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Research article

Prevalence, molecular detection, and therapeutic efficacy of Aspergillosis in indigenous chickens in Bangladesh

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Abstract

In Bangladesh, a significant challenge of raising traditional chicken breeds during the early stages of chicks is Aspergillosis, a fungal disease primarily caused by *Aspergillus* spp. A cross-sectional study was conducted in the Joypurhat district of Bangladesh from November 2022 to October 2023, involving a sample size of 394 birds. The primary focus was post-mortem analysis, involving species detection of Aspergillus through culturing on Sabouraud dextrose agar (SDA) medium, followed by PCR assays, identification of risk factors, and evaluation of therapeutic efficacy. The prevalence of Aspergillosis was found to be 12.18% (48/394), with rates of 11.24% (10/89) in Naked Neck breeds and 12.46% (38/305) in Native breeds. A higher prevalence was found among chicks aged 0-15 days (18.96%). *Aspergillus fumigatus* was identified as the most prevalent fungal species. Multivariate logistic regression analysis identified three significant risk factors: age (0–15 days, OR = 5.38), season (winter, OR = 3.55), and litter condition (Moist, OR = 14.88). In the experimental infection groups, The therapeutic measures indicated that Itraconazole, in combination with fungal toxin binder, NSAID drug (Paracetamol), and litter disinfectant (Blue vitriol), was the most effective against Aspergillosis in native chickens. The findings of this study hold promise for the development of native chicken breeds, offering insights that can be applied to achieve sustainable poultry production in Bangladesh.

Keywords: Aspergillosis, Indigenous Chicken, PCR Assay, Sabouraud Dextrose Agar Medium, Therapeutic Measures.

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INTRODUCTION

Numerous indigenous chicken breeds are vulnerable to various illnesses in their initial stages (0-30 days), with Aspergillosis, emerging as a significant issue (Arafat et al., 2022). Aspergillosis is a fungal infection caused by species of the genus Aspergillus. *Aspergillus fumigatus* is most commonly associated with Aspergillosis in poultry, including chickens (Abd El Tawab et al., 2015; Vahsen et al., 2021). However, other Aspergillus species like *A. niger, A. flavus, A. terreus, and A. glaucus* may also play a role in causing the disease (França et al., 2012).

This infection primarily affects the respiratory system, although it can also manifest in other organs. Aspergillus spores are ubiquitous in the environment, and birds, including native chickens, may inhale these spores, leading to the development of Aspergillosis under certain conditions (Arafat et al., 2022). Aspergillosis in chickens is characterized by respiratory distress, coughing, and nasal discharge, with advanced cases showing lethargy, weight loss, and even sudden death (Nururrozi et al., 2020; Ameji et al., 2020). Post-mortem examinations often reveal lesions in the air sacs and lungs, with white to yellowish plaques indicating fungal growth (Ameji et al., 2020). Early detection and intervention are crucial to mitigate its impact and prevent further spread within the flock. Aspergillosis presents in two forms: i) Acute or Insidious and ii) Chronic (Shaapan et al., 2024). Early detection and intervention are crucial to mitigate its impact and prevent further spread within the flock. Indigenous chickens in Bangladesh are reported to be more susceptible to Aspergillosis compared to commercial breeds due to several factors. They are often raised in free-range systems, exposing them to environmental Aspergillus spores in soil, decaying vegetation, and litter, especially in Bangladesh's humid and warm climate (Ahamad et al., 2018). Genetic predispositions, poor nutritional status, and traditional management practices like inadequate ventilation and sanitation further compromise their immune systems (Cafarchia et al., 2014). Additionally, these chickens face various stressors, such as handling and transportation, which increase their vulnerability to infections.

The prevalence of *Aspergillus* spp. *is* 44% in Bangladesh, and among the Aspergilli, *A. flavus* was detected in 10%, while *A. fumigatus* and *A. niger* were detected at 26% and 8%, respectively (Arafat et al., 2022). The mortality rate of Aspergillosis ranges from 4.5 to 90% in young birds (Arné et al., 2011).

Several factors contribute to this variation, including geographical location, climate, management practices, and bird population density (Abd El Tawab et al., 2015; Arafat et al., 2022). Numerous risk factors promote the spread and growth of Aspergillosis, such as warm and humid environment in sheds, unclean conditions, insufficient ventilation, contaminated feed and water, and damp litter (Hassanain et al., 2013).

Optimal treatment for Aspergillosis involves a comprehensive approach that includes antifungal medication, symptomatic relief, and supportive therapy (Krautwald-Junghanns et al., 2015; Jenks et al., 2018). The most favorable outcomes are achieved through the combination of two antifungal drugs. Various medications, such as Itraconazole, Ketoconazole, Clotrimazole, Miconazole, and Fluconazole, are commonly employed in commercial farms for treating Aspergillosis (Sultana et al., 2015). Therefore, a judicious combination of antifungal drugs and consideration of less toxic alternatives can significantly enhance the treatment approach for Aspergillosis caused by *Aspergillus* spp. (Tell et al., 2019).

In Bangladesh, research has focused on examining the prevalence of Aspergillosis in commercial chickens (Sultana et al., 2015; Arafat et al., 2022), but there is limited information on indigenous chickens regarding prevalence, risk factors, and suitable therapeutic measures. This study aims to address these gaps by investigating the prevalence of acute Aspergillosis in native chickens, identifying the species and risk factors associated with Aspergillosis, and examining the proper therapeutic strategy to cure experimental Aspergillosis.

MATERIALS AND METHODS

Ethical approval

This study was conducted in accordance with the research ethics of the Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur-5200, Bangladesh. Moreover, verbal permission was obtained from the poultry farmers.

Study Design and Location

A cross-sectional study was conducted from November 2022 to October 2023 to estimate the prevalence of Aspergillosis, supervised by the Department of Medicine, Surgery, and Obstetrics at Hajee Mohammad Danesh Science and Technology University (HSTU). Subsequently, a case-control study design based on the prevalence study was used to assess the determinants (risk factors) of Aspergillosis in selected farms in Joypurhat district, Bangladesh. For the case-control study assessing the risk factors of Aspergillosis at both the far and bird levels, a 1:1 case-to-control ratio was used. For every case, a control was randomly selected from a completely infection-free bird or farm. The study primarily took place in two Upazilas (sub-districts), Joypurhat Sadar and Akkelpur, from the Joypurhat district of Bangladesh, which was situated within the geographical coordinates of 24°51' to 25°17' north latitudes and 88°17' to 88°55' east longitudes, as shown in Figure 1.

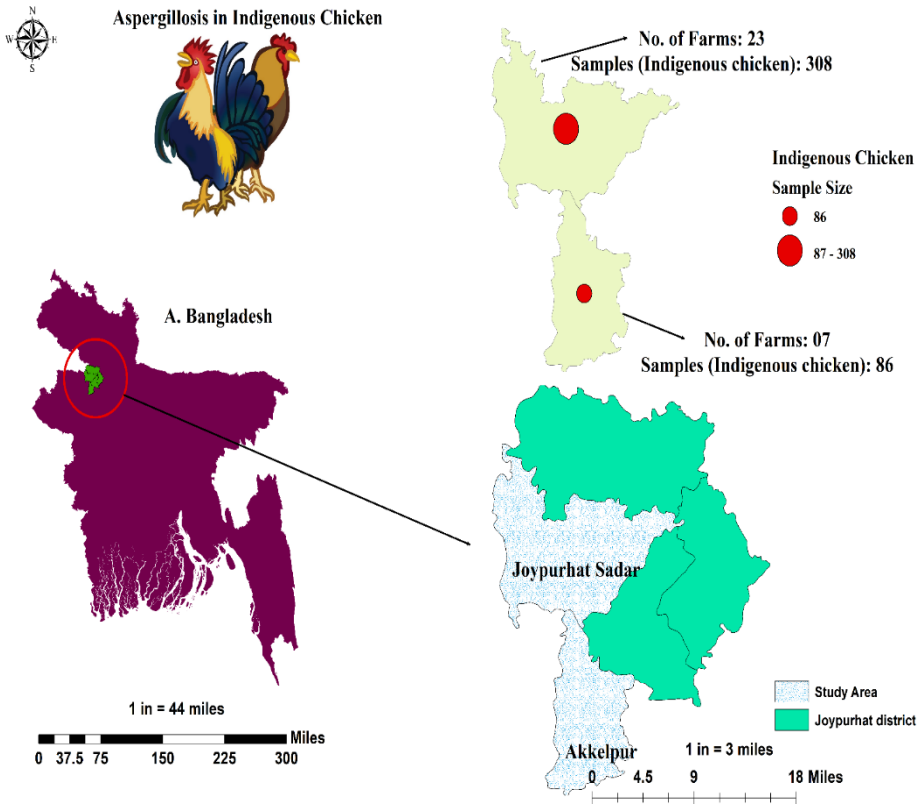

Figure 1 Geo-spatial mapping of the study area showing the selected upazila from Joypurhat district in Bangladesh (This map was generated by using ArcMap 10.7)

Determination of Sampling Population

For this study, a convenient sampling approach was employed to select chicken samples. Specifically, 30 farms that rear indigenous chickens (specifically, non-descriptive dwarf chickens) were chosen through a random selection process facilitated by the Joypurhat Rural Development Movement (JRDM). The list of eligible farms was obtained from JRDM, and the selection of farms was accomplished by employing simple random sampling techniques with the assistance of the Random Number Generator Application (RNG Plus App). Though there is a demand for indigenous chicken because of its good taste, it is expensive to common people. There are very few farmers who produce indigenous chicken commercially, as they are not so productive and profitable as commercial broiler and layer chicken. It was really hard to find the indigenous chicken farms, which had over 200 chickens. This is the major reason to choose specific farms and the sampling method as well as limitation in farm selection.

The sample size was estimated by using the formula for determining prevalence according to Rahman et al. (2024).

$$n = \frac{Z^2 \times Pexp \times (1 - Pexp)}{d^2}$$

Where, n = Desired sample size
Z = 1.96 for 95% confidence interval
P_{exp} = 0.5, Expected prevalence (50%)
d = 0.05, Desired absolute precision (5%)

As there is no reliable prior information on the prevalence of Aspergillosis in Bangladesh, a prevalence of 50% was chosen to optimize the sample size. Following the calculation, a total of 394 samples (minimum) were determined to be necessary for estimating Aspergillosis in Bangladesh. The study was then conducted using 394 samples (male = 185, female = 209) of different ages collected from 2 sub-districts across 30 farms.

Criteria for farm selection

The study carefully chose farms that met specific criteria, including:

- Farms that had been established at least 12 months before the investigation.
- Farms with a minimum of 200 birds.
- Farms that raised indigenous chicken breeds.

Collection of sample and data

Lungs and air sac samples were aseptically collected from the chickens under suspicion following a post-mortem examination. These samples were promptly transported to the microbiology laboratory of Hajee Mohammad Danesh Science and Technology University, Dinajpur, and the postgraduate laboratory of Sylhet Agricultural University, Sylhet, for further laboratory examination while maintaining a consistent cold chain to preserve their integrity. Data collection was carried out using a previously validated questionnaire. Anamnesis, which included information such as age, gender, flock size, physical history, and clinical findings, which were meticulously recorded. Determinants or risk factors were assessed through a combination of open-ended and closed-ended questions as well as through observation.

Diagnostic Procedure

Clinico-pathological Examination

The birds were clinically examined by clinical symptoms of diseased birds and postmortems of dead and sick birds. The presented clinical manifestations of various diseases of the flock were recorded, and the farmer's statement and response to treatment in relation to diseases were recorded carefully.

Clinical Signs were respiratory distress characterized by dyspnea (difficulty breathing), gasping, or open-mouth breathing; rales, which are clicking or bubbling noises when the birds breathe; central nervous dysfunction, including symptoms such as tremors, ataxia, and torticollis; increased thirst and reduced appetite (inappetence); somnolence, or a sleepy and lethargic state; and watery or foamy eye discharge.

Post-mortem lesions were caseous masses in the air sacs (indicative of Aspergillus air sacculitis), small nodules in the lungs (ranging from a few millimeters to centimeters), nodules on the air sacs and trachea, and nodules in other organs such as the liver and brain.

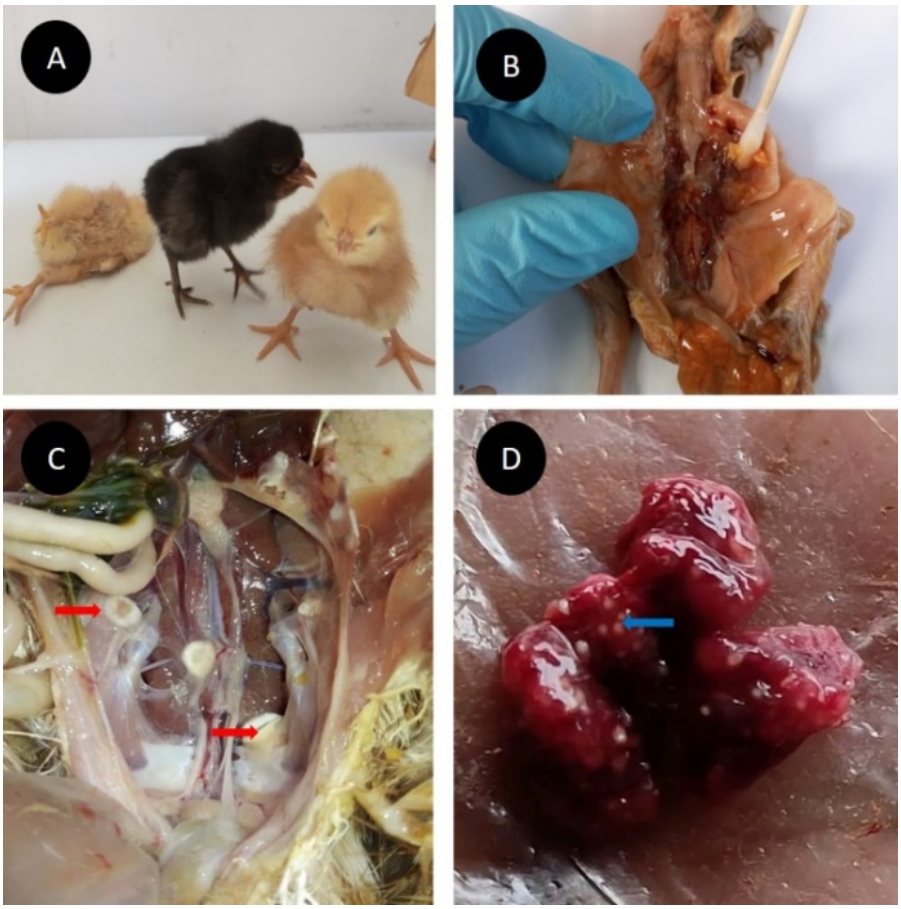

Figure 2 Findings of post mortem examination of suspected chicken. Difficult breathing (Fig. A); Cloudy air sac with caseous mass (Fig. B); Fungal nodular growth in abdominal air sac (Red arrow, Fig. C); Nodule present in lungs (Fig. D).

Laboratory Examination

Cultural examination

The source of sample was the lung swab of the affected bird to culure because this disease is airborne diesease, and typical lesion is found in lung mostly.

When the diesease was diagnosed tentatively through post mortem examination, lung sample was taken immediately by following appropriate protocol for performing lab examination for the confirmative diagnosis, and the lab examination was started the next morning. After obtaining lung swabs from the birds identified as potential cases and culturing them on SDA media.

Culture on Sabourauds dextrose agar (SDA)

Initially, an SDA agar plate was prepared in accordance with the manufacturer's instructions and stored for subsequent use. Following the aseptic collection of samples, the suspected specimens were promptly inoculated onto the SDA plates using a sterile loop. Subsequently, the SDA plates were incubated at 28°C for a period ranging from 3 to 5 days or until visible Aspergillus growth was observed. Daily monitoring was conducted to observe the fungal colony's morphology and color, and detailed characteristics were duly recorded. To establish pure cultures, colonies of *Aspergillus flavus, Aspergillus fumigatus* were sub-cultured onto new SDA plates.

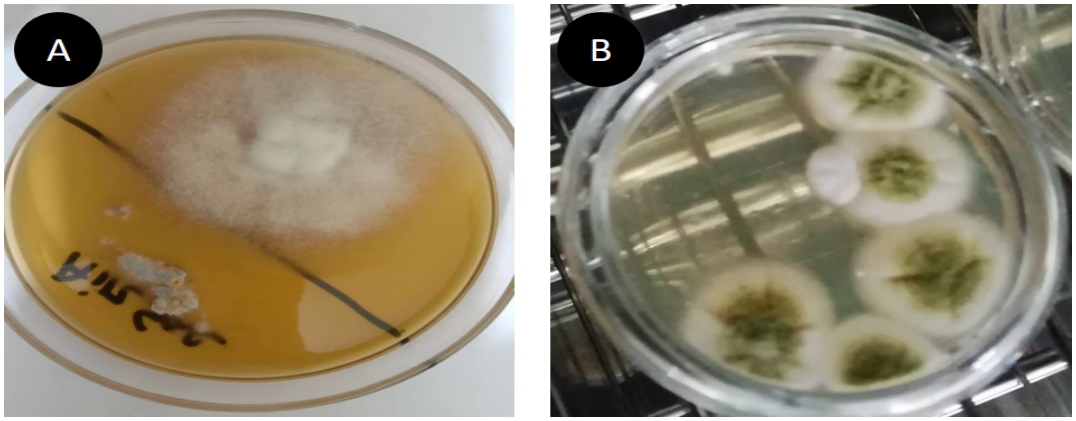

Figure 3 (A-B): Culture of *Aspergillus spp.* suspected samples on SDA agar plate. *Aspergillus fumigatus* after 72 hours of incubation (Fig. 3A); *Aspergillus flavus* 80 hours of incubation (Fig. 3B).

Molecular Assay

DNA extraction

For the extraction of DNA from the recently cultivated (young) sub-cultured Aspergillus colonies, sterile toothpicks were used to transfer the colonies into micro-centrifuge tubes containing nuclease-free water. The DNA extraction process described below was then employed to prepare the DNA samples for polymerase chain reaction (PCR).

First, 200 µl of colony was harvested by centrifugation at 13,000 rpm for 30 seconds, and the supernatant was discarded. 20 µl of Proteinase K solution (concentration: 20 mg/ml) was added to a 1.5 ml microcentrifuge tube. Then, 200µl of Lysis Solution was added to the same 1.5 ml micro-centrifuge tube, along with PBS if the sample volume was less than 200µl. 200 µl of binding solution was added to the sample tube and mixed by pulse-vortexing for 15 seconds. The mixture was incubated at 56°C for ten minutes. After that, 200 µl of absolute ethanol was added and mixed by pulse-vortexing for 15 seconds. Next, a quick spin-down was performed to collect any drips under the lid. The lysate was transferred carefully from the lower reservoir of the spin column to the top reservoir of the spin column. After centrifugation at 13,000 rpm for 1 minute, the flow-through was discarded, and the spin column was reassembled with a 2.0 ml collecting tube. Afterwards, 500µl of Washing 1 solution was added to the spin column with the collecting tube and centrifuged at 13,000 rpm for 1 minute. The flow-through was discarded. 500µl

of Washing 2 solution was added to the spin column with the collecting tube, and the apparatus was centrifuged at 13,000 rpm for 1 minute. Additional centrifugation removed any remaining ethanol in the spin column. The spin column was moved to a fresh 1.5-mL microcentrifuge tube. Then, 30µl of elution solution was added to the spin column, allowed to stand for at least one minute, and genomic DNA was extracted by centrifuging at 13,000 rpm for 1 minute. Finally, the eluted DNA samples were stored at -20°C until PCR analysis was performed.

Species specific identification by PCR

The molecular confirmation of suspected colonies involved the use of genus and species-specific genes. Specific primers designed for the targeted genes were utilized to perform both uniplex and multiplex PCR for amplifying *Aspergillus* spp. genes. Table 1 displays the reference primers and their corresponding amplicon sizes employed in this study. The previously extracted DNA was used as the template for the PCR test. The PCR assay was optimized in a 25-µL reaction mixture, comprising 2 µL of DNA, 12.5 µL of 2X master mix (GoTaq Promega Green Master Mix), 0.5 µL of primers (10 pmol/µL), and the remaining volume filled with nuclease-free water. The thermal cycling conditions for the PCR assay were based on the protocol described by Arafat et al. (2022).

Table 1 Primer used in this study for molecular identification of *Aspergillus* spp.

Target species	Primer	Primer sequence (5´-3´)	Amplicon Size (bp)	References
Aspergillus spp.	*ASAP*	F-CAGCGAGTACATCACCTTGG R-CCATTGTTGAAAGTTTTAACTGATT	521	(Sugita et al., 2004)
A. flavus	*fla*	F-GTAGGGTTCCTAGCGAGCC R-GGAAAAAGATTGATTTGCGTTC	497	(Al-Shuhaib et al., 2018)
A. fumigatus	*Fmi*	F-ACTACCGATTGAATGGCTCG R-CATACTTTCAGAACAGCGTTCA	310	(Sugita et al., 2004)

PCR amplification was performed using ASAP primers to specifically detect *Aspergillus* spp., resulting in 521 bp bands under UV exposure, confirming their presence (Figure 4A). Additionally, primers specific for *Aspergillus flavus* (fla) yielded 500 bp bands (Figure 4B), and primers for *Aspergillus fumigatus* (fmi) produced 310 bp bands (Figure 4C).

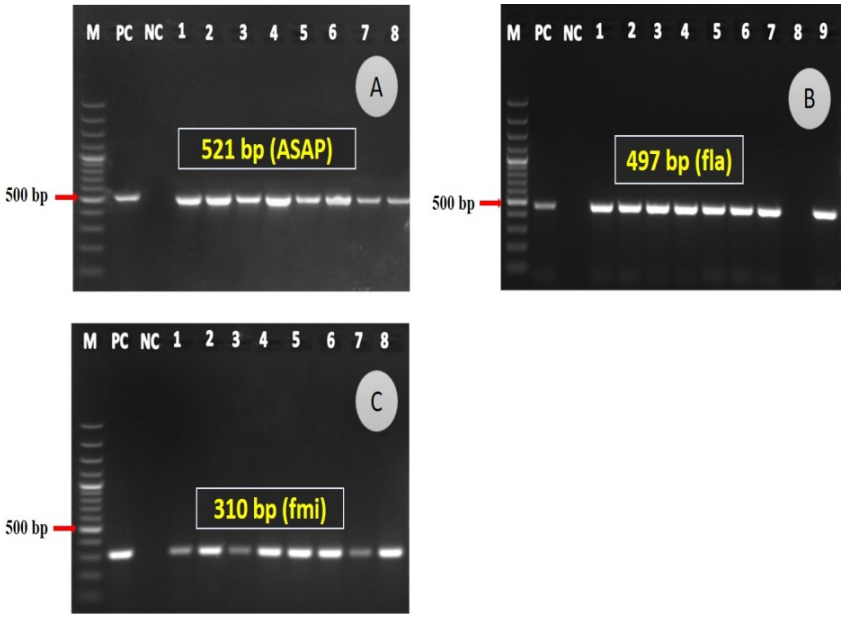

Figure 4 (A) Average daily gain (ADG) and (B) feed conversion ratio (FCR) of the experimental chickens

Any positive outcome for *Aspergillus* spp., including strains like *A. flavus* or *A. fumigatus*, was regarded as suggestive of Aspergillosis. It is noteworthy that every sample that exhibited a positive result in the culture testing also confirmed positive through PCR detection, as shown in Figure 4.

Therapeutic measures

After detecting Aspergillus-positive farms, three farms were chosen for therapeutic measures. A total of 270 birds were treated with three different therapeutic strategies, which were categorized into Treatment Group A (n = 90), Treatment Group B (n = 90), and Treatment Group C (n = 90), each group was further divided into three replications, with each replication consisting of 30 birds. Details about therapeutic groups are shown in Table 2. Once the birds looked clinically healthy, a representative sample of five birds (n = 5) was randomly chosen for culture to detect Aspergillosis. Upon obtaining negative results, the recovery time was recorded, and the mortality percentage was documented.

Statistical analysis

The data from the field and laboratory were gathered and organized into the Microsoft Excel 2013 (USA) spreadsheet. Subsequently, both the parametric and non-parametric data were coded and, sorted. The prevalence for Aspergillosis was calculated using the following formula stated by Mahen et al. (2024) and Asha et al. (2024):

$$\text{Prevalence} = \frac{\text{Number of current cases (new and preexisting) at a specified point in time}}{\text{Population at the same specified point in time}} \text{X} 100$$

The precision of these estimates was ensured by calculating the 95% confidence interval of the proportions followed by Naser et al. (2024). A chi-square goodness-of-fit test was performed among the explanatory variables of chicken. The P-value (<0.05) was considered the level of significance. Following this, the data were analyzed for multivariate regression and Spearman's correlation using SPSS v26 (IBM, USA) and R and RStudio, respectively. A one-way ANOVA was performed to estimate the mean recovery time and mortality percent, and the result was visualized using GraphPad Prism 8 software.

All the relevant independent variables associated with the dependent variable were chosen for univariate analysis. The univariate chi-square analysis of the "risk factor" was performed initially. Significant variables (p≤0.20) from the univariate analysis were selected for a multivariate logistic regression analysis (Hoque et al., 2023).

Table 2 Grouping of birds for therapeutic purpose

Group	No. of birds	Drug Group	Name of drugs	Trade Name & Composition	Dosages
A	90	Anti-Fungal	Fluconazole	Cap Candinil 200mg	4mg/kg B.W., drinking water, once daily for 7 days
		Toxin Binder	Sacharomyces cerevisae, organic acids,Vitamins & Minerals	Liquid Two-Plus Vet	1ml/liter of drinking water, twice daily for 7 days
		NSAID	Paracetamol	Powder Fast Vet, 500mg/g	1ml/2liter of drinking water, thrice daily for 7 days
		Litter Disinfectant	Blue vitriol		1gm/3liter CuSO4.5H2O, spray on litter, twice daily
B	90	Anti-Fungal	Itraconazole	Cap Itra 100	5mg/kg B.W., drinking water, twice daily for 7 days
		Toxin Binder	Sacharomyces cerevisae, organic acids,Vitamins & Minerals	Liquid Two-Plus Vet	1ml/liter of drinking water, twice daily for 7 days
		NSAID	Paracetamol	Powder Fast Vet, 500mg/g	1ml/2liter of drinking water, thrice daily for 7 days
		Litter Disinfectant	Blue vitriol		1gm/3liter CuSO4.5H2O, spray on litter, twice daily
C	90	Anti-Fungal	Nystatin	Tab Nystat	1lac unit/liter of drinking water, twice daily for 7 days
		Toxin Binder	Sacharomyces cerevisae, organic acids,Vitamins & Minerals	Liquid Two-Plus Vet	1ml/liter of drinking water, twice daily for 7 days
		NSAID	Paracetamol	Powder Fast Vet, 500mg/g	1ml/2liter of drinking water, thrice daily for 7 days
		Litter Disinfectant	Blue vitriol		1gm/3liter CuSO4.5H2O, spray on litter, twice daily

RESULTS

Overall Prevalence of Aspergillosis in indigenous chicken at the selected area

Overall prevalence was (12.18%) that shown in Table 3 which was calculated by equation for prevalence.

Table 3 Overall Prevalence of Aspergillosis in indigenous chicken at the selected area

No. of birds examined	Positive case	Prevalence % (95% CI)
394	48	12.18 (9.12-15.83)

Prevalence of Aspergillosis based on breed, age and sex

Table 4 presents the prevalence of Aspergillosis based on various bird-related factors. No significant variation in prevalence was observed among different bird breeds (p = 0.756). The prevalence in male birds was 10.27% (19 out of 185), while in females, it was higher at 13.88% (29 out of 209). Female chickens exhibited

a comparatively higher prevalence of Aspergillosis. Additionally, among different age groups, chicks aged between 0 and 15 days demonstrated a significantly higher prevalence of Aspergillosis (18.96%) with a p-value of less than 0.001.

Table 4 Prevalence of Aspergillosis according to the bird (n=394) related factors at the selected area

Predictor Variable	No. of sample tested	No. of positive	Prevalence %, (95% CI)	χ2 Value	P-value
Breed				0.096	0.756
Naked Neck	89	10	11.24% (5.52-19.69)		
Deshi	305	38	12.46% (8.97-16.70)		
Sex				1.192	0.275
Male	185	19	10.27% (6.30-15.57)		
Female	209	29	13.88% (9.49-19.32)		
Age				19.496	<0.001
0-15 days	211	40	18.96% (13.90-24.91)		
16-30 days	87	4	4.60% (1.21-11.36)		
31-50 days	96	4	4.17% (1.15-10.33)		

CI: Confidence Interval; Chi-square goodness of fit test

Prevalence based on Season

The prevalence of Aspergillosis exhibited a significant difference between winter and summer ($p < 0.05$), with a higher prevalence in rainy season at 17.24%, compared to 11.36% in winter as detailed in Table 5.

Table 5 Frequency of Aspergillosis based on season

Season	No. of sample tested	No. of positive	Prevalence %, (95% CI)	x2 Value	P-value
Winter (Nov-Feb)	132	15	11.36	0.036	0.036
Summer (Mar-June)	117	08	6.84		
Rainy (July-Dec)	145	25	17.24		

CI: Confidence Interval; Chi-square goodness of fit test

Farm level prevalence

This investigation identified certain farm-related factors contributing to the occurrence of Aspergillosis. Farms with moist litter conditions showed a significantly higher prevalence of Aspergillosis ($p = 0.002$). Specifically, the prevalence in dry litter conditions was 10.53%, whereas in moist litter conditions, it spiked to 63.64%. Furthermore, farms using sawdust, rice husk, and sand recorded prevalence rates of 42.86%, 27.78%, and 20.00%, respectively. In high stocking density conditions, the prevalence was 66.67%, while in low stocking density conditions, it was 10.00%. Farms with improper ventilation exhibited a prevalence of 37.50%, as outlined in Table 6. The farm-related associated factors are visually represented in Figure 5.

Species identification and mixed infection

Within the culture-positive samples, molecular assay identification revealed 31 samples as *A. fumigatus* and 16 samples as *A. flavus*. In the overall analysis, 7 samples tested positive for *Aspergillus* spp., encompassing both *A. fumigatus* and *A. flavus*. Notably, co-infection with both *A. flavus* and *A. fumigatus* was observed in two samples, as depicted in Figure 6.

Table 6 Frequency of Aspergillosis according to the farm related risk factors

Predictor Variable	No. of sample tested	No. of positive	Prevalence %, (95% CI)	x2 Value	P-value
Litter condition				9.358	0.002
Moist	11	07	63.64% (30.79-89.07)		
Dry	19	02	10.53% (1.30-33.14)		
Type of litter				1.308	0.52
Saw dust	07	03	42.86% (9.90-81.59)		
Rice husk	18	05	27.78% (9.69-53.48)		
Sand	05	01	20.00% (0.51-71.64)		
Stocking density				5.760	0.056
High	06	04	66.67% (22.28-95.67)		
Moderate	14	04	28.57% (8.39-58.10)		
Low	10	01	10.00% (0.25-44.50)		
Ventilation status				3.087	0.079
Proper	14	03	21.43% (4.66-50.80)		
Improper	16	06	37.50% (15.20-64.57)		

CI: Confidence Interval; Chi-square goodness of fit test

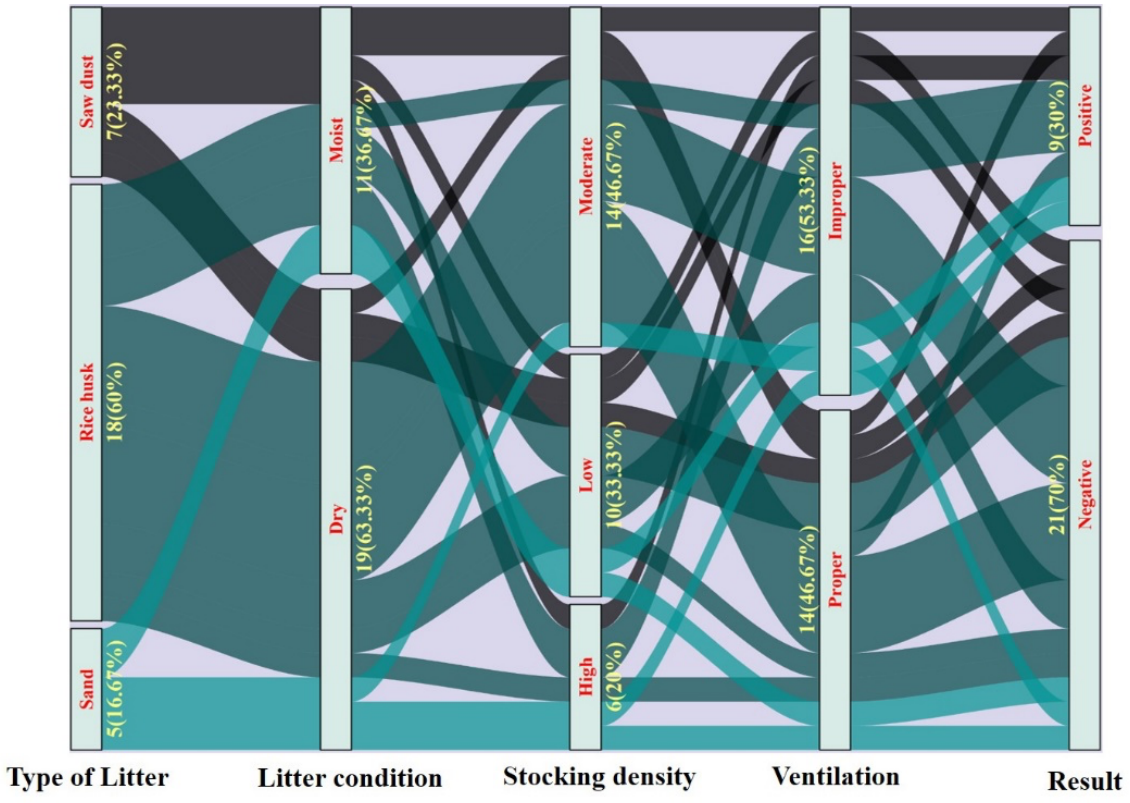

Figure 5 Sankey diagram showing the association among the farm level factors

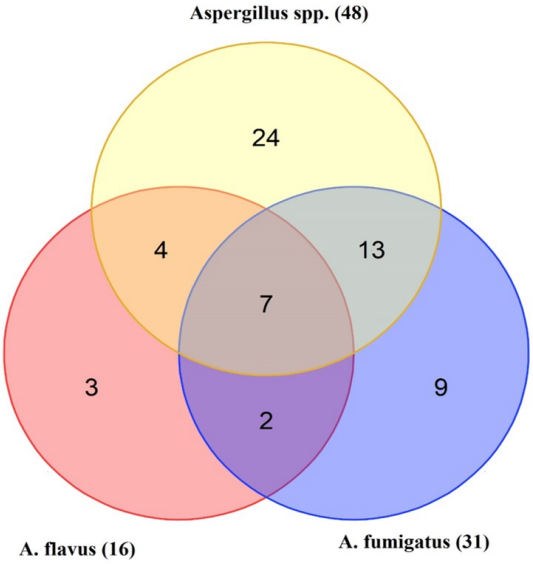

Figure 6 Venn diagram showing the interaction and mixed infection with different *Aspergillus* spp.

Determination of risk factors

Following multivariate logistic regression analysis, three significant risk factors were identified (refer to Table 7). Chicks in the 0–15-day age category were found to be 5.38 times more likely to experience Aspergillosis compared to chickens in the 30-+-day age group (OR = 5.38; P = 0.002). Additionally, chickens in winter were discovered to be more susceptible to Aspergillosis (OR = 3.55; P = 0.001). Concerning farm-related factors, farms with moist litter conditions exhibited significantly higher susceptibility to Aspergillosis (OR = 14.88; P = 0.006).

Table 7 Multivariate logistic regression analysis of risk factors associated with Aspergillosis in chicken

Factors	Predictors	Odds ratio	95% CI	P-value
Age				
	0-15 days	5.38	1.87-15.51	0.002
	16-30 days	1.11	0.27-4.57	0.887
	31-50 days	1		
Season				
	Winter	3.55	1.71-7.34	0.001
	Summer	1		
Litter condition				
	Moist	14.88	2.19-100.66	0.006
	Dry	1		

CI: Confidence Interval; Odds ratio 1 indicates reference category

Recovery time and Mortality

In group B, where the treatment involved Itraconazole with fungal toxin binder, NSAID drug (Paracetamol), and litter disinfectant (Blue vitriol), the recovery

time was (4.00±1.00) days, whereas in group C, the recovery time was 6.67±1.53 days.

The group with the highest mortality (mean) was observed in group C (7.78%), while the lowest mortality was found in group A (4.44%), as illustrated in Figure 7.

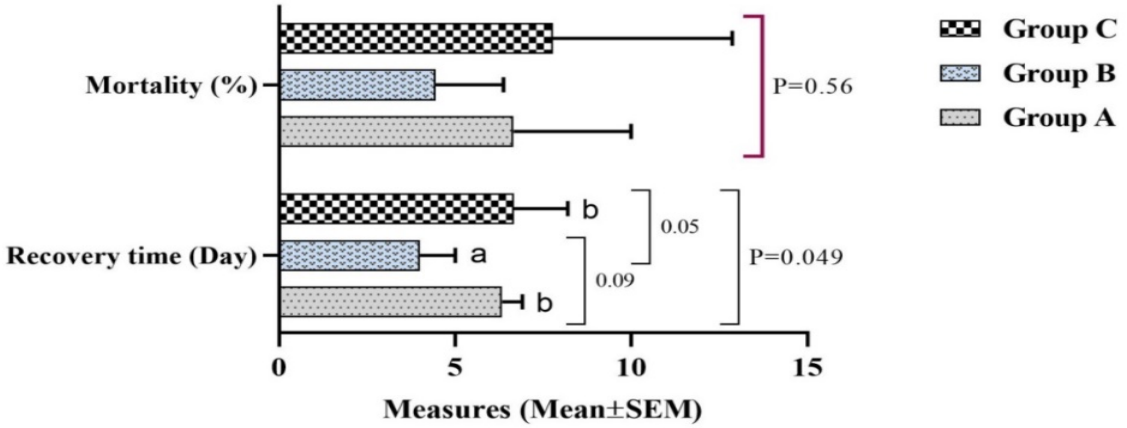

Figure 7 Mean recovery time and mortality (%) of birds after therapeutic treatment.

DISCUSSION

This investigation explores the prevalence, risk factors, and therapeutic measures associated with Aspergillosis in the Joypurhat district of Bangladesh. Our investigation focused on native chicken breeds, specifically non-descriptive Deshi chicken and naked neck, revealing a prevalence of 12.18%. Furthermore, the prevalence in Deshi chicken stood at 12.46%, while naked neck exhibited a prevalence of 11.24%. Comparisons were drawn with existing literature, including studies by Arafat et al. (2022), Eassa et al. (2017), and Sultana et al. (2015).

Interestingly, our findings indicated a slightly lower prevalence compared to the study conducted by Arafat et al. (2022), where the prevalence of Aspergillosis in commercial chicken in Gazipur district was notably higher at 44%. The variations in prevalence observed in native chicken breeds may be attributed to factors such as robust immunity, adaptability to harsh environments, species-specific differences, seasonal fluctuations, sample size, exposure levels, rearing practices, geographical location, and other associated risk factors. This study concentrated on native chicken breeds, such as deshi and naked neck varieties, which are renowned for their resilience and capacity to flourish in harsh environments. Geographical considerations, as highlighted by the study's location in Joypurhat district, may play a crucial role in influencing prevalence rates. This is in line with observations made by Eassa et al. (2017), who reported higher Aspergillosis prevalence in their respective studies. Conversely, Sultana et al. (2015) found a lower prevalence of 6.14% in Chattogram district, emphasizing the impact of geographical variations, seasonal impact, and age variation on disease prevalence.

Additional studies conducted in different districts of Bangladesh reported varying prevalence rates, such as 7.98% in Narshindi district (Uddin et al., 2010), 4.20% in Mymensingh district (Talha et al., 2002), 1.54% (Uddin et al., 2011), and 1.60% in Gaibandha district. These discrepancies may be attributed to differences in sample size, diagnostic methods employed, and the specific geographical locations where the studies were conducted. It is noteworthy that our study employed a comprehensive approach, combining post-mortem examination, culture, and PCR techniques for the precise identification of Aspergillus, enhancing

the accuracy of our diagnostic findings. This multidimensional approach strengthens the validity of our results and contributes valuable insights to the understanding of Aspergillosis prevalence in native chicken breeds in the Joypurhat district of Bangladesh.

The predominant cause of Aspergillosis in chickens is *Aspergillus fumigatus* rather than *Aspergillus flavus*, aligning with the findings of Arafat et al. (2022). Molecular assays were utilized for diagnosis, consistent with our study. Post-mortem investigations identified common pathological conditions, such as lung nodules, air sacculitis, and respiratory distress, echoing similar findings by Sultana et al. (2015). While the prevalence did not significantly differ by location, Sultana et al. (2015) reported significant variation in different upazilas of Chattogram district. Our study revealed seasonal variations, with the highest prevalence in the rainy season, followed by winter and summer, in agreement with Islam et al. (2003) and Sultana et al. (2015). However, Sajid et al. (2006) reported a higher prevalence in the summer, contrasting our findings. Climatic factors like humidity, temperature, and rainfall may contribute to these variations. Female chickens exhibited a higher susceptibility to Aspergillosis, attributed to their lower immune status compared to males. Age emerged as a significant factor, with chicks aged 0–15 days showing the highest prevalence, consistent with Sajid et al. (2006) and Sultana et al. (2015). Herd-level risk factors included litter material, with sawdust and rice husk contributing to prevalence, in line with Sultana et al. (2015). Moist litter conditions were a significant predisposing factor (OR = 14.88). High stocking density and poor ventilation were crucial factors, exacerbating Aspergillosis conditions.

In Bangladesh, deprived and poor people usually rear indigenous chickens, as they do not require investment of money for this farming. Indigenous chickens can find their own feed by grazing around the environment, and they are always in risk of warm and humid environment, unclean conditions, and contaminated feed and water. Henceforth, they are very prone to be affected to this disease.

Multivariate logistic regression analysis identified age (0–15 days), winter season, and litter condition as biologically plausible risk factors for Aspergillosis in native chickens. Therapeutically, various antifungal drugs, including Fluconazole, Itraconazole, and Nystatin, were evaluated with a fungal toxin binder, an NSAID drug (Paracetamol), and a litter disinfectant (Blue vitriol) in experimentally infected birds. Itraconazole showed superior efficacy, reducing recovery time and mortality, consistent with Jennings et al. (1993). The focus was on exploring the best therapeutic treatment to prevent Aspergillosis in chickens, with Itraconazole in combination with a fungal toxins binder, an NSAID drug (Paracetamol), and a litter disinfectant (Blue vitriol) showing promising results. Further investigation is warranted to isolate the specific effects of Itraconazole. Limitations of the study include a focus on acute Aspergillosis and being the first of its kind in Bangladesh for Naked Neck and Native chicken breeds regarding risk factors, prevalence, and therapeutic measures. This study had several limitations, including a small sample size of 30 farms in Joypurhat district, which limits the generalizability of the findings. Selection bias may have occurred due to reliance on voluntary participation, potentially excluding farms with different practices. Environmental factors, such as microclimates, were not considered, possibly affecting disease prevalence. Diagnostic methods were primarily post-mortem, missing subclinical or early-stage infections. Sabouraud dextrose agar, while standard, may not detect all cases, and molecular diagnostics are constrained by the quality of DNA extraction and PCR conditions.

Future research should include a larger, more diverse sample and expand geographically. Non-invasive diagnostics, such as serology, should be incorporated for early detection of subclinical infections. Longitudinal studies are needed to monitor disease trends, and research should investigate the impact of environmental and management practices on Aspergillosis prevalence. Advanced techniques, like next-generation sequencing, should be used to explore *Aspergillus*

spp. genetic diversity. Integrated disease management strategies, including improved environmental management and biosecurity, are essential. Additionally, educational programs for farmers on early detection and effective treatment are recommended.

CONCLUSIONS

The current study in Bangladesh addressed the underexplored issue of Aspergillosis in native chickens caused by Aspergillus spp. The research identified 12.18% prevalence, with higher susceptibility in chicks aged 0–15 days. Aspergillus fumigatus was the predominant species. Key risk factors included chick age, the winter season, and moist litter conditions. Itraconazole, in combination with fungal toxin binder, NSAID drug (Paracetamol), and litter disinfectant (Blue vitriol), proved most effective in reducing recovery time and mortality. These findings offer practical insights for sustainable poultry production and the development of resilient native chicken breeds in Bangladesh.

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AUTHOR CONTRIBUTIONS

Md Nurnoby Islam designed and supervised the study. Mst Aireen Akter, Md Shajedur Rahman, Nazmi Ara Rumi, Md Masuk Rahman Kingshuk, and Hemayet Hossain conducted the experiment. Mst Aireen Akter analyzed the data. Mst Aireen Akter and Md Masuk Rahman Kingshuk wrote the manuscript. Md Nurnoby Islam revised the manuscript.

CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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