ARTICLE

Seroprevalence and risk factors of Newcastle disease virus in unvaccinated backyard chickens in Bukidnon, Philippines

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ABSTRACT

Newcastle disease (ND) remains a significant threat to poultry production in the Philippines, particularly within smallholder backyard systems. This cross-sectional serological survey evaluated the seroprevalence of ND antibodies and assessed associated risk factors among unvaccinated backyard chickens in selected areas of Bukidnon. A total of 288 blood samples were collected from chickens in four study areas in Bukidnon: two municipalities (Manolo Fortich and Quezon) and two cities (Malaybalay City and Valencia City). An indirect enzyme-linked immunosorbent assay (ELISA) was employed to detect ND antibodies, and associations between seropositivity and potential risk factors were analyzed using chi-square tests and odds ratio estimates. The observed and true prevalence of ND antibodies were 12.50% (95% CI: 8.68–16.32%) and 12.71% (95% CI: 8.86–16.56%), respectively. However, no statistically significant differences in ND seroprevalence were found among the study areas ($p > 0.05$). Confined housing posed the greatest threat (OR = 10.23), followed by feeding through/bowl (OR = 3.22), and age segregation (OR = 2.63). The detection of ND antibodies in unvaccinated backyard chickens indicates natural exposure to ND virus within the surveyed areas. These findings are important in biosecurity, vaccination strategies, and enhanced ND surveillance in smallholder poultry systems to inform evidence-based husbandry and management practices, thereby mitigating viral transmission and preventing disease outbreaks.

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INTRODUCTION

Poultry production has been reported as the fastest-growing agricultural sector in the Philippines. Based on the July-September 2023 quarter, chicken output reached 464.97 thousand metric tons (PSA 2023), with national inventories growing to approximately 217 million birds by late 2025 amid post-pandemic recovery, rising demand, and expanded backyard operations (PSA 2025b). Backyard poultry farming underpins this growth by providing household income and a readily accessible source of animal protein for rural families. However, smallholder flocks are typically raised under minimal biosecurity, often with mixed-age birds and free-range housing. These conditions increase contact among domestic birds and with wild avifauna, creating an environment conducive to the introduction and spread of infectious diseases both within and between flocks (Sato and Wakenell 2020). As of 2023, Northern Mindanao was the third-largest chicken-producing region in the Philippines, recording a liveweight production of 178,403 metric tons and a total chicken inventory of 10,538,103 birds (Philippines Statistics Authority, 2024). Within the region, Bukidnon served as the primary poultry-producing province, contributing 79,351 metric tons or 44.5% of the regional output. By 2025, Northern Mindanao maintained the highest inventory of

KEYWORDS

Newcastle disease virus, seroprevalence, backyard poultry, ELISA, risk factors, Philippines

native chickens in the country, with 10.08 million birds (Philippines Statistics Authority, 2025a). The representativeness of the present study is supported by the substantial poultry populations within the selected study sites in Bukidnon. Valencia City had the largest chicken inventory (343,680 birds), followed by Malaybalay City (68,534 birds), Quezon (23,320 birds) and Manolo Fortich (23,257 birds) (Philippines Statistics Authority, 2025b). Given that 43.3% of the region's inventory consists of native or improved chickens and nearly all birds are raised on small-scale farms, these high-density areas present a significant baseline for potential viral transmission. The proximity of these smallholder operations necessitates a rigorous evaluation of current management and biosecurity practices to mitigate the risk of disease outbreaks in the area.

One of the most economically devastating and contagious viral infections in domestic and wild birds is Newcastle disease (ND), caused by *avian orthoavulavirus 1* and affecting domestic and wild birds. It leads to widespread illness, rapid mortality in poultry, mild flu-like symptoms, and conjunctivitis in humans (Dimitrov 2023). ND outbreaks have been documented across various poultry systems in the Philippines, including a notable outbreak among native chickens following poultry dispersal programs in Bohol, which caused substantial mortality and highlighted gaps in bird movement controls and vaccination compliance (Tapdasan, unpublished thesis). Outbreaks of velogenic Newcastle disease virus (subgenotype VIIi) in Central Luzon (Region 3) caused significant poultry losses following late 2015 typhoons, escalating into widespread mortality in commercial and backyard flocks by early 2016 (Umali et al., 2017; Beguas and Umali, 2018). Additionally, serological surveys in Batangas have revealed widespread exposure to ND virus among native chickens in live bird markets, indicating its circulation in unvaccinated populations (Resplandor and Umali, 2018). Environmental stressors, such as sudden temperature changes, high humidity, and heavy rainfall, further exacerbated mortality rates in affected flocks (Mwamberi, 2022). Despite the significant impact of ND, data on seroprevalence and associated risk factors in unvaccinated backyard chickens remain scarce in many Philippine provinces, including Bukidnon. Given the vulnerability of smallholder systems to disease outbreaks and the potential implications for food security and livelihoods, this cross-sectional survey assessed the seroprevalence of ND antibodies and identified associated risk factors among backyard chickens in selected areas of Bukidnon. The results are intended to inform evidence-based husbandry and biosecurity practices for smallholder farmers and to guide local policy interventions aimed at reducing disease transmission and improving flock health.

MATERIALS AND METHODS

Study Sites

In the Northern Mindanao region, 10,538,103 birds were predominantly smallholder-raised: 43.3% native/improved chickens, 34.5% broilers, and 22.2% layers (PSA, 2023). Bukidnon is a landlocked province in Northern Mindanao covering 10,498.59 km², comprising 20 municipalities and two cities, Malaybalay and Valencia (PhilAtlas, 2021). Bukidnon served as the primary poultry hub, contributing 79,351 metric tons (44.5%) of the regional output. This dominance of backyard systems, combined with Bukidnon's high altitude, moderated temperatures and distinct rainfall patterns, underscores the province's vulnerability to poultry diseases. These climatic conditions directly influence poultry health; for instance, high humidity and sudden temperature fluctuations are known triggers for Newcastle disease and other respiratory illnesses, as the causative pathogens persist and spread more readily in humid conditions. This study was conducted from March to April 2025 in two municipalities of Bukidnon: Manolo Fortich and Quezon, and two cities, Malaybalay City and Valencia City (Figure 1).

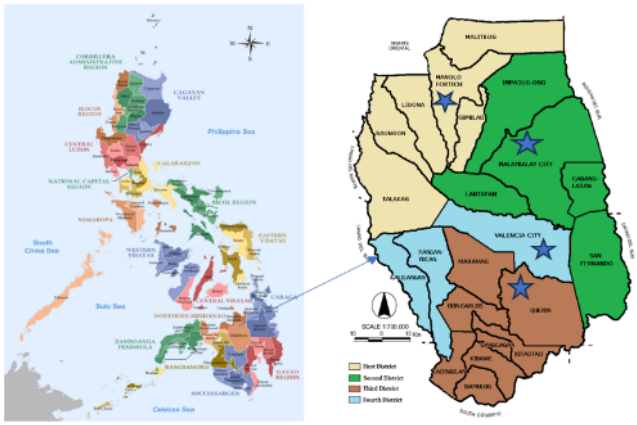

Figure 1: Map of Bukidnon Province indicating the municipalities included in the study on Newcastle disease virus seroprevalence among backyard chickens.

Study Population and Sampling Strategy

A cross-sectional study was conducted to sample 288 unvaccinated backyard chickens from two municipalities and two cities in Bukidnon Province. Within each municipality or city, two barangays were selected based on the presence of active backyard poultry production. Barangays were selected purposively based on the presence of active backyard poultry production, with additional consideration of accessibility and safety recommendations from the respective local government units (LGUs). The selected sites represented the diversity of smallholder backyard poultry production systems within the province and were characterized by relatively high backyard chicken populations, ensuring an adequate pool of potential study animals. Chickens were eligible for inclusion if they were at least one month of age, unvaccinated against Newcastle disease, and clinically healthy at the time of sampling. Birds younger than one month were excluded to minimize potential interference from maternally derived antibodies. Gamefowl were also excluded to maintain homogeneity in production type and management practices among the sampled birds.

Sample Size Determination

The required sample size was calculated using the formula for estimating disease prevalence in a large population. Computation was performed using the Epitools (Sergeant, 2018) to estimate a single proportion. The expected prevalence was was set at 9.33% based on a prior study (Adlawon *et al*., 2019), with a 95% confidence level and a desired precision of 5%. Under these assumptions, the minimum required sample size was 288 chickens. To ensure balanced geographic representation, the total sample was distributed equally across the two municipalities and two cities, resulting in 72 chickens sampled per study site.

Household and Bird Selection

Households with backyard chickens that agreed to participate in the study were purposively selected. A total of 80 respondents from the selected households were interviewed during the study, with 10 per barangay based on the predetermined criteria (at least 18 years old and handler of the sampled chickens). Chickens in confinement were randomly selected based on the flock size. Two chickens were sampled from each household with fewer than 10 birds, while four chickens were sampled from households with ≥ 10 birds.

Ethical Consideration

Ethical approval for the study was obtained from the Institutional Animal Care and Use Committee (IACUC, Approval No. 2025-168D), Institutional Ethics Review Committee (IERC), and University Biosafety and Biosecurity Committee (UBBC) of Central Mindanao University. Written informed consent was

secured from all participating farmers to ensure voluntary participation and adherence to animal welfare and research ethics guidelines. All animal procedures complied with institutional and national standards for the care and use of animals in research.

Blood Collection and Indirect ELISA

Blood samples (2–3 mL) were collected via the wing vein using sterile syringes and transferred to labeled plain vacutainer tubes. Samples were maintained in coolers with ice packs during transport to the Animal Disease Diagnostic Laboratory (ADDL), College of Veterinary Medicine, Central Mindanao University, Maramag, Bukidnon. Serum was separated by centrifugation at 3,000 rpm for 10 minutes and stored at –20 °C until analysis. Serological detection of Newcastle disease virus (NDV) antibodies was performed using an indirect enzyme-linked immunosorbent assay (ELISA; IDEXX® NDV Ab test kit), following the manufacturer's instructions (https://dimune.com/wp-content/uploads/2019/12/IDEXX_NDV_Ab_Test.pdf). Samples were run in duplicate alongside positive and negative controls. The sensitivity and specificity values were 91.5% and 99.0% for the IDEXX NDV Ab Test kit. (World Organization for Animal Health, 2021). Optical density readings were converted to antibody titers using the kit's formula, and a predetermined cut-off was applied to determine seropositivity.

$$S/P = \frac{\text{Sample mean} - \text{NCx}}{\text{PCx} - \text{NCx}}$$

Negative results = S/P ≤ 0.20 Positive results = S/P > 0.20

Where:

PCx = means of positive control

NCx= mean of negative control

S/P= sample to positive ratio

Risk Factors Analysis

The questionnaire was reviewed by the Institutional Ethics Review Committee. The questionnaire included questions on the owner's personal data and demographics, flock scale and management practices, and biosecurity framework. Interviewers were trained using a standardized script for consistency and uniformity before the interview process. A pilot study was conducted with 15 households to assess the clarity of terminology and the logistical feasibility of the interview format. A Cronbach's alpha coefficient > 0.70 was obtained, indicating acceptable reliability. A total of 80 respondents were interviewed during the study, with 10 per barangay based on the predetermined criteria (at least 18 years old and handler of the sampled chickens). Risk factors were assessed at the chicken level.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 27 (IBM Corp., Armonk, NY, USA). The association between sampling location and the seroprevalence of Newcastle disease virus (NDV) antibodies among unvaccinated backyard chickens was assessed using Pearson's chi-square test. Apparent seroprevalence and corresponding 95% confidence intervals (95% CI) were estimated using the exact binomial method. The true seroprevalence and its 95% CI were subsequently calculated: by adjusting the apparent prevalence based on the reported diagnostic sensitivity and specificity of the serological assay. Potential risk factors associated with NDV seropositivity were initially evaluated using Pearson's chi-square tests. Odds ratios (ORs) and their corresponding 95% confidence intervals were calculated to quantify the magnitude of association between each explanatory variable and NDV seropositivity. Two-tailed tests were used, with significance was determined using, with $p < 0.05$ as the threshold for statistical significance.

RESULTS

Seroprevalence of NDV

The overall apparent seroprevalence of ND antibodies was 12.50% (36/288; 95% CI: 8.68–16.32%); after adjusting for the diagnostic sensitivity (91.5%) and specificity (99.0%) of the indirect ELISA kit, the estimated true prevalence was 12.7%. As shown in Figure 2, seroprevalence varied by location, ranging from 15.28% (95% CI: 6.97-23.59%) in Malaybalay City to 9.72% (95% CI: 2.88–16.56%) in Quezon, with no significant differences ($\chi^2 = 1.27$, $p = 0.736$), suggesting broadly comparable exposure levels across the study areas.

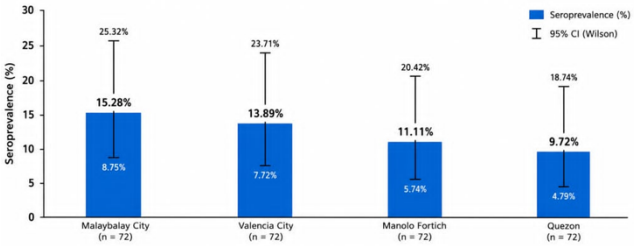

Figure 2: Seroprevalence (%) of Newcastle disease in backyard chickens by municipality in Bukidnon (n= 288).

Risk Factors for ND Seropositivity

As shown in Table 1, housing type, means of feeding, and age separation were all significantly associated with NDV seropositivity ($p < 0.05$). These results reveal counterintuitive risks in backyard systems: confined housing posed the greatest threat (OR = 10.23), followed by feeding bowl/trough (OR = 3.22) and age segregation (OR = 2.63).

Table 1: Risk factors associated with Newcastle disease seropositivity in backyard chickens in Bukidnon

Risk factor	Category	n	Positive	Prevalence % (95% CI)	OR (95% CI)	χ^2	p-value
Housing	Confined	230	35	15.22 (10.58-19.86)	10.23 (1.37-76.32)	7.71	0.005*
	Free-range (Ref)	58	1	1.72 (0.00-5.07)	1.00 (0.06, 16.38)		
Means of Feeding	Bowl/Trough	228	33	14.47 (9.90-19.04)	3.22 (0.95–10.87)	3.90	0.048*
	Ground (Ref)	60	3	5.00 (0.00-10.51)	1.00 (0.19-5.17)		
Age separation	Yes	208	31	14.9 (10.06-19.74)	2.63 (0.98-7.02)	3.96	0.047*
	No (Ref)	80	5	6.25 (0.95, 11.55)	1.00 (0.28-3.60)		

n = number of chickens examined; OR = odds ratio; CI = confidence interval; Ref = reference category

*Significant at p < 0.05

Descriptive Statistics of Backyard Poultry Management and Biosecurity Practices

Management practices showed mixed adoption: 66.7% used designated carcass disposal while others disposed of carcasses indiscriminately; 78.5% sourced tap water; and 55.6% provided health interventions (Figure 3). Age separation (72.2%), use of feeder/trough (79.2%) and utilization of commercial feed (88.9%) aimed to limit transmission, yet high-risk features predominated: 82.6% confined housing, 76.4% within 1 km of other farms, and 63.2% disease history (Figure 3). These patterns were associated with elevated ND seropositivity (Table 1).

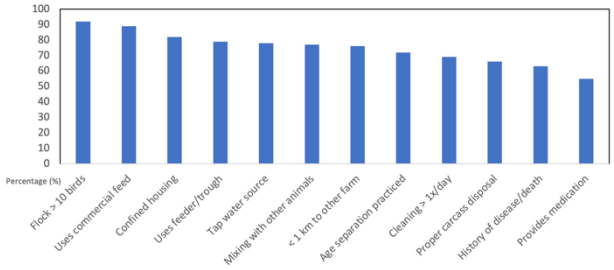

Figure 3: Percentage of reported backyard chicken management and biosecurity practices in the four study municipalities in Bukidnon (n=288)

DISCUSSION

This study detected an apparent NDV seroprevalence of 12.50% (36/288; 95% CI: 8.68–16.32%) and an estimated true prevalence of 12.71% (95% CI: 8.86–16.56%) among backyard chickens across selected municipalities and cities in Bukidnon, indicating prior viral exposure. Previous studies of NDV seroprevalence in Bukidnon have reported 40.7% (95% CI: 32.5-49.5%) in 2024 (Eledia, unpublished thesis), 8.00% (95% CI: 4.90-13.10%) in 2023 (Paraguas, unpublished thesis). Lower seroprevalence estimates reported elsewhere may reflect drier climates that limit viral persistence or stricter vaccination programs, in contrast to Bukidnon's humid tropical conditions and the minimal biosecurity observed in the mixed-age backyard flocks of this study. NDV antibodies in healthy, unvaccinated birds confirm subclinical exposure (Adebiyi et al., 2022; Alexander & Senne, 2008), primarily via aerosols, secretions, fomites, and bird movement (Sallam, 2024; Dimitrov et al., 2023). Uniform seropositivity across municipalities reflects consistent small flock sizes, husbandry, and low biosecurity (Olorunshola et al., 2022; Wodajo et al., 2023).

Among management practices, housing type was the strongest risk factor: confined systems increased seropositivity odds 10.23-fold compared to free-range (p = 0.048). High stocking density promotes NDV transmission via direct contact, aerosols, and crowding-induced immune suppression (Dawkins et al., 2004; Hussein et al., 2022). Optimizing housing design and flock density is thus critical for smallholder poultry disease control. Feeding free-range chickens through a bowl/trough increased NDV exposure (OR = 3.22, $p < 0.01$), with fomite transmission exacerbated by poor disinfection and wild-bird access (Khatun et al., 2022; Dimitrov, 2024). Routine hygiene and dedicated troughs per flock are vital for control in backyard systems. Despite protective intent, incomplete implementation enables transmission via density, fomites, and poor isolation—highlighting the need for optimized biosecurity over partial measures. Age segregation within flocks was also associated with increased seropositivity, with birds in age-segregated flocks exhibiting 2.63 times higher odds of NDV exposure. Although age separation aims to reduce disease transmission, its effectiveness depends on physical isolation and proper biosecurity. In backyard systems, the close proximity of age groups may permit aerosol-mediated spread. As stated by Wodajo et al., (2023) and Song et al. (2021), younger chicks (6–13 days old) are especially vulnerable due to their immature immune systems, whereas older birds gradually develop antibodies following repeated viral exposure.

Overall, these findings indicate that management practices, rather than geographic location, drive NDV exposure in backyard chickens. Confined housing, mixed-age housing, and shared feeding systems create circumstances favorable to viral circulation. The moderate seroprevalence observed implies on-going subclinical infection, which may compromise productivity and smallholder livelihoods in the absence of intervention. Efficient mitigation requires integrated biosecurity interventions, including improved housing design and reduced stocking density, strategic age-group management, sanitation of feeding equipment, and farmer education. Some limitations of this study include its cross-sectional design and purposive sampling, which limit causal inference; a small number of free-range positive cases, leading to wide confidence intervals. Use of a single ELISA test without molecular confirmation; exclusion of seasonal variation (the study was conducted only in March–April); and potential recall bias from farmer-reported data. Future research should incorporate longitudinal monitoring, virus isolation, and seasonal evaluation to better characterize NDV trends in backyard poultry systems.

CONCLUSION

Serological findings reveal widespread NDV antibody prevalence among backyard chickens across selected Bukidnon areas, indicating active viral circulation in smallholder flocks. Analysis identified housing type, shared feeding equipment, and age segregation as primary risk factors for seropositivity (OR > 2.5, $p < 0.05$), underscoring how sub-optimal management amplifies transmission in high-density, low-biosecurity settings. These results underscore the vulnerability of smallholder poultry to ND outbreaks, which threaten flock productivity and livelihoods. Targeted interventions are recommended, including redesigned housing for density control, rigorous age-based segregation, equipment sanitation protocols, regular ND vaccination, and enhanced biosecurity, paired with farmer training and community surveillance. This framework offers a scalable model for NDV control in Philippine backyard systems, supporting sustainable and disease-resilient smallholder poultry production.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Maria Lebena B. Montemayor conceptualized the study, curated the data, and wrote the manuscript; Jenny D. Montemayor collected and processed samples and wrote the first draft; Elaine M. Alkuino, Katherine Mae I. Caterial, Vingelle B. Jimenez, and Bernard V. Calo conceptualized the study and analyzed the data; Karla Cristine C Doysabas conceptualized the study and secured funding for the

research; Jose M. Obedencio, Jr. and Lotis M. Balala conceptualized the study, reviewed, and edited the manuscript.

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