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Research Article

Age-Related Patterns of Coccidial Oocyst Shedding and the Predictive Performance of Clinical Indicators in H'Mong Chickens

Nguyen Thi Chuc, Phan Nhan*

Faculty of Applied Biology, Tay Do University, 68 Tran Chien Street, Cai Rang Ward, Can Tho 900000, Vietnam.

Abstract | This study investigated age-related differences in coccidial oocyst shedding and evaluated the predictive performance of simple clinical indicators in H'Mong chickens raised on rice husk litter in Can Tho City, Vietnam. A total of 240 chickens aged 14 to 45 days were stratified into four age groups. Individual fecal samples were examined using the McMaster method, and oocyst per gram values were determined. For analytical purposes, high oocyst shedding was defined as OPG ≥ 10,000. Oocyst prevalence and shedding intensity declined progressively with age, with the highest levels observed in chickens aged 14 to 21 days. Clinical indicators including fecal consistency, feather condition, and crop fill were significantly associated with high oocyst shedding. Among individual indicators, fecal score showed the strongest discriminative ability, while a composite clinical index integrating all three parameters demonstrated improved overall performance (area under the curve = 0.85). Sensitivity and specificity varied according to the selected cut-off values, whereas negative predictive values remained consistently high. Multivariable logistic regression showed that clinical scores and younger age were independently associated with high oocyst shedding. These findings support the practical use of standardized clinical scoring as a screening tool for identifying chickens with relatively high oocyst shedding under litter-based production conditions.

Keywords | Coccidiosis, Oocyst shedding, Clinical scoring, H'Mong chickens, Rice husk litter, Diagnostic performance

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***Correspondence** | Phan Nhan, Faculty of Applied Biology, Tay Do University, 68 Tran Chien Street, Cai Rang Ward, Can Tho 900000, Vietnam; **Email:** pnhan@tdu.edu.vn
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INTRODUCTION

The poultry sector represents one of the fastest-growing and most dynamic components of global agriculture. Within broiler production systems, there is a continuous demand for essential inputs, among which litter material plays a critical role (Babu *et al.*, 2025). Litter is a fundamental requirement for broiler rearing, particularly in deep-litter housing systems, where it contributes significantly to maintaining suitable environmental and hygienic conditions (Farghly *et al.*, 2018). Coccidiosis continues to be one of the most widespread and economically important parasitic diseases affecting poultry production worldwide. The global economic losses associated with this disease have been estimated to exceed US$3 billion annually in the poultry industry (Blake and Tomley, 2014). The disease is caused by protozoan parasites of the genus *Eimeria*, which invade and multiply within the intestinal epithelial cells. This infection results in intestinal inflammation, reduced nutrient absorption, decreased growth performance, and in severe cases may lead to increased mortality. Despite the routine use of anticoccidial control programs, subclinical infections commonly persist, particularly in production systems that utilize litter bedding. In such environments, birds are repeatedly exposed to sporulated oocysts that accumulate within the bedding material. Various

materials have been investigated for their suitability as bedding or litter in poultry production systems (Burke *et al*., 1993; Grimes *et al*., 2002, 2006; Aktan and Sagdic, 2004; Hermes *et al*., 2004; Bennett *et al*., 2005; Atapattu and Wickramasinghe, 2007). Poultry litter typically represents a composite mixture consisting of the original bedding substrate together with feed remnants, manure, feathers, and other organic debris generated during the rearing process. The term 'litter' may therefore encompass both freshly introduced and reused bedding materials. The condition and quality of litter are known to exert substantial effects on poultry productivity, health, carcass characteristics, and animal welfare (Malone *et al*., 1982; Malone and Chaloupka, 1983). In many regions, broiler litter is reused for several consecutive production cycles before a complete removal and replacement are performed. This practice is primarily adopted to reduce the costs associated with purchasing fresh bedding materials and disposing of used litter (Wang *et al*., 2017; Abougabal, 2019). However, repeated reuse of litter can lead to marked changes in its chemical composition and microbial ecology. Poor litter management often results in increased moisture content, which may subsequently elevate ammonia concentration and pH levels. Such conditions also promote higher densities and greater diversity of enteric pathogens and parasites, including *Escherichia coli*, *Coliform bacteri*a, and *Eimeria* species (Omeira *et al*., 2006; Cressman *et al*., 2010). Alterations in the microbial community of reused litter can influence the composition of the gastrointestinal microbiota in chickens (Cressman *et al*., 2010). Early exposure of young chicks to different microbial populations and pathogenic organisms is recognized as an important factor shaping the development of the gut microbial ecosystem (Yin *et al*., 2010). Nevertheless, the microbial community in the chicken gastrointestinal tract is not stable and undergoes continuous changes over time, particularly in association with the age of the host (Oakley and Kogut, 2016). Microorganisms that colonize the intestinal tract during the early stages of life can influence the later establishment of other microbial populations by occupying ecological niches and limiting subsequent colonization.

In deep litter systems using rice husk, the dynamics of oocyst shedding are strongly influenced by age and environmental conditions. According to Nouri (2024), once chicks are introduced into commercial poultry houses where litter is used as bedding, they encounter various bacterial sources capable of entering their still-developing gastrointestinal tract. These external bacterial sources may originate from the litter material itself, as well as from feed, drinking water, and the surrounding air. Young chickens are generally more susceptible due to incomplete development of protective immunity, and oocyst excretion typically peaks during the early growing phase before gradually declining as partial resistance develops. Because the gastrointestinal tract of young chicks has a low capacity to resist microbial colonization, certain bacteria, particularly pathogenic species, can readily establish themselves in this environment (Torok *et al*., 2009; Wang *et al*., 2017). However, the magnitude and temporal pattern of shedding may vary depending on stocking density, litter moisture, ventilation, and management practices. Kim *et al*. (2015) reported that coccidial infection significantly reduced the population of resident microbiota while promoting the proliferation of numerous opportunistic pathogenic bacteria. Indigenous chicken breeds reared on rice husk litter are continuously exposed to environmental contamination, yet quantitative data describing age-related shedding patterns in these populations remain limited.

The global shift toward minimizing the use of anticoccidial drugs in poultry production has underscored the need for a more comprehensive understanding of the etiological agents and the underlying mechanisms of disease pathogenesis. Such knowledge is essential for the development of more effective and sustainable control strategies (Mesa-Pineda *et al*., 2021). Field diagnosis of coccidiosis commonly relies on fecal oocyst detection or postmortem lesion scoring. While quantitative techniques such as the McMaster method provide objective measurement of oocyst burden, laboratory-based fecal examination is not always readily available in routine farm practice. Consequently, field veterinarians and farmers often depend on observable clinical signs, including diarrhea, ruffled feathers, and reduced feed intake, to guide treatment decisions. However, the diagnostic reliability and predictive performance of these simple clinical indicators in identifying birds with high oocyst shedding under litter-based conditions have not been sufficiently quantified. H'Mong chickens are an indigenous Vietnamese breed commonly raised in small- and medium-scale production systems on rice husk litter. Under such conditions, accumulation and recirculation of oocysts within the litter may facilitate early exposure and repeated infection cycles. Nevertheless, systematic evaluation of age-related oocyst shedding and the practical value of clinical scoring systems in this breed remains limited.

The present study was conducted to characterize age-related differences in coccidial oocyst shedding in H'Mong chickens reared on rice husk litter between 14 and 45 days of age and to assess the diagnostic performance of simple clinical indicators and a composite clinical index for predicting high oocyst shedding under field conditions.

MATERIALS AND METHODS

Animals

A total of 240 H'Mong chickens were included in this study. The chickens were raised under a deep litter system using rice husk as bedding material at a commercial

poultry farm located in Dong Phuoc commune, Can Tho City, Vietnam. All chickens were managed under routine farm conditions, with feed and water provided ad libitum.

Chickens ranged from 14 to 45 days of age at the time of sampling and were categorized into four age groups: 14–21 days, 22–29 days, 30–37 days, and 38–45 days. Sixty chickens were selected from each age group. Only clinically observable birds without severe systemic disease unrelated to intestinal disorders were included.

Chickens had free access to clean drinking water throughout the study period. Water was supplied continuously using the farm's standard watering system to ensure unrestricted intake. All chickens were fed a commercial complete mixed diet formulated to meet the nutritional requirements of growing indigenous chickens. The diet contained approximately 3,000 kcal/kg of metabolizable energy and 19% crude protein. The feed was formulated using major ingredients including maize, wheat bran, rice bran, fish meal, meat meal, soybean meal, and other minor ingredients commonly used in poultry feed formulations. The calcium content ranged from 0.8% to 1.5%, and total phosphorus ranged from 0.5% to 1.2%, depending on the growth phase. Feed was provided ad libitum according to routine farm management practices.

Experimental design

This study was designed as a cross-sectional field investigation conducted within a single production cycle to evaluate age-related variations in coccidial oocyst shedding and to determine the diagnostic performance of simple clinical indicators for identifying birds with high oocyst excretion.

H'Mong chickens raised on rice husk litter were stratified into four predefined age groups: 14–21 days, 22–29 days, 30–37 days, and 38–45 days. From each age group, 60 chickens were randomly selected, resulting in a total sample size of 240 chickens. Sampling was performed within the same rearing flock to minimize inter-flock variability. Chickens were selected from different locations within the house to reduce potential spatial clustering associated with litter contamination.

For each selected chicken, clinical assessment and fecal sampling were performed at the same time point. Quantitative oocyst counts were subsequently used to characterize age-specific shedding patterns and to classify chickens into two outcome categories: high oocyst shedding (OPG ≥ 10,000) and non-high shedding (OPG < 10,000). This classification served as the reference standard for evaluating the predictive performance of individual clinical indicators and the composite clinical index.

Clinical assessment

Each selected bird underwent standardized clinical evaluation prior to fecal sampling. Three observable clinical indicators were recorded: fecal consistency, feather condition, and crop fill status.

Fecal consistency was scored on a four-point scale: 0 = normal, well-formed feces; 1 = slightly soft feces; 2 = loose feces; 3 = watery or mucoid feces, with or without visible blood. Ruffled feather condition was scored as 0 = normal plumage, 1 = mildly ruffled feathers or reduced activity, and 2 = clearly ruffled feathers with evident depression. Crop fill was evaluated by gentle palpation and scored as 0 = empty or nearly empty crop, 1 = moderately filled crop, and 2 = full crop.

A composite clinical index (CI) was calculated for each bird by summing the three scores, resulting in a total score ranging from 0 to 7.

To assess inter-observer agreement, a subset of 40 birds was independently scored by two trained observers, and Cohen's kappa coefficient was calculated.

Fecal sampling and oocyst quantification

Individual fresh fecal samples (approximately 3–5 g per bird) were collected immediately after defecation to ensure accurate individual identification. Samples were placed in labeled containers and stored at 4–8°C until examination, which was performed within 48 hours.

Oocyst quantification was conducted using the McMaster technique. Briefly, fecal samples were weighed, homogenized in flotation solution, filtered, and loaded into a McMaster counting chamber. Oocysts were counted under light microscopy, and oocyst per gram (OPG) values were calculated according to the dilution factor of the method. Oocyst counts were used as a quantitative measure of infection intensity; however, species-level identification of *Eimeria* was not performed in this study.

For the purpose of this study, high oocyst shedding was operationally defined as OPG ≥ 10,000. This threshold was used to distinguish chickens with relatively higher fecal oocyst output under field conditions and to support risk stratification in the diagnostic analysis.

Statistical analysis

All statistical analyses were performed at the chicken level. Oocyst per gram (OPG) values were non-normally distributed and were summarized as median and interquartile range (IQR). Oocyst positivity (OPG > 0) and high oocyst shedding (defined as OPG ≥ 10,000) were expressed as percentages. Differences in oocyst positivity

among age groups were analyzed using the chi-square or Fisher's exact test, while OPG levels were compared using the Kruskal–Wallis test followed by Dunn's post-hoc test with Bonferroni adjustment. Clinical scores were compared between high and non-high shedders using the Mann–Whitney U test. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of individual clinical indicators and the composite clinical index for predicting high oocyst shedding, and the area under the curve (AUC) with 95% confidence intervals (CI) was calculated. Optimal cut-off values for the composite index were determined using the Youden index, and sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were estimated. Multivariable logistic regression analysis was conducted to estimate adjusted odds ratios (OR) and 95% CI for predictors of high oocyst shedding, including age group and clinical indicators as explanatory variables. The composite clinical index was not entered simultaneously with its component scores to avoid multicollinearity. Model fit was assessed using the Hosmer–Lemeshow test. All tests were two-sided and statistical significance was set at $p < 0.05$.

RESULTS

To investigate age-related patterns of coccidial oocyst shedding

Age-related differences in coccidial oocyst shedding are presented in Table 1 and Figure 1. Overall, more than half of the examined chickens were positive for oocysts, while a smaller proportion exhibited high-level shedding.

Table 1: Prevalence and oocyst shedding intensity of coccidia by age group in H'Mong chickens (n = 240).

Age group (days)	n	Oocyst positive (%)	Median OPG (IQR)	OPG ≥ 10,000 (%)
14–21	60	68.3	4,600 (700–15,800)	25.0
22–29	60	60.0	2,900 (300–10,900)	20.0
30–37	60	46.7	1,100 (0–6,300)	13.3
38–45	60	36.7	600 (0–2,400)	8.3
Overall	240	52.9	1,800 (200–9,700)	16.7

A consistent decline in both oocyst positivity and shedding intensity was observed with increasing age. The youngest chickens showed the highest levels of infection, whereas progressively lower values were recorded in older groups. This pattern was evident not only in prevalence but also in oocyst output, with younger birds exhibiting higher and more variable shedding compared with older birds.

Analogously, the proportion of chickens classified as high shedders decreased across age groups, indicating a reduction in parasite burden as birds matured. In addition, variability in oocyst excretion appeared to diminish with age, suggesting more stable infection levels in older chickens.

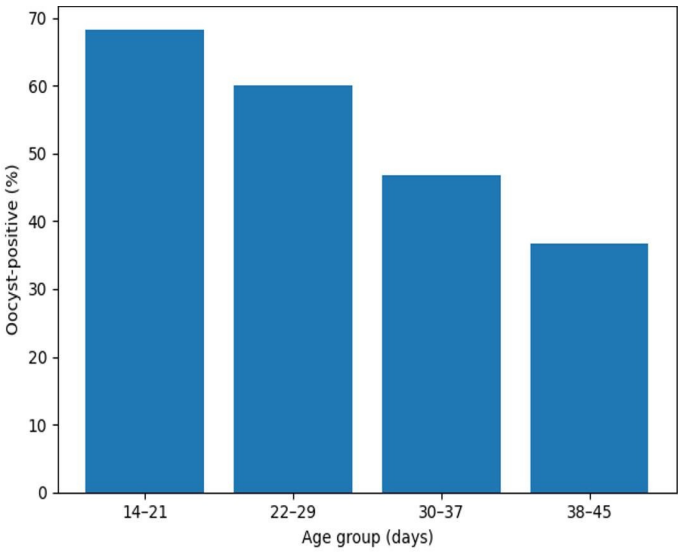

Figure 1: Age-related variation in oocyst positivity (%) among H'Mong chickens.

Broadly, these findings demonstrate clear age-associated differences in coccidial infection burden, with younger chickens showing higher levels of exposure and shedding under the same field conditions.

To evaluate the predictive performance of simple clinical indicators for detecting high oocyst shedding

The distribution of clinical indicators according to oocyst shedding status is presented in Table 2. Chickens classified as having high oocyst shedding showed consistently poorer clinical profiles compared with those in the non-high shedding group.

Table 2: Distribution of clinical indicators according to high oocyst shedding status (OPG ≥ 10,000).

Clinical indicator	OPG < 10,000 (n = 200)	OPG ≥ 10,000 (n = 40)	p value
Fecal score (median, IQR)	1 (0–1)	2 (1–3)	<0.001
Ruffled feathers score (median, IQR)	0 (0–1)	1 (1–2)	<0.001
Crop fill score (median, IQR)	2 (1–2)	1 (0–2)	0.003
Total clinical index (0–7)	1 (0–3)	4 (3–6)	<0.001

Higher fecal scores and more pronounced feather ruffling were observed among chickens with elevated oocyst output, whereas crop fill tended to be reduced. These differences indicate a general deterioration in clinical condition associated with increased oocyst shedding. Notably, The composite clinical index showed a clear

separation between groups, with higher scores observed in chickens with greater oocyst output. All indicators differed significantly between groups, supporting the consistency of these associations. Notably, these differences align with the analytical threshold used to define high oocyst shedding, indicating that this cutoff corresponds to meaningful clinical distinctions under field conditions.

The diagnostic performance of individual clinical indicators and the composite clinical index is presented in Table 3 and Figure 2. Overall, all indicators showed measurable discriminative ability for identifying chickens with higher oocyst shedding, although their performance varied.

Table 3: Diagnostic performance of individual clinical indicators and composite clinical index for predicting high oocyst shedding (OPG ≥ 10,000).

Predictor	AUC (95% CI)
Fecal score	0.80 (0.73–0.87)
Ruffled feathers score	0.71 (0.62–0.80)
Crop fill score	0.67 (0.58–0.76)
Composite clinical index	0.85 (0.79–0.91)

Among the individual parameters, fecal score demonstrated the strongest discriminative capacity, while ruffled feathers and crop fill showed more moderate performance. In comparison, the composite clinical index provided the highest overall discrimination, indicating that combining multiple clinical observations improves the ability to distinguish between groups.

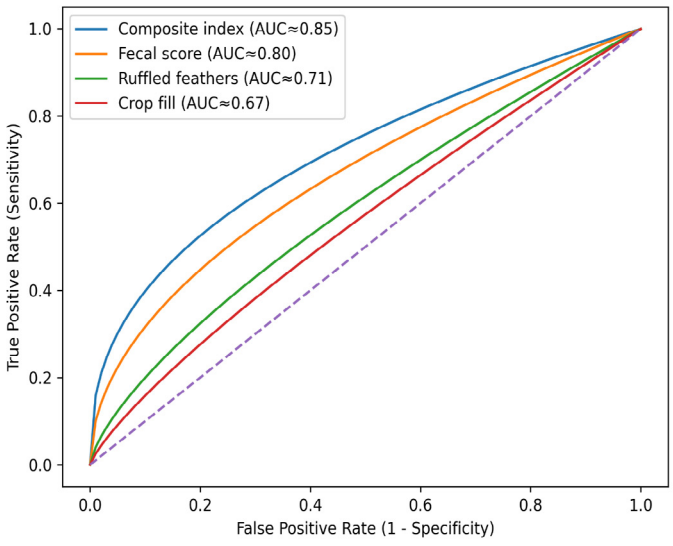

Figure 2: ROC curves for predicting high oocyst shedding in H'Mong chickens.

As illustrated in Figure 2, the composite index consistently remained above the individual indicators across the range of false-positive rates, suggesting more stable performance under different classification thresholds. However, the differences between indicators should be interpreted as relative rather than absolute, as their performance may be influenced by field conditions and the prevalence of high oocyst shedding in the study population.

Table 4: Sensitivity, specificity, and predictive values of the composite clinical index at different cut-off points.

Clinical index cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
≥ 2	92.5	54.0	27.8	97.3
≥ 3	82.5	70.5	36.7	94.7
≥ 4	72.5	82.5	45.3	93.2
≥ 5	55.0	89.5	51.2	90.8

The diagnostic characteristics of the composite clinical index at different cut-off values are presented in Table 4 and Figure 3. As the cut-off increased, sensitivity declined while specificity improved, reflecting the expected trade-off between identifying true positives and reducing false-positive classifications.

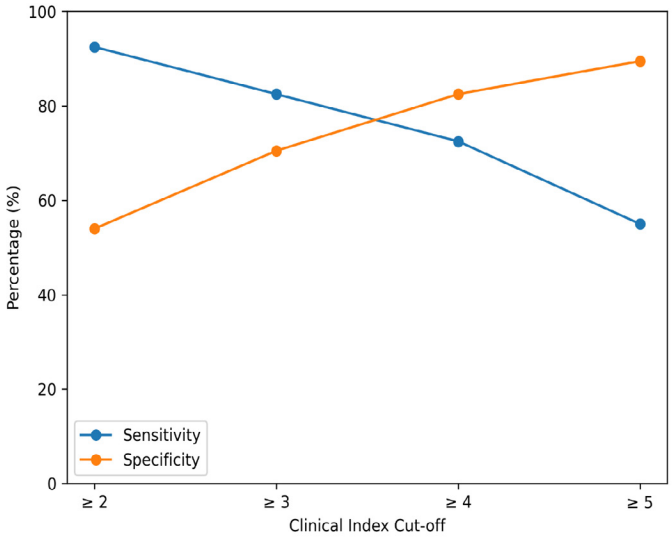

Figure 3: Sensitivity and specificity of the composite clinical index at different cut-off values.

Lower cut-offs were associated with higher sensitivity, whereas higher cut-offs yielded greater specificity. Across all thresholds, negative predictive values remained consistently high, indicating that chickens with low clinical scores were unlikely to have high oocyst shedding under the conditions of this study. In contrast, positive predictive values were more moderate and increased with higher cut-offs.

It should be noted that the relatively high negative predictive values are partly influenced by the low prevalence of high oocyst shedding in the study population. Therefore, these values should be interpreted in the context of the observed prevalence rather than as intrinsic properties of

the clinical index alone.

The results of the multivariable logistic regression analysis are presented in Table 5 and Figure 4. After adjustment for age group, all clinical indicators remained significantly associated with high oocyst shedding.

Table 5: Multivariable logistic regression analysis for predicting high oocyst shedding (OPG ≥ 10,000).

Variable	Odds ratio (OR)	95% CI	p value
Fecal score (per 1-point increase)	2.15	1.55–2.98	<0.001
Ruffled feathers score (per 1-point increase)	1.55	1.05–2.30	0.028
Crop fill score (per 1-point decrease)	1.40	1.02–1.94	0.038
Age group 14–21 days (vs 38–45 days)	2.60	1.10–6.20	0.031

Higher fecal scores were associated with increased odds of high oocyst shedding, showing the strongest relationship among the evaluated indicators. Ruffled feathers and reduced crop fill were also associated with higher likelihood of elevated oocyst output, although with more moderate effect sizes.

In addition, chickens in the youngest age group showed higher odds of high oocyst shedding compared with older birds. These findings indicate that both clinical condition and age are independently associated with differences in oocyst shedding levels under field conditions.

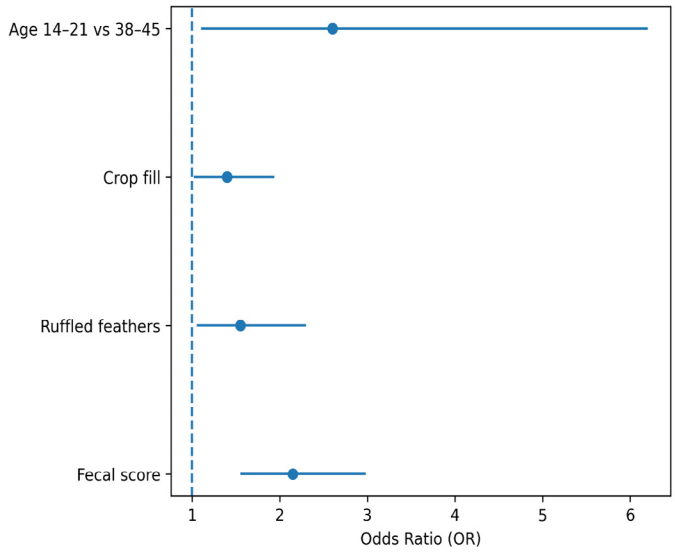

Figure 4: Forest plot of multivariable logistic regression analysis for predictors of high oocyst shedding in H'Mong chickens.

Inter-observer agreement for the clinical scoring system is presented in Table 6. Agreement levels across the evaluated parameters were consistently within the substantial to near-perfect range, indicating strong concordance between observers.

Table 6: Inter-observer agreement for clinical scoring (n = 40 Chickens).

Clinical parameter	Cohen's Kappa
Fecal score	0.80
Ruffled feathers score	0.72
Crop fill score	0.65

The highest level of agreement was observed for fecal scoring, suggesting that alterations in fecal consistency represent a relatively objective and easily recognizable clinical parameter under field conditions. Feather ruffling also demonstrated substantial reproducibility, although with slightly lower concordance, likely reflecting a degree of subjective interpretation in assessing behavioral and postural changes. Crop fill scoring showed comparatively lower agreement, which may be attributable to variability in palpation technique and the dynamic nature of gastrointestinal filling status.

Overall, the magnitude of kappa coefficients indicates that the clinical scoring system is methodologically reliable and reproducible when applied by trained observers. This level of consistency supports the internal validity of subsequent analyses linking clinical indicators to high oocyst shedding.

DISCUSSION

This study provides field-based evidence on age-related differences in coccidial oocyst shedding and highlights the practical value of simple clinical indicators for identifying chickens with higher levels of oocyst excretion in a rice husk litter system. The findings consistently show that both the occurrence and intensity of oocyst shedding decrease across age groups, and that a small set of easily observable clinical parameters can effectively distinguish chickens with relatively higher oocyst output under routine farm conditions. Clark *et al.* (2016) reported that Eimeria species are widely distributed and that most broiler chickens are likely exposed to one or more species during their lifetime. Although severe coccidiosis can result in mortality, morbidity is more frequently observed. Such infections often lead to malabsorptive and haemorrhagic enteric disorders that impair nutrient absorption and reduce body weight gain (Sakkas *et al.*, 2018).

The age-related differences observed in Table 1 and Figure 1 demonstrate a clear decline in oocyst positivity, median OPG, and the proportion of high shedders from younger to older age groups. These findings should be interpreted as comparisons between age groups rather

than true longitudinal changes within individual birds, given the cross-sectional design of the study. Nevertheless, the observed trend is biologically consistent with the development of partial protective immunity following early exposure in litter-based systems. After chicks are transferred to the farm, they encounter a considerably more complex microbial environment. Colonization of the chicken gastrointestinal tract by bacterial species begins shortly after hatching, typically from the first day of life (Ballou *et al*., 2016). The composition of this microbial community continues to change during the bird's life as certain bacterial populations are replaced by newly established taxa. Nevertheless, several bacterial groups, including *Lactobacillus*, *Escherichia coli*, and other coliform bacteria, are commonly present in high abundance within the intestinal tract. These microorganisms play important roles in influencing digestive health, immune function, and overall production performance in poultry (Waite and Taylor, 2015; Shang *et al*., 2018; Yan *et al*., 2019). In rice husk litter housing, repeated contact with sporulated oocysts is expected, particularly during early growth stages when birds explore the litter and hygiene is challenging to maintain perfectly. The progressive reduction in high shedding prevalence across age groups, as shown in Table 1 and Figure 1, supports the interpretation that susceptibility is greater during the early post-brooding period, followed by a gradual decline in parasite replication and oocyst output as immune competence increases. The narrowing of the IQR in older age groups further suggests reduced variability in oocyst excretion, which is consistent with a more stable host–parasite equilibrium once partial immunity is established.

The distribution of clinical scores observed in Table 2 is consistent with the expected pathophysiological features of coccidiosis. Chickens with higher oocyst shedding showed increased fecal scores and more pronounced feather ruffling, together with reduced crop fill. Elevated fecal scores likely reflect impaired intestinal integrity and altered nutrient absorption associated with Eimeria infection, while feather ruffling and reduced crop fill may indicate systemic responses, decreased comfort, and reduced feed intake. Notably, the composite clinical index demonstrated a clear shift between high and non-high shedders, suggesting that the combined assessment of multiple clinical signs provides a more comprehensive representation of clinical status than individual indicators alone. The consistent statistical differences across all parameters (Table 2) support the internal coherence of the clinical scoring system in capturing variation in oocyst shedding levels under field conditions. Although poultry have been reported to ingest litter equivalent to approximately 4% of their total feed intake, the direct effects of litter material on the intestinal microbiota of poultry remain poorly understood (Malone *et al*., 1983).

The ROC analysis presented in Table 3 and Figure 2 provides further insight into the relative contribution of individual clinical indicators. Among these, fecal score showed the strongest discriminative performance, which is consistent with the role of diarrhea as a direct manifestation of intestinal damage. However, the composite clinical index demonstrated superior overall discrimination, indicating that combining multiple clinical signs improves classification compared with reliance on a single parameter. From a practical perspective, fecal consistency alone may be influenced by factors such as diet, water quality, and environmental stress, whereas the composite index integrates multiple dimensions, including enteric output, general condition, and feeding behavior. This combined approach likely contributes to more stable performance under variable field conditions, as reflected in the ROC curves (Figure 2). Environmental context may also play an important role in shaping these observations. Fries *et al*. (2005) suggested that microbial communities in poultry litter tend to become relatively uniform regardless of bedding material, indicating that litter-associated microbial exposure may be broadly similar across systems. In addition, the persistence of oocysts in the environment is well documented. Sporulated oocysts can survive for extended periods outside the host, potentially up to 602 days (Fatoba and Adeleke, 2018), while unsporulated oocysts may remain viable for several months within the host. Following excretion, oocysts undergo sporulation under suitable environmental conditions, becoming infective. A typical Eimeria oocyst contains four sporocysts, each with two sporozoites, whereas Isospora oocysts contain two sporocysts with four sporozoites each (Belli *et al*., 2006). Differences in oocyst morphology, including size, shape, and internal structure, have been used to distinguish species (Castañón *et al*., 2007). These factors highlight the complexity of environmental exposure and parasite biology, which may influence the observed variability in clinical presentation and oocyst shedding under field conditions.

The cut-off analysis presented in Table 4 and Figure 3 provides practical guidance for on-farm application. Lower thresholds are associated with higher sensitivity, which is appropriate when the priority is to avoid missing chickens with elevated oocyst shedding, whereas higher thresholds improve specificity and reduce false-positive classifications. The cutoff of OPG ≥ 10,000 used in this study should be interpreted as a practical threshold for identifying chickens with relatively high oocyst excretion under field conditions rather than as a definitive marker of clinical coccidiosis severity. Although direct production losses and intestinal lesion scores were not measured, chickens above this threshold consistently showed less favorable clinical indicator profiles, supporting its use for screening and risk stratification. Across all cut-off

levels, negative predictive values remained consistently high (Table 4), indicating that chickens with low clinical index scores are unlikely to have high oocyst shedding under the conditions of this study. However, this finding should be interpreted in the context of the relatively low prevalence of high shedders in the study population, which contributes to elevated NPV values. In contrast, positive predictive values were more moderate and increased with higher thresholds, suggesting that positive classifications should be interpreted as indicative of increased likelihood rather than definitive diagnosis. Under field conditions, this supports the use of the clinical index as a screening tool, with confirmatory fecal examination where necessary. Lesion scoring can provide additional information regarding the Eimeria species involved and the extent of intestinal damage at a given time point. However, the window for detecting peak lesions is relatively narrow and depends on the prepatent period of the infecting species (Chapman *et al*., 2013). In turkeys, visible intestinal lesions typically occur within a limited time frame, often between 5 and 7 days post-infection, although variation may exist (Vrba and Pakandl, 2014). Furthermore, lesion scores can be influenced by parasite strain, resulting in differences in both lesion severity and anatomical distribution (Barrios *et al*., 2017; El-Sherry *et al*., 2019). These factors limit the use of lesion scoring alone as a reliable indicator of infection severity or treatment response. Therefore, lesion scoring is best considered alongside other diagnostic measures, including oocyst counts per gram of feces, to provide a more comprehensive assessment of coccidial infection and control strategies (Chasser *et al*., 2020).

The multivariable regression analysis presented in Table 5 and Figure 4 further supports the consistency of the observed associations. Fecal score, feather ruffling, and crop fill remained significantly associated with high oocyst shedding after adjustment for age, indicating that these clinical indicators provide information beyond age-related differences alone. The higher odds of high oocyst shedding observed in the youngest age group are consistent with the descriptive findings and reinforce the importance of early-life stages in the epidemiology of coccidial infection. These results suggest that the early post-brooding period represents a critical window during which chickens are more likely to exhibit elevated oocyst output under field conditions. From a practical standpoint, these findings support closer clinical monitoring during the early growth phase, particularly between 14 and 29 days of age, when the likelihood of higher oocyst shedding appears to be greater. Management practices during this period, including litter condition and general husbandry, may therefore play an important role in limiting environmental contamination and subsequent transmission.

The reliability of the clinical scoring system is an important methodological consideration for field-based application. The level of agreement observed in Table 6 indicates that the scoring approach is reproducible when applied by trained observers. The highest agreement for fecal scoring suggests that fecal consistency is relatively objective in practice, whereas the slightly lower agreement for crop fill likely reflects the dynamic nature of crop content and variability in palpation. Overall, these findings support the internal consistency of the scoring system and suggest that observer-related variation is unlikely to account for the observed differences in clinical indicators and oocyst shedding. Several limitations should be considered when interpreting the findings. Oocyst quantification in this study was based solely on total OPG and did not include identification of *Eimeria* species. Because different Eimeria species vary in their pathogenicity and clinical expression, similar oocyst counts may not necessarily reflect the same level of biological impact. Therefore, the findings should be interpreted as reflecting overall coccidial burden rather than species-specific infection dynamics. In addition, environmental factors related to litter conditions, such as moisture, pH, and oocyst accumulation, were not measured and may have influenced oocyst shedding levels, representing a potential source of confounding. Despite these limitations, total OPG remains a practical and widely used measure in field studies, particularly when the objective is to assess infection intensity and its association with observable clinical signs under routine production conditions.

The study was conducted within a single farm and a single production cycle; therefore, extrapolation to other farms with different litter management, stocking densities, or anticoccidial programs should be made with caution. In addition, because the study used a cross-sectional design, temporal relationships at the individual level cannot be established. However, the age-stratified approach and consistent associations observed across multiple analyses provide useful evidence for practical interpretation. Future studies across multiple farms and production cycles, incorporating environmental measurements such as litter moisture and spatial variation within housing systems, would help to refine predictive performance and improve generalizability. Overall, the findings support a practical framework for field monitoring of coccidiosis in H'Mong chickens raised on rice husk litter. Oocyst shedding was higher in younger age groups and decreased with age, while a simple composite clinical index based on fecal consistency, feather condition, and crop fill provided useful discrimination for identifying chickens with relatively higher oocyst excretion. This approach may assist in timely decision-making and targeted fecal testing, particularly in settings where access to laboratory diagnostics is limited.

CONCLUSIONS

In H'Mong chickens raised on rice husk litter, coccidial oocyst shedding was highest during the early growing phase and declined progressively with age, consistent with the development of partial protective immunity. Simple clinical indicators, particularly fecal consistency, were significantly associated with high oocyst excretion. A composite clinical index that integrates fecal score, feather condition, and crop fill demonstrated good discriminative ability and acceptable reproducibility under field conditions. These findings support the practical application of standardized clinical scoring as a screening approach for identifying chickens at risk of high oocyst shedding in litter-based production systems.

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NOVELTY STATEMENT

This study provides field-based evidence on age-related differences in coccidial oocyst shedding in H'Mong chickens raised on rice husk litter under practical farm conditions. It demonstrates that a simple composite clinical index, based on fecal consistency, feather condition, and crop fill, can be used as a practical tool to identify chickens with higher oocyst shedding. The study also highlights the applicability of clinical scoring for on-farm screening in resource-limited settings, where routine laboratory diagnostics may not be readily available.

AUTHOR'S CONTRIBUTION

Nguyen Thi Chuc: Conceptualization, formal analysis and writing original draft. Phan Nhan: Methodology, data curation, and Writing review and editing. All authors reviewed and approved the final manuscript.

Ethical approval

This study involved only non-invasive clinical observation and collection of freshly voided fecal samples from chickens under routine farm management conditions. No experimental infection, invasive procedures, handling causing injury, or euthanasia were performed. Therefore, formal ethical approval and an approval number were not required according to institutional guidelines for observational animal studies. All procedures were conducted in accordance with the Law on Animal Husbandry of Vietnam (No. 32/2018/QH14) and standard animal welfare principles. The farm owner provided permission for access to the flock and for collection of observational and fecal data.

Generative AI and AI-assisted technology statement

Generative AI tools were not used to generate any scientific content. Any AI assistance was limited to minor language editing, and all ideas, interpretations, and conclusions are solely those of the authors.

Conflict of interest

The authors have declared no conflict of interest.

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