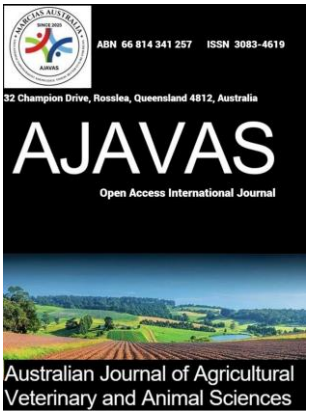

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The prevalence and clinicopathological alterations of chicken coccidiosis in Thai Nguyen Province, Vietnam

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ABSTRACT: *Poultry coccidiosis remains a major parasitic disease causing significant economic losses worldwide. Although previous studies have reported its occurrence in Vietnam, there is a lack of region-specific epidemiological and clinicopathological data under distinct agro-ecological and production conditions in Thai Nguyen Province. This study determined the prevalence, associated risk factors, and clinicopathological alterations of coccidiosis in chickens in this region. A cross-sectional survey was conducted from January to December 2024 in six communes of Phu Binh District, with 300 faecal samples examined using the flotation technique for Eimeria oocysts. Infection intensity was semi-quantitatively assessed, while clinical signs, gross lesions (n = 75), and haematological parameters (10 infected vs. 10 healthy chickens) were evaluated. Prevalence differences were analysed using the Chi-square test, and haematological parameters were compared using independent-samples t-tests ($P < 0.05$). The overall prevalence was 49.00%, with significantly higher rates in chickens aged 1-3 months (67.05%) and in free-range systems (86.90%). Seasonal variation showed higher prevalence in summer (63.04%) and spring (53.57%) compared to winter (33.82%). Infected chickens exhibited diarrhoea, ruffled feathers, lethargy, and anorexia. Haematological analysis revealed reduced red blood cell counts and haemoglobin levels, alongside increased white blood cell counts, heterophils, and eosinophils ($P < 0.05$). Gross lesions included haemorrhagic enteritis, intestinal ballooning, and mucosal thickening. These findings highlight the high prevalence of coccidiosis in Thai Nguyen Province and provide region-specific evidence to support targeted control strategies and improved biosecurity measures.*

Keywords: Chicken coccidiosis, prevalence, haematology, Thai Nguyen Province

Highlights

- Coccidiosis remains highly prevalent in chickens with significant associations between infection and age, season, and husbandry system in Thai Nguyen Province, Vietnam.
- Region-specific epidemiological and clinicopathological evidence herein can support the development of targeted control strategies.
- To better understand the epidemiology and pathogenicity of coccidiosis in this region, future studies should incorporate *Eimeria species* identification and larger sample sizes.

1.0 Introduction

Coccidiosis is one of the most economically important parasitic diseases affecting poultry production worldwide, caused by intracellular protozoa of the genus *Eimeria*. The disease results in intestinal epithelial damage, impaired nutrient absorption, reduced growth performance, and, in severe cases, mortality (Chapman et al., 2013; Quiroz-Castaneda and Dantan-Gonzalez, 2015; Blake et al., 2020; Geng et al., 2021). Globally, the economic impact of coccidiosis has been estimated to exceed billions of dollars annually due to production losses, treatment costs, and preventive measures (Blake et al., 2020). In addition, coccidial infection predisposes chickens to secondary bacterial infections, further exacerbating productivity losses in both commercial and smallholder production systems (Chapman et al., 2013).

In Vietnam, poultry production plays a crucial role in food security and rural livelihoods. Thai Nguyen province is one of the key poultry-producing areas in northern Vietnam, characterised by diverse farming systems, including intensive, semi-intensive, and traditional free-range production (Burgos et al., 2007). These systems are often associated with varying levels of biosecurity, environmental exposure, and management practices, all of which can influence the epidemiology of infectious diseases such as coccidiosis (Razmi and Kalideri, 2000; Shirzad et al., 2011). Previous studies in Vietnam have reported that coccidiosis is widely distributed; however, these studies are often limited by restricted geographical scope, outdated datasets, or a lack of integration between epidemiological patterns and clinicopathological findings.

Despite the recognised importance of coccidiosis, there remains a lack of comprehensive, region-specific data in Thai Nguyen province that simultaneously evaluate prevalence, associated risk factors (such as age, season, and husbandry system), and clinicopathological alterations under local agro-ecological conditions. In particular, few studies have integrated parasitological findings with clinical manifestations and hematological responses, which are essential for understanding disease dynamics and improving diagnostic and control strategies (Razmi and Kalideri, 2000; Shirzad et al., 2011). Furthermore, ongoing changes in poultry production practices and environmental conditions may alter the epidemiological patterns of coccidiosis, highlighting the need for updated and context-specific investigations.
We hypothesized that the prevalence and severity of coccidiosis in chickens in Thai Nguyen province are significantly influenced by host-related factors (age), environmental conditions (season), and management systems (husbandry practices), and that infection is associated with measurable clinicopathological and hematological alterations.
Therefore, the present study aimed to (i) determine the prevalence and infection intensity of coccidiosis in chickens in Thai Nguyen province, (ii) evaluate the effects of key risk factors, including age, season, and husbandry system, and (iii) assess the associated clinical signs, gross pathological lesions, and hematological changes in infected chickens. The findings of this study provide region-specific evidence to support improved disease management and targeted control strategies in poultry production systems.

2.0 Materials and methods

2.1. Study Site

The study was conducted in Phu Binh District, Thai Nguyen Province, northern Vietnam, from January to December 2024. This area is characterised by a monsoonal and humid subtropical climate with distinct seasonal variations, including spring (February-April), summer (May-August), autumn (September-October), and winter (November-January). Poultry production in this district is dominated by smallholder farming systems, including cage, semi-free-range, and free-range production, which differ in management practices and biosecurity levels. The study population consisted of farming households raising crossbred meat-type chickens, which are commonly used in smallholder production systems in the region. The study was conducted in six communes of Phu Binh district, namely Bao Ly, Tan Khanh, Tan Thanh, Tam Kim, Duong Thanh, and Diem Thuy.

2.2. Study design and sampling strategy

A cross-sectional study design was employed to investigate the prevalence of coccidiosis and associated risk factors in chickens. A total of 300 faecal samples was collected from chickens across six communes, comprising 50 samples per commune. In each commune, 10 farms or large-scale poultry households were selected, and five chickens were sampled per farm or household. Farms were selected using a convenience sampling approach based on accessibility and farmers' willingness to participate, which is commonly applied in field epidemiological studies in rural settings. Within each farm, chickens were randomly selected to minimize selection bias. Chickens were categorized into three age groups: <1 month, 1–3 months, and >3 months. Information on potential risk factors, including age, season, and husbandry system, was recorded at the time of sampling. Only clinically healthy or naturally exposed chickens were included, while chickens under active anticoccidial treatment were excluded.

2.3. Parasitological examination

Faecal samples were collected directly from the cloaca or as freshly voided droppings and transported to the laboratory under appropriate conditions. Samples were examined for the presence of *Eimeria* oocysts using a flotation technique with saturated sodium chloride solution. Briefly, approximately 2 g of faeces were homogenised with flotation solution, filtered through a sieve to remove debris, and transferred into a test tube. The tube was filled to form a convex meniscus and covered with a coverslip and allowed to stand for 10–15 minutes to facilitate oocyst flotation. The coverslip was subsequently placed on a glass slide and examined under a light microscope at ×10 magnification (Yu and Heo, 2021). Infection intensity was assessed semi-quantitatively based on the number of oocysts observed per microscopic field. The degree of infection was classified into four levels adapted from the criteria described by Yu and Heo (2021): low (+, <30 oocysts/field), moderate (++, 30-50 oocysts/field), high (+++, 50-80 oocysts/field), and very high (++++, >80 oocysts/field). To minimize observer bias, all samples were examined by the same investigator. Although this method does not provide absolute oocyst counts per gram (OPG), it is widely applied in field-based epidemiological studies for rapid assessment of infection intensity under resource-limited conditions.

2.4. Clinical and pathological examination

Clinical signs were recorded in chickens during field observations. A total of 75 chickens positive for *Eimeria* infection were randomly selected and subjected to necropsy to assess gross pathological lesions. Particular attention was given to intestinal changes, including hemorrhage, ballooning, and mucosal thickening, which are characteristic of coccidiosis.

2.5. Haematological analysis

Blood samples were collected from 10 infected chickens and 10 apparently healthy chickens (control group) for hematological analysis. Parameters measured included red blood cell (RBC) count, haemoglobin (Hb), white blood cell (WBC) count, and differential leukocyte counts (heterophils and eosinophils), using an automated haematology analyser.

2.6. Statistical analysis

All statistical analyses were performed using statistical software (Minitab version 16, Minitab Inc., State College, PA, USA). The prevalence of coccidiosis was calculated as the proportion of positive samples among the total examined. Differences in prevalence among groups (age, season, and husbandry system) were analysed using the Chi-square test. Haematological parameters between infected and healthy chickens were compared using independent samples t-tests. Statistical significance was set at $P < 0.05$.

3.0 Results

3.1. The prevalence and infection intensity of chicken coccidiosis

The prevalence and infection intensity of chicken infected with coccidiosis across the six surveyed communes are summarized in Thai Nguyen Province in Table 1.

Table 1. The prevalence and infection intensity of chicken coccidiosis in Thai Nguyen Province

Communes	No. of chickens examined	No. of positive chickens	Rate (%)	Infection intensity (OPM)							
				Mild		Moderate		Heavy		Very Heavy	
				n	%	n	%	n	%	n	%
Bao Ly	50	28	56.00[a]	13	46.43	7	25.00	5	17.86	3	10.71
Tan Khanh	50	31	62.00[ab]	14	45.16	8	25.81	6	19.35	3	9.68
Tan Thanh	50	21	42.00[ac]	10	47.62	6	28.57	3	14.29	2	9.52
Tam Kim	50	29	58.00[a]	13	44.83	8	27.59	5	17.24	3	10.34
Duong Thanh	50	18	36.00[bd]	9	50.00	6	33.33	2	11.11	1	5.56
Diem Thuy	50	20	40.00[ac]	11	55.00	5	25.00	3	15.00	1	5.00
Total	300	147	49.00	70	47.62	40	27.21	24	16.33	13	8.84

* Means within a column with different superscripts differ significantly (P<0.05).

Out of 300 samples examined, 147 were positive for *Eimeria* oocysts, representing an overall prevalence of 49.00%. The prevalence varied significantly among communes (P < 0.05), ranging from 36.00% in Duong Thanh to 62.00% in Tan Khanh. Regarding infection intensity, the majority of infections were mild. Specifically, 47.62% of positive cases were mild, followed by 27.21% were moderate, 16.33% were heavy, and 8.84% were very heavy.

The prevalence and infection intensity of coccidiosis stratified by age group are presented in Table 2.

Table 2. Variations due to age in the prevalence and infection intensity of chicken coccidiosis

Ages (months)	No. of chickens examined	No. of positive chickens	Rate (%)	Infection intensity (OPM)							
				Mild		Moderate		Heavy		Very Heavy	
				n	%	n	%	n	%	n	%
Less than 1 month	92	47	51.09[a]	22	46.81	13	27.66	8	17.02	4	8.51
1 - 3 months	88	59	67.05[ab]	26	44.07	17	28.81	10	16.95	6	10.17
>3 - 6 months	64	26	40.63[b]	13	50.00	7	26.92	4	15.38	2	7.69
Over 6 months	56	15	26.79[c]	9	60.00	3	20.00	2	13.33	1	6.67
Total	300	147	49.00	70	47.62	40	27.21	24	16.33	13	8.84

* Means within a column with different superscripts differ significantly (P<0.05).

The study revealed that chickens of all ages were susceptible to coccidiosis, but the prevalence significantly differed by age group (P < 0.05). The highest infection rate was observed in chickens aged 1–3 months (67.05%), followed by those under 1 month (51.09%). The lowest rate was found in chickens over 6 months old (26.79%). The peak prevalence observed in the 1–3 month age group likely coincides with the transition from brooding to free-range environments, exposing immunologically immature chickens to contaminated soil and vectors.

The influence of different husbandry methods on the prevalence and intensity of coccidiosis is summarised in Table 3.

Table 3. Variations in husbandry method in the prevalence and infection intensity of chicken coccidiosis

Husbandry method	No. of chickens examined	No. of positive chickens	Rate (%)	Infection intensity (OPM)							
				Mild		Moderate		Heavy		Very Heavy	
				n	%	n	%	n	%	n	%
Cage	96	18	18.75[a]	12	66.67	4	22.22	2	11.11	0	0.00
Semi-free-range	120	56	46.67[ab]	29	51.79	17	30.36	8	14.29	2	3.57
Free-range	84	73	86.90[b]	29	39.73	19	26.03	14	19.18	11	15.07
Total	300	147	49.00	70	47.62	40	27.21	24	16.33	13	8.84

* Means within a column with different superscripts differ significantly (P<0.05).

Farming systems had a significant impact on coccidiosis prevalence (P < 0.05). Free-range chickens exhibited the highest infection rate (86.90%), followed by semi-free-range chickens (46.67%), while caged chickens had the lowest rate (18.75%). The significantly higher prevalence in free-range systems is likely due to continuous contact with soil and environmental pathogens. In contrast, caged systems allow for better waste management and biosecurity, breaking the faecal-oral transmission cycle.

The prevalence and intensity of coccidiosis varied significantly across different seasons, as detailed in Table 4.

Table 4. Seasonal variations in the prevalence and infection intensity of chicken coccidiosis

Season	No. of chickens examined	No. of positive chickens	Rate (%)	Infection intensity (OPM)							
				Mild		Moderate		Heavy		Very Heavy	
				n	%	n	%	n	%	n	%
Spring	56	30	53.57[a]	13	43.33	9	30.00	5	16.67	3	10.00
Summer	92	58	63.04[ab]	24	41.38	14	24.14	13	22.41	7	12.07
Autumn	84	36	42.86[b]	19	52.78	10	27.78	5	13.89	2	5.56
Winter	68	23	33.82[c]	14	60.87	7	30.43	1	4.35	1	4.35
Total	300	147	49.00	70	47.62	40	27.21	24	16.33	13	8.84

* Means within a column with different superscripts differ significantly (P<0.05).

Seasonal analysis showed that coccidiosis was prevalent throughout the year but peaked in Summer (63.04%) and Spring (53.57%). The prevalence was lower in Autumn (42.86%) and lowest in Winter (33.82%). The high prevalence during Spring and Summer is likely associated with warm and humid conditions in Northern Vietnam, which provide an ideal environment for oocyst sporulation and survival. Conversely, the colder temperatures in Winter may inhibit oocyst development.

3.2. Clinicopathological examination and haematological analysis

The frequency of clinical symptoms observed in the affected flocks is detailed in Table 5.

Table 5. The rate and clinical symptoms of chicken infected with coccidiosis

No. of infected chickens	No. of symptomatic chickens	Rate (%)	Clinical symptoms	No. of chickens manifested	Rate (%)
147	64	43.54	Lethargy, reduced motility, ruffled feathers, drooping wings, and huddling behavior	59	92.19
			Emaciation, anorexia (or reduced feed intake), pyrexia, and polydipsia.	53	82.81
			Mucoid (waxy) feces, pasted vent.	42	65.63
			Haemorrhagic diarrhoea (or Bloody diarrhoea).	28	43.75
			Dehydration (dry shanks), leg paralysis.	14	21.88

Among infected birds, 43.54% displayed clinical symptoms. The most common signs included lethargy (92.19%), anorexia, ruffled feathers, and diarrhea with waxy or bloody stools. Necropsy of 75 infected chicken revealed that 86.67% (65/75) had macroscopic lesions in the digestive tract. Typical lesions included ballooning of intestinal segments, petechial hemorrhages in the small intestine, thickened mucosa, and prominent mesenteric blood vessels. In severe cases, the ceca were filled with blood, and the carcasses appeared pale and anaemic. These lesions result from the destruction of epithelial cells and blood vessels by replicating parasites.

The comparison of haematological indices between healthy and infected chickens is presented in Table 6.

Table 6. Haematological parameters of healthy and coccidial chickens

Haematological parameters	Healthy chickens $(\bar{x} \pm m_{\bar{x}})$ n = 10	Coccidial chickens $(\bar{x} \pm m_{\bar{x}})$ n =10	P Value
Red blood cells (10^6/mm^3)	2.86 ± 0.16	1.59 ± 0.05	P< 0.05
White blood cells (10^3/mm^3)	28.72 ± 0.12	39.15 ± 0.38	P< 0.05
Haemoglobin (g%)	12.56 ± 0.14	8.10 ± 0.13	P< 0.05
Heterophils (%)	27.97 ± 0.10	36.89 ± 0.69	P < 0.05
Eosinophils (%)	4.01 ± 0.15	6.03 ± 0.35	P < 0.05
Basophils (%)	5.78 ± 0.05	2.12 ± 0.24	P < 0.05
Lymphocytes (%)	58.17 ± 0.14	51.19 ± 0.49	P < 0.05
Monocytes (%)	5.79± 0.31	3.34± 0.33	P < 0.05

Infected chickens exhibited a significant reduction in RBC counts (1.59 x 10^6/mm^3) and haemoglobin levels (8.10 g/dL) compared to healthy controls ($P < 0.05$), indicating severe anemia. Conversely, the total WBC count significantly increased in infected chickens (39.15 x 10^3/mm^3) compared to healthy ones (28.72 x 10^3/mm^3). Regarding the differential leukocyte count, infected chickens showed significantly higher percentages of heterophils and eosinophils

($P < 0.05$). Specifically, eosinophils increased to 6.03%, consistent with the immune response to parasitic infection. However, a significant decrease was observed in lymphocytes, monocytes, and basophils.

4.0 Discussion

The present study provides updated, region-specific data on the prevalence, risk factors, and clinicopathological alterations associated with coccidiosis in chickens in Thai Nguyen Province, Vietnam. The overall prevalence of 49.00% indicates that coccidiosis remains a highly prevalent parasitic disease in the study area. This finding is consistent with previous reports from Vietnam and other tropical regions, where warm and humid environmental conditions favour the sporulation and survival of *Eimeria* oocysts (Attree et al., 2021; Ahad et al., 2015). However, the relatively high prevalence observed in this study may also reflect local production practices, particularly the widespread use of free-range systems with limited biosecurity measures. A significant association between age and infection was observed, with the highest prevalence recorded in chickens aged 1-3 months. This age-related pattern can be explained by the gradual development of immunity to *Eimeria* infection (McDougald and Fitz-Coy, 2008). Younger chickens, especially those in the early post-weaning period, are more susceptible due to their immature immune systems and increased exposure to contaminated environments. As birds age, repeated exposure to low levels of oocysts may lead to the development of partial protective immunity, resulting in lower infection rates in older chickens. Similar age-dependent trends have been widely reported in previous epidemiological studies (Razmi and Kalideri, 2000).

The markedly higher prevalence in free-range systems compared to confined systems highlights the important role of husbandry practices in disease transmission. In free-range systems, chickens are more likely to come into contact with contaminated soil, feces, and environmental reservoirs, which facilitate the ingestion of infective oocysts. In contrast, confined systems, although not free from infection, may benefit from improved hygiene, controlled feeding, and reduced environmental exposure. This finding is consistent with previous studies highlighting the role of management systems in coccidiosis epidemiology (Razmi and Kalideri, 2000). Seasonal variation was also evident, with higher prevalence during the summer and spring compared to winter. This pattern is likely associated with increased temperature and humidity, which are optimal for oocyst sporulation and survival in the environment (Attree et al., 2021; Ahad et al., 2015). Conversely, lower temperatures during winter may reduce oocyst development and transmission. These findings highlight the influence of climatic factors on the epidemiology of coccidiosis and suggest that control strategies should be adapted according to seasonal risk patterns. Gross pathological examination revealed lesions typical of coccidiosis, including haemorrhagic enteritis, intestinal ballooning, and mucosal thickening. These lesions are consistent with the destructive intracellular life cycle of *Eimeria*, which leads to epithelial cell rupture, inflammation, and tissue damage (Freitas et al., 2023). The integration of parasitological, clinical, and haematological findings in this study provides a more comprehensive understanding of the disease compared to studies that focus solely on prevalence. The haematological findings of this study revealed reduced red blood cell counts and haemoglobin levels in infected chickens, indicating anemia. This condition is likely associated with intestinal haemorrhage and mucosal damage caused by *Eimeria* infection, which impairs nutrient absorption and may lead to blood loss (Chapman et al., 2013; Awais et al., 2012). In addition, significant increases in white blood cell counts, heterophils, and eosinophils were observed. The elevation of heterophils reflects an acute inflammatory response to intestinal tissue damage, while increased eosinophils are typically associated with parasitic infections and may play a role in host immune responses against *Eimeria* (Chapman et al., 2013). These haematological alterations provide further evidence of the systemic impact of coccidiosis beyond the gastrointestinal tract and highlight their potential value as supportive diagnostic indicators.

Despite these findings, several limitations should be acknowledged. First, the identification of *Eimeria* species was not performed, which limits the ability to assess species-specific pathogenicity and epidemiological patterns. Second, the semi-quantitative method used to assess oocyst load does not provide precise measurements compared to standard techniques such as oocysts per gram (OPG). Third, the relatively small sample size used for hematological analysis may limit the generalisability of these findings and should be interpreted with caution. Future studies should incorporate molecular identification of *Eimeria* species, quantitative parasitological methods, and larger sample sizes to enhance the robustness of the results.

Overall, this study expands current knowledge by providing integrated epidemiological and clinicopathological data on coccidiosis in a specific agro-ecological context in northern Vietnam. The findings have important practical implications for disease control, particularly in highlighting the roles of age, husbandry system, and seasonal factors in shaping

infection dynamics. Targeted interventions, including improved hygiene, strategic use of anticoccidial agents, and enhanced biosecurity in free-range systems, are essential to reduce the burden of coccidiosis in the region.

5.0 Conclusion

This study demonstrated that coccidiosis remains highly prevalent in chickens in Thai Nguyen province, Vietnam, with significant associations observed between infection and age, season, and husbandry system. Higher prevalence in young chickens and free-range production systems highlights the important role of management practices and environmental exposure in disease transmission. Infected chickens exhibited clinical signs and characteristic intestinal lesions, accompanied by haematological alterations, including reduced erythrocytic indices and increased leukocyte counts, indicative of anaemia and inflammatory responses. These findings provide region-specific epidemiological and clinicopathological evidence that can support the development of targeted control strategies, particularly through improved biosecurity, optimized management practices, and seasonal disease prevention. However, further studies incorporating *Eimeria* species identification and larger sample sizes are needed to better understand the epidemiology and pathogenicity of coccidiosis in this region.

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Ethics Approval Statement: All research procedures were conducted in compliance with Article 72 of the Law on Livestock Production (Law 32/2018/QH14) regarding humane treatment and animal welfare and followed the National Regulation (TCVN 12448:2018) on animal welfare management. Faecal and blood samples were collected during routine veterinary health checks. Pathological samples were obtained from carcasses of chickens that were culled by farmers due to severe illness. Verbal informed consent was obtained from all farm owners involved in the study.

Data Availability Statement: All the relevant data that support the findings of this study are available from the corresponding author upon reasonable request.

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