein intake in rural areas of Bangladesh is 7.19 gm (11.35%) per capita, of which poultry meat and eggs provide the superior portion, around 77.33% (5.56 gm) of the proteins [6]. However, the introduction, outbreak, and the existence of different viral diseases resulted in a reduced production system, hindering the expected production of backyard chickens. High morbidity and mortality and degradation in chickens' growth performance occurred due to the introduction of vulnerable viral diseases are slowing down the economic growth [7]. Several viral diseases, e.g., Newcastle disease, infectious bronchitis, infectious bursal disease, avian influenza, avian reovirus (ARV) infection, avian leucosis, and fowl pox, hamper the poultry production tremendously in Bangladesh [8,9]. These viral diseases, among which ARV infection, play a notable role in economic losses, leading to an adverse impact on the poultry sector.

ARV, the causal agent of ARV infection in chickens, plays a crucial role in reducing egg production [10]. Among the 15 genera of the *Reoviridae* family, genus *Orthoreovirus* belongs to two main principle groups, namely ARV and mammalian reovirus [11–13]. Although ARV is inactive at 56°C for <1 h and stable at pH 3.0–9.0, they can survive on feathers, galvanized metals, wood shavings, rubbers, and glass with limited effects on infectivity for 10 weeks [14]. Due to the divergence in ARV pathogenicity, a wide range of avian species, including broilers, broiler breeders, turkey, layer breeds, and backyard chickens, are affected by them [15–17]. In most cases, they appear harmless, but commercial and backyard chickens show great susceptibility to them.

Additionally, ARV can be isolated from affected tissues and organs [18]. Because of having diversified pathogenicity, ARV may cause various diseases, disease conditions, and abnormalities in domestic poultry, including enteric-respiratory diseases, hepatitis, myocarditis, pericarditis, and hydropericardium [19]. Of these diseases, viral arthritis or tenosynovitis is the disease condition that causes lameness in the chickens. It is characterized by the swelling of joints and lesions on the gastrocnemius tendon [20,21].

Along with causing diseases, ARV can create significant labyrinths in feed conversion ratio and weight gain of chickens, leading to severe economic losses [22]. In several cases, ARV does not develop any clinical signs and symptoms because of it being asymptomatic or subclinical [23]. However, exposure to ARV may depend on several risk factors constituting geographical location, age, sex, breed, immune status of the host, virus exposure route, pathotypes of the virus, and the presence of co-infecting pathogens [24].

For the detection of ARV antibodies, the enzyme-linked immunosorbent assay (ELISA) test has been regarded as a sensitive test and is used mostly. Because of it being readily available in the market, ELISA serves as an expedient test to examine a large number of serum samples [25]. In Bangladesh, the Animal Research Division, Bangladesh Livestock Research Institute had first reported on ARV in June 1997 [26]. After the first report, a few more studies were conducted on ARV infection in chickens in Bangladesh, e.g., Salam et al. [25] conducted their study on commercial layers in Dinajpur district, Neepa et al. [27] conducted on commercial broilers and layers in Mymensingh and Gazipur districts, and Biswas et al. [28] conducted on chickens in smallholdings in the northern parts of Bangladesh. To the best of our knowledge, there is no report on ARV in backyard chickens in the Mymensingh district in Bangladesh. Besides, there is a scarcity of knowledge and information on ARV infection occurring in backyard chickens in Bangladesh. However, it is now an urgent demand to check the prevalence of ARV infection in backyard chickens in Bangladesh. Therefore, isolation and identification of ARV at both serological and molecular levels should be performed. Because of this, the current study was undertaken to check the seroprevalence of ARV antibodies in backyard chickens relating to several important risk factors, including areas, ages, sex, source, breed, health status, hygienic conditions, and housing system in Mymensingh district in Bangladesh.

Materials and Methods

Ethical approval

Expert veterinarians collected the blood samples from the birds considering the ethical standards and animal welfare issues. Verbal permission was taken from farmers before sample collection.

Sample size calculation and study area selection

The sample size from backyard chickens for ARV detection was determined by the following assumption that the seroprevalence was 50% and the confidence interval was 95%. The formula used to calculate the sample size was as follows [29]: $n = Z^2 \, pq/d^2$, where n = desired sample size, Z = the standard normal deviation, usually set at 1.96 at the 5% level, which corresponds to 95% confidence level, p = prevalence (we assume 50% or 0.5), q = 1−p = (1−0.5) = 0.5, d = precision (5%, so d = 0.05). So, $n = (1.96)^2*0.5*0.5/(0.05)^2 = 384$. For adjusting non–response, 10% more samples were taken and then sample size was = (384+10% of 384) chickens = (384 + 38) = 422 chickens. Eight Upazilas (Fig. 1) of Mymensingh district (24.7539° N, 90.4073° E) in Bangladesh were selected for the study. Therefore, we selected 50 backyard chickens from each Upazila, while 110 samples from Mymensingh Sadar Upazila as its higher population size. Finally, a total of 460 chickens were selected for blood collection.
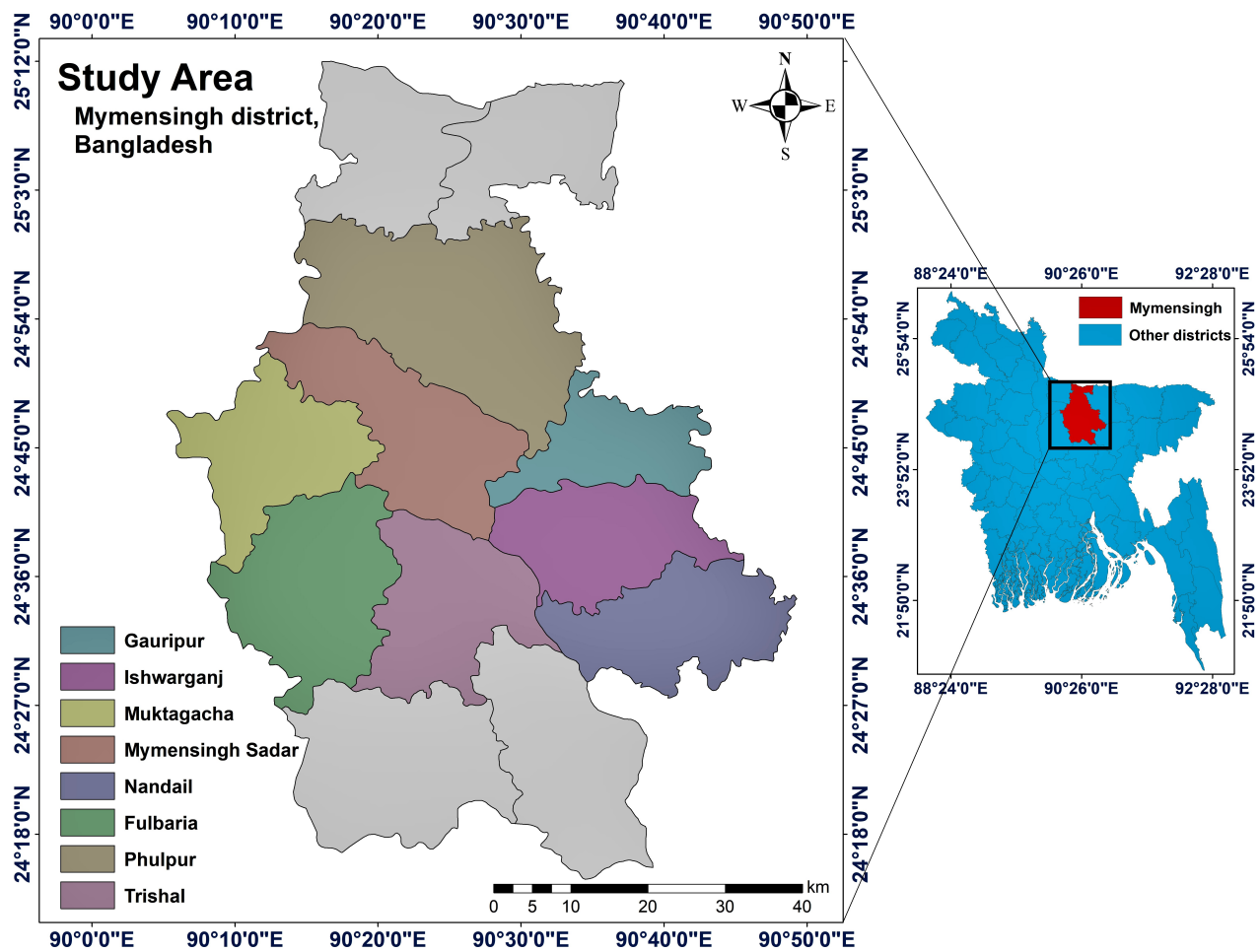
Figure 1. Study area map of different Upazilas of Mymensingh district, Bangladesh. Images were extracted from DIVA-GIS (http://www.diva-gis.org/) using the geographical information system (GIS), and finally, the map was processed with ArcMap 10.7 Software.

Selection of households and data collection

Households selected in the current study were based on their previous history of backyard chickens rearing, the current number of chickens keeping, and eagerness to take part in the study. Considering the above-mentioned point, a total of 65 households were included in this research. During sample collection, a structured questionnaire was carried to collect the information from owners. The questionnaire comprised the owner name, location, sources of birds (home-reared or brought from the market), sex (male or female), breed types (non-descriptive indigenous breed or crossbreed chickens, which included the Naked neck, Aseel or hilly chickens), age, health status at the time of sample collection (healthy or unhealthy), hygienic conditions, vaccination status, and housing system (free-ranging or in a separate house built with brick or tin made). Regarding age,

birds were categorized into three groups, such as 2–8 weeks, 9–16 weeks, and 17–20 weeks. Based on hygienic conditions, chickens were divided into three types as a good condition: supply feed and water in a separate bowl and clean the house daily; moderate condition: provide feed and water in a single bowl and occasionally clean the house, and poor: provide feed spreading on the ground and birds consume water from nearby the kitchen wastewater streams. The data were taken from January to June 2019. Table 1 represents the distribution of sample size based on selected variables.

Blood collection and serum preparation

For the detection of antibody titer, a total of 460 blood samples were collected from selected backyard chickens considering several risk factors. Initially, blood samples were taken from the wing vein of chickens using 3-ml sterile

Table 1. Sample size distribution based on different variables.

Variables	Categories	Frequency (%)
Areas/Location(Upazilas)	Mymensingh Sadar	110 (23.91)
	Trishal	50 (10.87)
	Muktagacha	50 (10.87)
	Fulbaria	50 (10.87)
	Phulpur	50 (10.87)
	Ishwarganj	50 (10.87)
	Nandail	50 (10.87)
	Gauripur	50 (10.87)
Sex	Female	300 (65.22)
	Male	160 (34.78)
Age group(Weeks)	2–8	150 (32.61)
	9–16	250 (54.35)
	17–20	60 (13.04)
Source of birds	Home reared	410 (89.13)
	Brought from market	50 (10.87)
Breed type	Non-descriptive indigenous	400 (86.96)
	Crossbreed	60 (13.04)
Health Status	Healthy	390 (84.78)
	Unhealthy	70 (15.22)
Hygienic conditions	Poor	150 (32.61)
	Moderate	230 (50.00)
	Good	80 (17.39)
Housing system	Free-ranging	300 (65.22)
	Separate house	160 (34.78)

syringes, followed by keeping them in a slanting position at room temperature to clot. The collected blood samples were kept in the icebox after clotting within the syringes and transported to the Virology Laboratory, Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, by maintaining a cool chain. After transporting to the laboratory, a sterile needle was used to remove clotted blood gingerly, and the sera were poured into clean sterilized 1.5-ml Eppendorf tubes. Subsequently, using the centrifuge machine, the sera were subjected to spin at 3,000 rpm for 5 min to remove the remaining red blood cells, clots, and other materials. Finally, clear sera were separated and collected into new 0.5-ml sterilized Eppendorf tubes, followed by storing at −20°C to conduct indirect ELISA.

Enzyme-linked immunosorbent assay

Using indirect ELISA, the collected and processed sera were analyzed. The ARV antibody test kit (ID Screen® ARV Indirect, ID Vet, Grables, France) was used to detect antibody titers directed against ARV in chicken serum. However, the indirect ELISA was performed according to the procedure, which was previously described by Neepa et al. [27].

Statistical analysis

Data were incorporated into the Microsoft Excel 2010 (Microsoft Corporation, *Redmond*, WA) and analyzed using Statistical Package for the Social Sciences (SPSS) software (IBM SPSS-25.0, Armonk, NY). Descriptive statistics were conducted to calculate the prevalence. Pearson's Chi-square test was conducted to assess the significant relationship of ARV seropositivity with different variables, such as location, age, sex, source of birds, breed type, health status, hygienic conditions, and housing system. The probability (p) value less than 0.05 was considered as significant.

Results

Seroprevalence of ARV based on areas

Of the 460 samples, 321 (69.78%) samples were positive for ARV antibodies. Regarding areas, 74.55% (82/110) seroprevalence was exhibited as the highest in Mymensingh Sadar, whereas 64% (32/50) was the lowest in Gauripur Upazila. Within Mymensingh Sadar, the highest seropositive was found in Nodir Par 85.71% (30/35) and the lowest was in both Kachari and Sankipara 68% (17/25). In other Upazilas of Mymensingh district, including Trishal, Muktagacha, Fulbaria, Phulpur, Ishwarganj, and Nandail, the seropositivity of ARV was 68% (34/50), 70% (35/50), 72% (36/50), 68% (34/50), 70% (35/50), and 66% (33/50), respectively (Table 2). Study areas were not significantly varied with ARV seroprevalence ($p > 0.05$).

Seroprevalence of ARV based on sex groups

Table 3 represents the seroprevalence of ARV in backyard chickens based on sex groups. After sera examination, 271 (90.33%) out of 300-female and 50 (31.25%) out of 160-male chickens showed positive for ARV antibodies. Statistical analysis revealed that the difference in ARV seroprevalence between female and male chickens was highly significant ($p < 0.05$).

Seroprevalence of ARV based on age groups

Regarding age groups, the detailed seroprevalence of ARV infection in backyard chickens was 59.33% (89/150) within 2–8 weeks, 82% (205/250) within 9–16 weeks, and 45% (27/60) within 17–20 weeks of age groups, respectively (Table 3). According to statistical analysis, backyard chickens of 9–16 weeks of age groups exhibited a significantly higher seroprevalence of ARV within the selected age groups ($p < 0.05$).

Table 2. Seroprevalence of ARV in backyard chickens based on different Upazilas of Mymensingh district

Upazila	Areas	No. of sera tested	No. of positive sera	Seroprevalence according to areas (%)	Seroprevalence according to Upazila (%)	95% Confidence Interval (CI)	Average of Seropositive (%) with 95% CI	*p* value
Mymensingh Sadar	Nodir Par	35	30	85.71				
	Kachari	25	17	68.00	74.55	65.63–81.80		
	Shomvugang	25	18	72.00				
	Sankipara	25	17	68.00				
Trishal		50	34		68.00	54.13–79.30		
Muktagacha		50	35		70.00	56.17–80.97	69.78 (65.43–73.80)	0.921
Fulbaria		50	36		72.00	58.24–82.62		
Phulpur		50	34		68.00	54.13–79.30		
Ishwarganj		50	35		70.00	56.17–80.97		
Nandail		50	33		66.00	52.11–77.61		
Gauripur		50	32		64.00	50.11–75.90		

p-value less than 0.05 is considered as statistically significant.

Table 3. Seroprevalence of ARV in backyard chickens based on different parameters.

Variables	Categories	No. of sera tested	No. of positive sera	Seroprevalence (%)	95% Confidence Interval (CI)	*p* value
Sex	Female	300	271	90.33	86.42–93.22	0.001
	Male	160	50	31.25	24.57–38.81	
Age group (Weeks)	2–8	150	89	59.33	51.33–66.87	
	9–16	250	205	82.00	76.74–86.29	0.001
	17–20	60	27	45.00	33.09–57.51	
Source of birds	Home reared	410	283	69.02	64.39–73.31	0.331
	Brought from market	50	38	76	62.45–85.84	
Breed type	Non-descriptive indigenous	400	278	69.5	64.82–73.81	0.773
	Crossbreed	60	43	71.67	59.16–81.56	
Health status	Healthy	390	266	68.21	63.42–72.63	0.082
	Unhealthy	70	55	78.57	67.50–86.67	
Hygienic conditions	Poor	150	120	80	72.84–85.66	0.001
	Moderate	230	161	70	63.78–75.56	
	Good	80	40	50	39.30–60.70	
Housing system	Free ranging	300	220	73.33	68.05–78.03	0.023
	Separate house	160	101	63.12	55.42–70.22	

P-value less than 0.05 is considered as statistically significant.

Seroprevalence of ARV based on the source of birds

The indirect ELISA test was conducted on the collected sera, following the source of chickens: home-reared and brought from the market. In the case of home-reared, out of 410 samples, 283 samples (69.02%) were positive, whereas 76% of samples (38/50) were positive for ARV antibodies in backyard chickens brought from the market

(Table 3). There was no significant difference between backyard chickens considering their source.

Seroprevalence of ARV based on breed type

Table 3 represents the seroprevalence of ARV infection in backyard chickens selected on the breed. By serological test, the seropositive of ARV infection was detected

in 69.5% (278/400) non-descriptive native chickens, whereas 71.67% (43/60) in crossbreed chickens. No significant difference was observed in the detection of seroprevalence of ARV between selected breeds.

Seroprevalence of ARV based on health status

The health status of backyard chickens was categorized into healthy and unhealthy for the detection of antibody titer against ARV. In the case of healthy backyard chickens, 266 samples out of 390 samples showed positive (68.21%) and, on the contrary, 78.57% of samples showed positive (55/70) in case of unhealthy backyard chickens (Table 3).

Seroprevalence of ARV based on hygienic conditions

Backyard chickens keeping in several hygienic conditions were selected. The hygienic conditions were separated as good, moderate, and poor categories. The ARV seropositivity was highest (80%) for chickens reared in poor hygienic conditions. On the other hand, ARV seropositivity was comparatively lower in good (50%) and moderate (70%) hygienic conditions (Table 3). Hygienic conditions were significantly varied with ARV infection in backyard chickens ($p < 0.05$).

Seroprevalence of ARV based on housing systems

The collected sera were categorized into two types based on housing systems, such as free-ranging and in separate houses. In a free-ranging housing system, out of 300 sera samples, 220 (73.33%) was positive for ARV antibodies (Table 3). On the contrary, in separate houses, of 160 serum samples, 101 (63.12%) showed positive for ARV-specific antibodies in the indirect ELISA test. Moreover, the ARV seropositivity was significantly ($p < 0.05$) higher in those who reared backyard chickens in the free-ranging system compared to separate houses.

Discussion

Backyard chickens play a significant role in rural economics, but further amelioration is hindered by the existence of viral diseases. ARV is recognized as a precarious disease for the reduction of growth and production in backyard chickens, resulting in salient economic losses. Backyard chickens, along with broilers and layers, can be infected with ARV over the globe. As the pathogenicity of ARV is heterogeneous, its strains are closely associated with poultry diseases and disease conditions, including tenosynovitis, malabsorption syndrome, and viral arthritis [30]. Interestingly, ARV can be isolated from asymptomatic chickens [31]. However, there is a shortage of information on the ARV status in backyard chickens in Mymensingh, as well as other areas of Bangladesh. In this pivotal situation, the present study was designed to detect ARV antibodies by a serological study using indirect ELISA.

In the current study, a total of 460 serum samples were collected from backyard chickens of different areas of Mymensingh, of which 321 (69.78%) samples showed seropositive for ARV infection. Area-wise, the highest seropositive was found in Mymensingh Sadar, conversely lowest was in Gauripur Upazila. Previously, several studies were conducted on ARV infection in Bangladesh, e.g., Biswas et al. [28] performed a study in the northern parts of Bangladesh with 47% seroprevalence of ARV in smallholding chickens. However, the findings were lower than that in our present study. Another study was carried out by Neepa et al. [27] on broilers and layers in Mymensingh and Gazipur districts in Bangladesh that reported 39.5% ARV seropositive, which is also lower than the current results. This variation may be occurring due to the difference in the selection of poultry species, e.g., Neepa el al. [27] detected ARV antibodies from exotic chickens, whereas we identified from the backyard chickens.

Additionally, in the Dinajpur district in Bangladesh, Salam et al. [25] conducted a study in layers and recorded 93.33% seroprevalence of ARV much higher than our findings. Several studies from other countries also performed on ARV infection previously, e.g., Baksi et al. [22] reported only 8% seropositivity of ARV infection in broilers in some parts of India, Nham et al. [21] showed 91% seroprevalence rate of ARV in broilers in Ontario, Canada, and Pu et al. [32] found 92% in unvaccinated chickens in China. Furthermore, Gottdenker et al. [33] and Soos et al. [34] reported 68% and 73.9% seroprevalence of ARV, respectively, in native birds in Ecuador. These findings are almost similar to our present study. However, variations occurred, and these might be due to the specificity and sensitivity of applied diagnostic tools along with some salient factors such as geographical differences, sample size, or selection procedures. Furthermore, poor sanitary conditions, nutritional deficiency, contact with other chickens, and absence of vaccination might also be the cause of a higher prevalence of ARV antibodies in backyard chickens.

With regard to sex groups, female backyard chickens showed a significantly higher seroprevalence of ARV antibodies compared to male chickens. The variation of seroprevalence between female and male chickens may be occurring due to immunological and physiological differences between the two sexes [35]. Additionally, the vulnerable reproductive system of females compared to males and some immeasurable risk factors, e.g., behaviors, can increase the risk of occurrence of viral diseases, like ARV infection [35]. With respect to age groups, the lowest seroprevalence was found within 17–20 weeks of age and the highest was within 9–16 weeks of age groups of backyard chickens. Chickens within 9–16 weeks of age groups showed highly significant to ARV infection. The variation might be due to the immune system of backyard chickens.

Chickens become increasingly resistant to ARV with age and in later stages of age, they show the highest resistance because of the full development of their immune system [36].

Sources, breed types, and health status of backyard chickens had no significant relationship with ARV antibodies. Based on sources, the prevalence was higher in brought from market birds than home-reared birds. In the open-air market, birds brought from different areas come in contact with other birds, which are brought back to various areas can spread the ARV pathogen to domestic chickens. Indigenous chickens consist of non-descriptive Deshi chickens as well as crossbreeds such as Naked neck, Aseel, Hilly and Yasine [37]. The study revealed that the ARV seroprevalence was higher in crossbred than non-descriptive chickens. It is not clear why the ARV detection was higher in crossbred; however, it might be their origin because few birds were brought from the hilly area. Similar findings were also observed by Soos et al. [34], who found higher ARV seroprevalence in backyard chickens in the Galapagos Islands when it foraged with wild birds.

Regarding health status, the high prevalence in unhealthy birds might be due to weak immunity. According to hygienic conditions, the highest seroprevalence of ARV was recorded in backyard chickens due to poor conditions (80%); in another point of view, good hygienic condition showed the lowest (50%) seroprevalence among chickens. In Bangladesh, feed is given to birds on the ground, and only a few farmers supply feed and water through feeders (25%) and drinkers (12%) [38]. Sil et al. [39] reported that predisposing factors, such as cleaning, ventilation system, litter conditions, and overcrowding could influence the spreading of infection to the poultry. Besides, we conducted the current study to know the association of different housing systems for the spread of ARV infection. In Bangladesh, backyard chickens are usually reared in free-ranging systems, but few farmers kept them in brick and tin-made houses in the night time [37]. The seroprevalence of ARV in backyard chickens of the free-ranging housing system was significantly higher (73.33%) than in a separate housing system (63.12%). This significant variation may be held due to rapid movement of chickens and direct contact with other migratory or wild birds or may be due to the connection with rodents.

Conclusion

The present study reveals the widespread occurrence of ARV infection in backyard chickens in different areas of the Mymensingh districts in Bangladesh, considering several significant risk factors. Although the variations in place of origin, sources, breed types, and health status had no significant association with the occurrence of ARV infection,

other risk factors, such as sex, age, hygienic conditions, and housing system showed substantial associations. Therefore, to reveal the exact status of ARV along with their epidemiology, pathology, and pathogenesis among backyard chickens, further investigations should be conducted in different areas of Bangladesh at epidemiological, serological, and molecular levels. It is recommended that the implementation of prevention and control strategies, application of effective biosecurity measures, and the improvement of awareness among people are inevitable to minimize the intensification of ARV infection in backyard chickens, ultimately minimizing the economic losses in the poultry sector in Bangladesh.

Conflict of interest

The authors declare that there is no conflict of interest in the publication of this article.

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