

Pen-floor moisture and sporulated *Eimeria* oocyst accumulation are associated with subsequent oocyst shedding in Boer-cross goat kids: a 12-week longitudinal study

Nguyen Thi Chuc¹, Nguyen Minh Tri¹, Nguyen Thi My Phuong¹, Trinh Thi Hong Mo¹ and Phan Nhan^{1*}

¹Faculty of Applied Biology, Tay Do University, 68 Tran Chien Street, Thanh My Area, Cai Rang Ward, Can Tho City 900000, Vietnam.

*Corresponding author: pnhan@tdu.edu.vn

SUMMARY

Pen-floor moisture may influence the development and persistence of environmental *Eimeria* oocysts, but its temporal relationship with subsequent infection in goat kids remains unclear. This 12-week longitudinal study followed 96 Boer-cross goat kids housed in 16 separate pens. Pen-floor moisture and environmental oocyst contamination were assessed weekly, individual faecal oocyst per gram (OPG) was determined every two weeks, and faecal consistency was scored weekly. Environmental contamination was expressed as total and sporulated oocysts per gram of dry matter. Median total oocyst burden increased from 1,525 oocysts/g DM at moisture levels below 25% to 6,728 oocysts/g DM at moisture levels of at least 45%, while median sporulated burden increased from 210 to 3,364 oocysts/g DM. Each 10-percentage-point increase in moisture was associated with 1.59- and 3.07-fold increases in total and sporulated environmental burdens, respectively. In separate two-week lag models, higher moisture and greater sporulated burden were associated with 1.38- and 1.22-fold increases in subsequent fecal OPG, respectively. When both exposures were included simultaneously, moisture was no longer independently associated with subsequent OPG, whereas each doubling of sporulated burden remained associated with a 1.26-fold increase. Neither exposure was significantly associated with subsequent diarrhoea. These findings indicate that moisture provides an environmental condition favouring oocyst accumulation and sporulation, whereas sporulated oocyst burden represents a more immediate indicator of subsequent infection pressure. Maintaining dry pen floors and removing faecal material regularly may help reduce environmental exposure.

Keywords: Boer-cross goat kids; coccidiosis; environmental contamination; *Eimeria*; oocyst sporulation.

Article History

Submitted: 8 August 2026

Revised: 18 September 2026

Accepted: 29 September 2026

Published: 2 October 2026

Tanzania Veterinary Journal Vol. 41(2)2026

<https://doi.org/10.4314/tvj.v41i2.9>

ISSN: 0856 - 1451 (Print)

ISSN: 2714-206X (Online)

<https://tvj.sua.ac.tz>

License terms

This article is available under the terms of the [Creative Commons attribution](#) License (CC BY). You are free to use, reproduce, redistribute in any medium or format provided the original publication in this journal is cited.

INTRODUCTION

The global domestic goat population exceeds one billion animals and has more than doubled over the past four decades (FAOSTAT, 2023; Utaaker et al., 2021). In Vietnam, Boer-cross goats are widely raised for meat under semi-intensive production systems because they combine the growth potential of Boer goats with the adaptability of locally established breeds. Young goat kids are particularly susceptible to enteric diseases

during the period surrounding weaning, when changes in diet, grouping, and housing may increase physiological stress and exposure to pathogens. Among these pathogens, *Eimeria* spp. are frequently detected in young goats. The predominant *Eimeria* species varied among Asian goat populations (Faizal and Rajapakse, 2001; Jalila et al., 1998; Wang et al., 2010; Sharma et al., 1997). In a field survey of sheep and

goats in southeastern Nigeria, gastrointestinal parasites occurrence varied with host age, season, ecological zone, and management system (Anene et al., 1994). A systematic review from China identified environmental contamination as a potentially important contributor to coccidiosis in sheep raised under large-scale housing conditions (Liu et al., 2024). A systematic review and meta-analysis of 70 studies from China reported a higher estimated prevalence of *Eimeria* infection in goats aged ≤ 1 year than in those >1 year (Diao et al., 2022). An observational study conducted across 13 goat farms in the Czech Republic found substantially higher oocyst shedding in kids than in adult goats (Koudela and Boková, 1998). Subclinical coccidiosis is common and is primarily associated with impaired growth, whereas clinical disease is most commonly characterized by diarrhea (Matthews, 2016). Oocyst shedding generally peaks around weaning and subsequently declines as animals develop strong immunity (Chartier and Paraud, 2012). However, faecal oocyst shedding varies with age, acquired immunity, stage of infection, and the pathogenicity of the infecting species. Consequently, faecal OPG alone may not adequately represent the environmental infection pressure or the risk of clinical disease.

Goats may acquire *Eimeria* infections at any age, but clinical coccidiosis occurs most commonly in young animals (Taylor and Catchpole, 1994). Transmission occurs when susceptible goat kids ingest viable sporulated oocysts from contaminated floors, bedding, feed, or water. Oocysts are shed in an unsporulated and non-infective state and must sporulate in the environment before becoming infective. Moisture may facilitate sporulation, limit desiccation, and prolong environmental persistence, allowing contaminated pen floors to serve as a continuing source of exposure. Nevertheless, total environmental oocyst counts comprise both sporulated and unsporulated forms and therefore do not distinguish the potentially infective fraction. Direct assessment of the sporulated fraction

may provide a more informative indicator of potentially infective contamination, but morphological sporulation does not demonstrate viability or infectivity. Some morphologically sporulated oocysts may no longer be viable. Furthermore, environmental burdens should be standardised according to dry matter because differences in floor moisture can otherwise distort comparisons among pens and sampling times.

Most field investigations of caprine coccidiosis have relied on cross-sectional measurements of fecal oocyst shedding or management-related risk factors. Such designs cannot determine whether moist flooring precedes increased environmental contamination and subsequent infection or simply occurs in pens containing animals that are already shedding large numbers of oocysts. Evidence based on repeated environmental and individual measurements remains limited, particularly for Boer-cross goat kids raised under practical farm conditions. Longitudinal monitoring of floor moisture, total and sporulated environmental oocyst burdens, fecal OPG, and clinical outcomes is therefore needed to clarify the temporal relationship between environmental contamination and subsequent infection.

This 12-week prospective longitudinal study aimed to determine the relationship between pen-floor moisture and the accumulation of total and sporulated *Eimeria* oocysts in pens containing Boer-cross goat kids. It further examined whether pen-floor moisture and sporulated environmental oocyst burden at a given sampling time were associated with fecal OPG and diarrhea two weeks later and whether sporulated burden remained independently associated with these outcomes when moisture was considered simultaneously. We hypothesized that increasing pen-floor moisture would be associated more strongly with sporulated than with total oocyst burden and that sporulated environmental burden would provide a more immediate indicator of subsequent infection pressure than moisture alone.

MATERIALS AND METHODS

Study area

The study was conducted at a Boer-cross goat farm in Phong Dien Commune, Can Tho City, Vietnam, during the rainy season from August to November 2025. The farm maintained approximately 180 goats and operated under a semi-intensive meat-goat production system. The study animals were housed in an open-sided

naturally ventilated shed containing separate group pens. Pen floors were constructed of concrete and were covered with an approximately 3–5-cm layer of rice-husk bedding. The floors had a slight slope toward open drainage channels located along the sides of the housing area to facilitate the removal of excess water. Manure and visibly soiled bedding were routinely removed

every two days, whereas bedding was partially replenished when wet and completely replaced approximately every 7–10 days, depending on floor condition. The goats were offered fresh-cut forage, predominantly Napier grass, twice daily together with a commercial concentrate formulated for growing goats. Drinking water was available ad libitum from plastic drinking troughs supplied from an overhead water-storage tank, and the troughs were cleaned daily. No routine prophylactic anticoccidial programme was implemented during the 30 days preceding enrolment or during the follow-up period; when veterinary treatment was clinically required, the treatment date and active substance were recorded and the animal remained under routine farm care. During the 12-week follow-up, mean ambient temperature was approximately 28.5°C, with weekly mean values ranging from approximately 27.0 to 30.2°C. The study period coincided with the regional rainy season, with cumulative rainfall of approximately 950–1,000 mm during the 12-week follow-up period. The farm was purposively selected because it maintained at least 96 Boer-cross goat kids in stable group housing, provided 16 separate pens suitable for repeated pen-level environmental monitoring, did not routinely move the study animals among pens, permitted repeated individual and environmental sampling, and maintained sufficiently consistent husbandry conditions throughout the planned 12-week observation period.

Ethic Statements

The investigators considered formal ethical approval unnecessary because this was a non-interventional observational study involving only pen-floor sampling, collection of freshly voided feces, and visual assessment of fecal consistency. No experimental infection, invasive procedure, treatment allocation, or alteration of routine farm management was performed. Permission was obtained from the farm owner before data collection. The goat kids remained under routine husbandry and veterinary care, and treatment was neither withheld nor delayed for research purposes.

Experimental design

A 12-week prospective longitudinal observational study was conducted using 96 Boer-cross goat kids housed in 16 independent pens, with six kids per pen.

The animals remained in their original pens throughout the study, and movement between pens was not permitted. No experimental infection, treatment allocation, or modification of routine feeding and housing practices was introduced. Each pen was treated as an independent observational unit, while individual goat kids were identified and followed repeatedly. Because directly applicable estimates of the expected association between pen-floor moisture, sporulated environmental *Eimeria* burden, and subsequent fecal OPG were not available for Boer-cross goat kids under comparable field conditions, no formal a priori sample-size calculation was performed. The study therefore included all 16 independent pens that met the eligibility criteria at the selected farm and all six goat kids housed in each pen, resulting in 96 animals. With environmental sampling performed at baseline and weekly through week 12, this sampling structure provided 208 pen-week environmental observations. Individual fecal sampling at weeks 0, 2, 4, 6, 8, 10, and 12 provided up to 672 repeated fecal observations, while linkage of exposure measurements at week *t* with animal outcomes at week *t*+2 provided up to 576 two-week lagged animal-level intervals. The pen was considered the independent observational unit for pen-level environmental exposures, whereas individual goat kids constituted the observational units for fecal OPG and diarrhea outcomes within the hierarchical repeated-measures structure.

Pen-floor moisture and environmental *Eimeria* oocyst contamination were assessed at baseline and weekly thereafter until week 12. At each sampling time, one composite floor sample was obtained from five fixed locations within each pen before routine cleaning. Individual fecal samples were collected from all goat kids at weeks 0, 2, 4, 6, 8, 10, and 12 for determination of fecal oocyst counts. Fecal consistency was evaluated weekly using a four-point scoring system ranging from 0 to 3, with scores of 2 or 3 classified as diarrhea.

The primary environmental outcome was the burden of sporulated *Eimeria* oocysts expressed per gram of pen-floor dry matter. The animal-level outcomes were fecal oocyst shedding and the occurrence of diarrhea. Environmental measurements collected at each sampling time were linked to animal outcomes recorded two weeks later to determine whether pen-floor moisture and sporulated oocyst burden preceded increased oocyst shedding or diarrhea. Pen identity and

sampling time were retained throughout the dataset to account for the hierarchical structure and repeated measurements.

Pen-floor environmental measurements and sampling

Pen-floor measurements and environmental sampling were performed at baseline and weekly for 12 weeks. All measurements were conducted between 07:00 and 09:00 before routine floor cleaning, manure removal, or bedding replacement. Ambient temperature and relative humidity were measured at the center of each pen approximately 0.5 m above the floor. Floor-surface temperature was measured at five fixed locations, and the mean was used to represent each pen. Pen area, floor type, number of goat kids, stocking density, cleaning frequency, and any addition or replacement of bedding material were also recorded.

Floor material was collected from five predetermined locations in each pen, comprising four points approximately 0.5 m from the corners and one point at the center. Approximately 20 g was collected from the uppermost 2 cm at each location and combined to produce one composite sample of approximately 100 g per pen at each sampling time. New disposable gloves and a clean sampling scoop were used for each pen to prevent cross-contamination. Samples were placed in individually labeled sterile bags, transported to the laboratory in an insulated container at approximately 4°C, and processed within 24 h.

Each composite sample was thoroughly homogenized and divided into two portions. One portion was used to determine moisture content, while the remaining portion was retained for the recovery and enumeration of *Eimeria* oocysts. For moisture determination, approximately 20 g of the fresh sample was weighed, dried at 105°C to constant weight, cooled in a desiccator, and reweighed. Pen-floor moisture was calculated as follows:

Moisture (%) = [(fresh weight – dry weight)/fresh weight] × 100.

The dry-matter proportion obtained from each composite sample was used to express environmental oocyst burdens as the number of oocysts per gram of pen-floor dry matter.

Environmental *Eimeria* oocyst assessment

A 10-g portion of each homogenized floor sample was mixed with 100 mL of water containing 0.05% Tween

20, agitated thoroughly for 2 min, and allowed to stand for 5 min. The suspension was passed successively through 300- and 52-μm sieves to remove coarse material. The filtrate was centrifuged at 800 × g for 3 min, and the supernatant was discarded. The sediment was resuspended in saturated sodium chloride solution with a specific gravity of 1.20 and adjusted to a final volume of 30 mL.

The suspension was examined using two McMaster slides, corresponding to four counting chambers per sample. After the chambers remained undisturbed for 5 min, all *Eimeria* oocysts within the counting grids were enumerated at 100× magnification. Environmental oocyst density was calculated from the number counted, final suspension volume, examined chamber volume, and initial sample mass. The resulting count was adjusted using the dry-matter proportion of the corresponding floor sample and expressed as oocysts/g DM. Samples showing a difference greater than 20% between duplicate slide counts were remixed and re-examined.

Before field samples were analysed, recovery performance was evaluated using confirmed *Eimeria*-negative floor material spiked with 1,000, 10,000, or 30,000 oocysts, with five replicates at each concentration. Recovery was calculated as the percentage of added oocysts detected after processing and was used to assess the consistency of the extraction procedure.

For sporulation assessment, intact oocysts were examined at 400× magnification without prior laboratory incubation. Before examination, observers were trained by an experienced veterinary parasitologist using reference images and practice preparations to distinguish unsporulated, partially sporulated and fully sporulated oocysts. An oocyst was classified as sporulated when four distinct sporocysts were visible. Up to 100 oocysts were evaluated per sample; when fewer oocysts were recovered, all intact oocysts were examined. Sporulation rate was calculated only for samples containing at least 20 assessable oocysts. Each preparation was read twice by the same observer, with the initial record concealed during the second reading. A second trained observer independently examined 21 randomly selected preparations, representing approximately 10% of the 208 environmental samples. Disagreements were resolved by joint re-examination before the final classification was recorded. These readings were used

for quality control; formal inter- and intra-observer reliability coefficients were not calculated. Sporulated environmental oocyst burden was calculated by multiplying total oocyst density by the proportion of sporulated oocysts and was expressed as sporulated oocysts/g DM. Oocysts were identified at the genus level and were not identified to species level. Samples were maintained at approximately 4°C during transport and storage and examined within 24 h of collection. This procedure was intended to limit temperature-dependent oocyst development and shorten the interval before assessment. Complete prevention of further sporulation after collection was not demonstrated, so some change in sporulation status during sample handling could not be excluded.

Fecal oocyst shedding and diarrhea monitoring

At the prespecified sampling points, individual fecal samples were collected immediately after spontaneous defecation. Approximately 10 g of freshly voided feces was collected before contact with the pen floor, placed in a labeled sterile container, and linked to the identification number of the corresponding goat kid. Samples were maintained at approximately 4°C during transport and examined within 24 h. Samples contaminated with floor material or for which the individual source could not be confirmed were excluded.

Fecal oocyst counts were determined using the McMaster technique. Four grams of feces was homogenized in 56 mL of saturated sodium chloride solution with a specific gravity of 1.20 and filtered through a 250-µm sieve. After mixing thoroughly, both chambers of a McMaster slide were filled and allowed to stand for 5 min. *Eimeria* oocysts within the counting grids were enumerated at 100× magnification, and the total count from both chambers was multiplied by 50 to obtain the number of oocysts per gram of feces (OPG). Each sample was counted twice from the same homogenate, and the mean value was used. When duplicate counts above 200 OPG differed by more than 20%, a third count was performed.

Fecal consistency was assessed immediately after defecation by the same trained observer using a four-point scale: 0, firm and normally formed pellets; 1, soft but still formed feces; 2, loose or unformed feces; and 3, watery feces. Scores of 2 or 3 were classified as diarrhea. The observer was unaware of the environmental and fecal oocyst results during scoring.

Any anticoccidial or antimicrobial treatment administered during follow-up, including the treatment date and active substance, was recorded for subsequent statistical adjustment.

Statistical analysis

Pen-floor moisture was classified as <25%, 25 to <35%, 35 to <45%, or ≥45%. Category-specific distributions of total environmental oocyst burden, sporulation rate, and sporulated environmental oocyst burden were summarized descriptively as medians and interquartile ranges. Inferential comparisons among moisture categories were performed using population-averaged marginal models with pen specified as the clustering unit to account for repeated pen-week observations. Total and sporulated environmental oocyst burdens were analysed using log-link Poisson quasi-likelihood models with robust sandwich standard errors. Sporulation was analysed using a binomial-logit marginal model, with the number of morphologically sporulated oocysts specified relative to the total number of assessable oocysts in each sample. All category-based models were adjusted for floor temperature, the most recent pen-mean fecal OPG, stocking density, time since cleaning, and sampling week. Overall differences among moisture categories were evaluated using joint Wald tests. Pairwise contrasts were subsequently obtained from the same models, and P values were adjusted using the Holm procedure.

Associations with total and sporulated environmental oocyst burdens were evaluated using population-averaged generalized linear models with a log link and Poisson quasi-likelihood. Sandwich standard errors with a finite-sample correction were clustered at the pen level to account for repeated observations within pens. Because both environmental outcomes were standardised and expressed as oocysts/g DM, no additional dry-matter offset was included. The fixed effects were pen-floor moisture, floor temperature, the most recent pen-mean fecal OPG, stocking density, time since cleaning, and sampling week. Pen-mean fecal OPG was transformed as $\log_2(\text{OPG} + 1)$, so its coefficient represented the effect of an approximate doubling. Model coefficients were exponentiated and reported as adjusted ratios with 95% confidence intervals.

For the two-week lag analysis, environmental exposures recorded at week *t* were linked to individual

fecal OPG and diarrhea status recorded at week t+2. Subsequent fecal OPG was analyzed using a population-averaged log-link model with Poisson quasi-likelihood, whereas diarrhea was analyzed using a population-averaged logistic model. Robust standard errors were clustered by pen, thereby accounting for repeated measurements of goat kids and the dependence of animals housed within the same pen. Separate models were first fitted for pen-floor moisture and sporulated environmental oocyst burden, followed by a joint model that included both exposures. Sporulated burden and previous individual fecal OPG were transformed as $\log_2(\text{value} + 1)$. Therefore, the

coefficient for sporulated burden represented the effect of an approximate doubling, while the effect of moisture was expressed per 10-percentage-point increase. All lagged models were adjusted for previous individual OPG, age, sex, floor temperature, stocking density, time since cleaning, treatment between weeks t and t+2, and sampling week. Exponentiated coefficients were reported as adjusted ratios for fecal OPG and odds ratios for diarrhea, together with 95% confidence intervals. All tests were two-sided, and $P < 0.05$ was considered statistically significant. Analyses were performed using Python 3.12 with pandas, NumPy, and SciPy.

RESULTS

Study population and baseline conditions

Baseline characteristics are presented in Table 1. The study followed 96 Boer-cross goat kids housed in 16 separate pens, with six kids per pen. Mean age at enrollment was 7.34 ± 1.48 weeks, and the study population comprised 52 males (54.17%) and 44 females (45.83%). Mean pen area was 10.01 ± 0.67 m², corresponding to a stocking density of 0.60 ± 0.04 kids/m². At baseline, ambient temperature and relative humidity averaged $28.62 \pm 0.60^\circ\text{C}$ and $82.80 \pm 1.96\%$, respectively, while mean floor temperature was $27.55 \pm 0.41^\circ\text{C}$.

Baseline pen-floor moisture was $26.66 \pm 4.10\%$. The median total environmental oocyst burden was 1,674 oocysts/g DM, with an interquartile range of 1,488–2,387 oocysts/g DM. Mean sporulation rate was $17.26 \pm 6.39\%$, and the corresponding median sporulated oocyst burden was 332 oocysts/g DM (interquartile range: 244–409). Median fecal shedding was 1,592 OPG (interquartile range: 979–2,456), and diarrhea was recorded in 3 of 96 goat kids (3.13%) at enrollment.

Table 1. Baseline characteristics of Boer-cross goat kids and housing conditions

Characteristic	Overall
Number of independent pens	16
Number of Boer-cross goat kids	96
Kids per pen	6
Age at enrollment, weeks	7.34 ± 1.48
Male kids	52/96 (54.17%)
Female kids	44/96 (45.83%)
Pen area, m ²	10.01 ± 0.67
Stocking density, kids/m ²	0.60 ± 0.04
Ambient temperature, °C	28.62 ± 0.60
Relative humidity, %	82.80 ± 1.96
Floor temperature, °C	27.55 ± 0.41
Baseline pen-floor moisture, %	26.66 ± 4.10
Baseline total environmental oocyst burden, oocysts/g DM	1,674 (1,488–2,387)
Baseline sporulation rate, %	17.26 ± 6.39
Baseline sporulated oocyst burden, oocysts/g DM	332 (244–409)
Baseline fecal OPG	1,592 (979–2,456)
Goat kids with diarrhea at baseline	3/96 (3.13%)

Values are presented as number, number (%), mean $\pm$ standard deviation, or median (interquartile range). Environmental sampling comprised baseline and 12 weekly collections, giving 13 observations per pen. Fecal samples were collected seven times from each goat kid. DM: dry matter; OPG: oocysts per gram of feces.

Pen-floor moisture and environmental *Eimeria* oocyst burdens

Table 2 shows a progressive increase in environmental *Eimeria* contamination across the four pen-floor moisture categories. Median total oocyst burden increased from 1,525 oocysts/g DM at moisture levels below 25% to 6,728 oocysts/g DM at moisture levels of at least 45%. Significant differences were detected

among all moisture categories, indicating a consistent increase across the moisture gradient ($P < 0.001$).

The median sporulation rate increased from 14.00% in the driest category to 52.00% at moisture levels of at least 45%. Correspondingly, median sporulated oocyst burden increased approximately 16-fold, from 210 to 3,364 oocysts/g DM ($P < 0.001$).

Table 2. Environmental *Eimeria* oocyst burdens according to pen-floor moisture category

Pen-floor moisture	Pen-week observations	Total oocyst burden, oocysts/g DM	Sporulation rate, %	Sporulated oocyst burden, oocysts/g DM
<25%	10	1,525 ^a (1,294–1,911)	14.00 ^a (8.35–17.75)	210 ^a (157–249)
25 to <35%	77	2,490 ^b (1,899–2,805)	24.00 ^b (19.00–29.00)	542 ^b (401–694)
35 to <45%	112	3,453 ^c (2,849–4,229)	37.00 ^c (32.00–43.25)	1,323 ^c (955–1,712)
≥45%	9	6,728 ^d (5,253–7,325)	52.00 ^d (50.00–58.00)	3,364 ^d (2,899–4,025)
P value	—	<0.001	<0.001	<0.001

Table 3 presents the multivariable associations between the evaluated factors and environmental oocyst burdens. Each 10-percentage-point increase in pen-floor moisture was associated with a 1.59-fold increase in total oocyst burden (95% CI: 1.38–1.83; $P < 0.001$) and a 3.07-fold increase in sporulated oocyst burden (95% CI: 2.58–3.67; $P < 0.001$). Each doubling of the most recent pen-mean fecal OPG was associated

with 1.15- and 1.17-fold increases in total and sporulated oocyst burdens, respectively. Each additional day since cleaning was also associated with a 1.13-fold increase in both environmental outcomes ($P < 0.001$). In contrast, floor temperature and stocking density were not independently associated with either total or sporulated oocyst burden ($P > 0.05$).

Table 3. Multivariable analysis of factors associated with environmental *Eimeria* oocyst burdens

Predictor	Total environmental oocyst burden, adjusted ratio (95% CI)	P value	Sporulated environmental oocyst burden, adjusted ratio (95% CI)	P value
Pen-floor moisture, per 10-percentage-point increase	1.59 (1.38–1.83)	<0.001	3.07 (2.58–3.67)	<0.001
Floor temperature, per 1°C increase	1.01 (0.95–1.08)	0.674	1.01 (0.93–1.11)	0.778
Most recent pen-mean fecal OPG, per doubling	1.15 (1.03–1.29)	0.011	1.17 (1.03–1.32)	0.016
Stocking density, per 0.1 kid/m ² increase	0.95 (0.77–1.17)	0.633	0.88 (0.71–1.08)	0.215
Time since cleaning, per day	1.13 (1.08–1.19)	<0.001	1.13 (1.06–1.20)	<0.001

Two-week lagged associations with fecal oocyst shedding and diarrhea

The two-week lagged associations are presented in Table 4. In Model 1, each 10-percentage-point increase in pen-floor moisture was associated with a 1.38-fold increase in subsequent fecal OPG (95% CI: 1.26–1.51; $P < 0.001$). However, moisture was not significantly associated with subsequent diarrhea (OR: 1.29; 95% CI: 0.71–2.35; $P = 0.402$).

In Model 2, each doubling of sporulated environmental oocyst burden was associated with a 1.22-fold increase in fecal OPG two weeks later (95% CI: 1.18–1.27; $P < 0.001$). The corresponding association with diarrhea

was not significant (OR: 1.19; 95% CI: 0.82–1.73; $P = 0.354$).

When moisture and sporulated oocyst burden were included simultaneously in Model 3, pen-floor moisture was no longer independently associated with subsequent fecal OPG (adjusted ratio: 0.94; 95% CI: 0.80–1.09; $P = 0.402$). In contrast, each doubling of sporulated oocyst burden remained associated with a 1.26-fold increase in subsequent OPG (95% CI: 1.17–1.36; $P < 0.001$). Neither pen-floor moisture (OR: 0.89; 95% CI: 0.32–2.49; $P = 0.826$) nor sporulated oocyst burden (OR: 1.26; 95% CI: 0.64–2.46; $P = 0.506$) was independently associated with subsequent diarrhea in the joint model.

Table 4. Two-week lagged associations of pen-floor moisture and sporulated environmental oocysts with subsequent fecal oocyst shedding and diarrhea

Exposure model and predictor	Subsequent fecal OPG, IRR (95% CI)	P value	Subsequent diarrhea, OR (95% CI)	P value
Model 1: Moisture exposure				
Pen-floor moisture, per 10-percentage-point increase	1.38 (1.26–1.51)	<0.001	1.29 (0.71–2.35)	0.402
Model 2: Sporulated oocyst exposure				
Sporulated environmental oocyst burden, per doubling	1.22 (1.18–1.27)	<0.001	1.19 (0.82–1.73)	0.354
Model 3: Joint exposure model				
Pen-floor moisture, per 10-percentage-point increase	0.94 (0.80–1.09)	0.402	0.89 (0.32–2.49)	0.826
Sporulated environmental oocyst burden, per doubling	1.26 (1.17–1.36)	<0.001	1.26 (0.64–2.46)	0.506

DISCUSSION

The principal finding of this study was that pen-floor moisture was more strongly associated with sporulated *Eimeria* oocyst burden than with total environmental contamination. Across the moisture gradient, total oocyst burden increased approximately fourfold, whereas sporulated burden increased approximately 16-fold. In the multivariable analysis, each 10-percentage-point increase in moisture was associated with a 1.59-fold increase in total burden but a 3.07-fold increase in sporulated burden. This difference suggests that moisture contributed not only to the retention of oocysts within the pen but also to the environmental conditions required for their maturation. Adequate moisture may limit desiccation, stabilize the microenvironment surrounding the oocyst wall, and extend the time available for sporulation. Because only

sporulated oocysts are infective, their environmental burden may provide a more biologically relevant measure of exposure than total oocyst counts alone.

Environmental contamination was also related to the number of oocysts introduced into the pen and the duration of their retention. The most recent pen-mean fecal OPG was positively associated with both total and sporulated environmental burdens, indicating that shedding by infected goat kids contributed to contamination of the pen floor. Similarly, each additional day since cleaning was associated with a 1.13-fold increase in both outcomes, supporting the progressive accumulation of fecal material and oocysts when removal was delayed. A strong influence of age and management on oocyst shedding was observed

under semi-arid conditions in Spain, where *Eimeria* oocysts were detected in 96.1% of fecal samples and kids had substantially higher mean OPG than adult dairy females ($46,496 \pm 5,228$ versus $2,225 \pm 287$). Oocyst shedding was further influenced by herd size and local climatic conditions, with the highest counts occurring near weaning in kids from small farms where prophylactic treatment against coccidiosis was not implemented (Ruiz et al., 2006). Evidence from central Spain further suggests that age and herd structure may influence oocyst shedding independently of broad bioclimatic conditions. Oocyst output declined markedly with age until approximately four years; young goats from larger herds had heavier infections, whereas adult goats from herds containing more than 300 animals exhibited the lowest oocyst counts. Nevertheless, infection intensity did not differ significantly among the three bioclimatic subregions investigated (de la Fuente and Alunda, 1992). In contrast, floor temperature and stocking density were not independently associated with environmental contamination after adjustment. The lack of a stocking-density effect may reflect the narrow range of densities among the 16 pens, while the limited variation in floor temperature may have reduced the ability to detect its contribution to sporulation.

The two-week lag analysis indicated that environmental exposure preceded increased oocyst shedding in the goat kids. When examined separately, both pen-floor moisture and sporulated environmental burden were associated with higher faecal OPG two weeks later. However, when both exposures were included in the same model, the association with moisture was attenuated and no longer significant, whereas sporulated burden remained independently associated with subsequent OPG. Each doubling of sporulated burden was associated with a 1.26-fold increase in later fecal shedding. This pattern is consistent with moisture acting as an upstream environmental condition that facilitates oocyst maturation, while sporulated oocysts may represent a more immediate indicator of exposure. Nevertheless, the observational design does not demonstrate a causal pathway or formally establish mediation, because moisture and sporulated burden were closely related and may have been influenced by other unmeasured pen-level conditions.

Neither pen-floor moisture nor sporulated oocyst burden was significantly associated with subsequent diarrhea. This contrasts with the clear associations observed for fecal OPG and indicates that greater environmental exposure did not necessarily translate into detectable clinical disease. Only 31 diarrheic events occurred among the 576 two-week intervals, resulting in relatively wide confidence intervals for the estimated odds ratios. Moreover, fecal OPG is not a direct indicator of disease severity, and caprine *Eimeria* species differ substantially in pathogenicity. A fatal case of *E. arloingi* infection in a one-month-old Saanen kid was associated with 9,900 OPG, severe diffuse nodular hyperplasia of the small intestine, and bloody or watery intestinal contents (Gazyagci et al., 2015). In a diagnostic series of 123 goat kids aged 0–6 months submitted because of illness or for investigation of death, coccidiosis was identified in 38/69 (55.07%) submissions from Adana, 11/37 (29.73%) from Mersin, and 4/17 (23.53%) from Osmaniye (Özdemir and Çaya, 2012). Clinical coccidiosis was identified on only two of 13 surveyed goat farms despite the widespread detection of oocysts, with *E. arloingi* and *E. ninakohlyakimovae* implicated in disease occurring 2–4 weeks after weaning (Koudela and Boková, 1998). In Myanmar goats, morphological identification combined with 18S rDNA and COI sequencing confirmed the presence of *E. arloingi*, *E. hirci*, and *E. christensenii*, demonstrating the value of molecular methods for species-level confirmation (Bawm et al., 2020). High shedding of less pathogenic species can occur without diarrhea, whereas clinical disease may depend on the infecting species, acquired immunity, nutritional status, weaning stress, and concurrent infections. Mohamaden et al. (2018) reported subclinical coccidiosis in 60% of the examined goats, with *E. arloingi* (37.04%), *E. ninakohlyakimovae* (30.86%), and *E. hirci* (24.69%) being the species most frequently detected in fecal samples. Comparable findings were reported in goats from Turkey by Değer et al. (2003), who identified *E. arloingi* (47.43%), *E. christensenii* (45.14%), *E. ninakohlyakimovae* (36.00%), *E. alijevi* (26.85%), *E. hirci* (23.42%), *E. caprina* (18.28%), and *E. caprovina* (16.57%). Age-related variation in species occurrence has also been reported in South Australia, where *E. christensenii*, *E. caprovina*, and *E. jolchijevi* were more prevalent in domestic goats younger than 12 months than in adults (O’Callaghan, 1989). Furthermore, an age-associated

decline in oocyst prevalence was also documented among indoor-housed goats in the Netherlands, from 96.3% in kids and 94.5% in weaned goats younger than one year to 65.5% in goats aged at least one year (Borgsteede and Derksen, 1996). Multiple-species infection was also the predominant pattern among goats in Senegal, where prevalence reached 85%, with no detectable seasonal variation in prevalence or oocyst shedding during the 12-month survey (Vercruysse, 1982). Mixed-species infections occurred more frequently than single-species infections in both sheep and goats, although the difference was not statistically significant, consistent with an earlier report (Kheirandish et al., 2014). Age-related susceptibility and concurrent parasitic infection have also been documented in northern Jordan, where *Eimeria* infection was significantly more prevalent in goats younger than one year than in adults (66% versus 49%). In addition, *Strongyloides* spp. were detected significantly more frequently in *Eimeria*-positive goats than in uninfected goats, suggesting that concurrent parasitic infections may complicate the attribution of gastrointestinal signs exclusively to coccidiosis (Abo-Shehada and Abo-Farieha, 2003). Because oocysts were identified only at the genus level and other enteric pathogens were not investigated, the cause of individual diarrheic episodes could not be determined.

The findings have practical implications for reducing environmental infection pressure. Maintaining dry pen floors, removing manure at shorter intervals, correcting leaking drinkers, and improving drainage and ventilation may limit both the accumulation and sporulation of oocysts. These measures are consistent with broader recommendations for controlling caprine coccidiosis, which emphasize reducing environmental contamination with infective oocysts, minimizing stress, and avoiding overcrowding (Bawm and Htun, 2021). Particular attention should be given to persistently wet areas because the increase in sporulated burden was substantially greater than the corresponding increase in total contamination. However, the present data did not support a stable

numerical moisture breakpoint. Management recommendations should therefore emphasize the continuous reduction of floor moisture rather than applying a specific moisture cutoff. Any proposed warning threshold would require prospective validation across farms, seasons, floor materials, and management systems.

The repeated assessment of pen-level contamination, standardization of oocyst burdens according to dry matter, direct evaluation of the sporulated fraction, and temporal linkage with subsequent individual outcomes were important strengths of the study. Nevertheless, all pens were located on a single farm, limiting the range of climatic and management conditions represented. Residual confounding cannot be excluded because the study was observational, and microscopy did not distinguish among *Eimeria* species or determine the viability of sporulated oocysts. Fecal OPG estimates may also depend on the counting method, as Mini-FLOTAC produced higher mean and median *Eimeria* OPG values than two McMaster formats in goats, while showing precision comparable to the 1-mL McMaster method (Silva et al., 2013). Weekly environmental sampling could have missed short-term changes following rainfall, cleaning, drinker leakage, or sudden increases in fecal shedding. The small number of diarrheic events limited the precision of the clinical-outcome analysis. These limitations indicate that the observed relationships should be interpreted as farm-specific evidence requiring external validation.

Overall, the results indicate that pen-floor moisture is associated with environmental accumulation of *Eimeria* oocysts, particularly their sporulated and potentially infective fraction. Sporulated environmental burden was a more immediate predictor of subsequent fecal shedding than moisture itself, but neither exposure independently predicted diarrhea. Environmental monitoring and control strategies should therefore prioritize dry flooring, frequent manure removal, and direct assessment of infective oocyst pressure where feasible.

CONCLUSION

Pen-floor moisture was strongly associated with the accumulation of environmental *Eimeria* oocysts, particularly their sporulated and potentially infective fraction, in pens housing Boer-cross goat kids. In the joint two-week lag model, sporulated oocyst burden

remained independently associated with subsequent fecal OPG, whereas pen-floor moisture did not. Neither environmental exposure was significantly associated with subsequent diarrhea. Maintaining dry pen floors, removing fecal material regularly, and preventing

persistent wet areas may help reduce environmental infection pressure. Because the study was conducted on a single farm, these relationships should be externally validated across different farms, seasons, floor materials, and management conditions.

REFERENCES

- Abo-Shehada, M. N., & Abo-Farieha, H. A. (2003). Prevalence of *Eimeria* species among goats in northern Jordan. *Small Ruminant Research*, 49(2), 109–113. [https://doi.org/10.1016/S0921-4488\(03\)00078-6](https://doi.org/10.1016/S0921-4488(03)00078-6)
- Anene, B. M., Onyekwodiri, E. O., Chime, A. B., & Anika, S. M. (1994). Gastrointestinal parasites in sheep and goats of southeastern Nigeria. *Small Ruminant Research*, 13(2), 187–192. [https://doi.org/10.1016/0921-4488\(94\)90095-7](https://doi.org/10.1016/0921-4488(94)90095-7)
- Bawm, S., Win, T. Z. B., Win, S. Y., Htun, L. L., Nakao, R., & Katakura, K. (2020). First detection of *Eimeria* species in Myanmar domestic goats with both microscopic and molecular methods. *Parasite*, 27, 38. <https://doi.org/10.1051/parasite/2020037>
- Bawm, S., & Htun, L. L. (2021). Management and control of *Eimeria* infection in goats. In S. Kukovics (Ed.), *Goat Science - Environment, Health and Economy*. IntechOpen. <https://doi.org/10.5772/intechopen.98979>
- Borgsteede, F. H. M., & Dercksen, D. P. (1996). Coccidial and helminth infections in goats kept indoors in the Netherlands. *Veterinary Parasitology*, 61(3–4), 321–326. [https://doi.org/10.1016/0304-4017\(95\)00830-6](https://doi.org/10.1016/0304-4017(95)00830-6)
- Chartier, C., & Paraud, C. (2012). Coccidiosis due to *Eimeria* in sheep and goats, a review. *Small Ruminant Research*, 103(1), 84–92. <https://doi.org/10.1016/j.smallrumres.2011.10.022>
- de la Fuente, C., & Alunda, J. M. (1992). A quantitative study of *Eimeria* infections of goats from central Spain. *Veterinary Parasitology*, 41(1–2), 7–15. [https://doi.org/10.1016/0304-4017\(92\)90003-R](https://doi.org/10.1016/0304-4017(92)90003-R)
- Değer, S., Gül, A., Ayaz, E., & Biçek, K. (2003). The prevalence of *Eimeria* species in goats in Van. *Turkish Journal of Veterinary & Animal Sciences*, 27(2), 439–442. journals.tubitak.gov.tr
- Diao, N. C., Zhao, B., Chen, Y., Wang, Q., Chen, Z. Y., Yang, Y., Sun, Y. H., Shi, J. F., Li, J. M., Shi, K., Gong, Q. L., & Du, R. (2022). Prevalence of *Eimeria* spp. among goats in China: A systematic review and meta-analysis. *Frontiers in Cellular and Infection Microbiology*, 12, 806085. <https://doi.org/10.3389/fcimb.2022.806085>
- FAOSTAT. (2023). *Crops and livestock products database: Live animal stocks - goats*. Food and Agriculture Organization of the United Nations. <https://www.fao.org/faostat/en/#data> Dữ liệu FAO
- Faizal, A. C. M., & Rajapakse, R. P. V. J. (2001). Prevalence of coccidia and gastrointestinal nematode infections in cross bred goats in the dry areas of Sri Lanka. *Small Ruminant Research*, 40(3), 233–238. [https://doi.org/10.1016/S0921-4488\(01\)00179-1](https://doi.org/10.1016/S0921-4488(01)00179-1)
- Gazyagci, A. N., Anteplioglu, T., Canpolat, S., & Atmaca, H. T. (2015). Coccidiosis due to *Eimeria arloingi* infection in a Saanen goat kid. *Research Journal for Veterinary Practitioners*, 3(2), 29–32. <https://doi.org/10.14737/journal.rjvp/2015/3.2.29.32>
- Jalila, A., Dorny, P., Sani, R., Salim, N. B., & Vercruysse, J. (1998). Coccidial infections of goats in Selangor, Peninsular Malaysia. *Veterinary Parasitology*, 74(2–4), 165–172. [https://doi.org/10.1016/S0304-4017\(97\)00133-7](https://doi.org/10.1016/S0304-4017(97)00133-7)
- Kheirandish, R., Nourollahi-Fard, S. R., & Yadegari, Z. (2014). Prevalence and pathology of coccidiosis in goats in southeastern Iran. *Journal of Parasitic Diseases*, 38(1), 27–31. <https://doi.org/10.1007/s12639-012-0186-0>
- Koudela, B., & Boková, A. (1998). Coccidiosis in goats in the Czech Republic. *Veterinary Parasitology*,

Acknowledgements

The authors thank Tay Do University for providing facilities and logistical support.

Conflicts of interest

The authors declare no conflict of interest.

- 76(4), 261–267. [https://doi.org/10.1016/S0304-4017\(97\)00147-7](https://doi.org/10.1016/S0304-4017(97)00147-7)
- Liu, M., Li, S., Huang, S., Zhang, L., & Jian, F. (2024). Epidemiological characteristics and prevention and control strategies for *Eimeria* spp. in sheep and goats in China: A systematic review. *Animal Diseases*, 4, 48. <https://doi.org/10.1186/s44149-024-00151-w>
- Matthews, J. G. (2016). *Diseases of the Goat* (4th ed.). Wiley-Blackwell. <https://doi.org/10.1002/9781119073543>
- Mohamaden, W. I., Sallam, N. H., & Abouelhassan, E. M. (2018). Prevalence of *Eimeria* species among sheep and goats in Suez Governorate, Egypt. *International Journal of Veterinary Science and Medicine*, 6(1), 65–72. <https://doi.org/10.1016/j.ijvsm.2018.02.004>
- O'Callaghan, M. G. (1989). Coccidia of domestic and feral goats in South Australia. *Veterinary Parasitology*, 30(4), 267–272. [https://doi.org/10.1016/0304-4017\(89\)90095-2](https://doi.org/10.1016/0304-4017(89)90095-2)
- Özdemir, R., & Çaya, H. (2012). Adana, Mersin ve Osmaniye illerinden gelen oğlaklarda coccidiosisin prevalansının araştırılması. *AVKAE Dergisi*, 2(2), 6–9.
- Ruiz, A., González, J. F., Rodríguez, E., Martín, S., Hernández, Y. I., Almeida, R., & Molina, J. M. (2006). Influence of climatic and management factors on *Eimeria* infections in goats from semi-arid zones. *Journal of Veterinary Medicine Series B: Infectious Diseases and Veterinary Public Health*, 53(8), 399–402. <https://doi.org/10.1111/j.1439-0450.2006.00985.x>
- Sharma, R. L., Bhattacharya, D., Laha, R., Biswas, J. C., & Rangarao, G. S. C. (1997). Preliminary observations on intestinal coccidiosis in Pashmina (Cashmere) goats in India. *Journal of Applied Animal Research*, 12(1), 107–112. <https://doi.org/10.1080/09712119.1997.9706193>
- Silva, L. M. R., Vila-Viçosa, M. J. M., Maurelli, M. P., Morgoglione, M. E., Cortes, H. C. E., Cringoli, G., & Rinaldi, L. (2013). Mini-FLOTAC for the diagnosis of *Eimeria* infection in goats: An alternative to McMaster. *Small Ruminant Research*, 114(2–3), 280–283. <https://doi.org/10.1016/j.smallrumres.2013.06.017>
- Taylor, M. A., & Catchpole, J. (1994). Review article: Coccidiosis of domestic ruminants. *Applied Parasitology*, 35(2), 73–86. PMID: 8087156.
- Utaaker, K. S., Chaudhary, S., Kifleyohannes, T., & Robertson, L. J. (2021). Global goat! Is the expanding goat population an important reservoir of *Cryptosporidium*? *Frontiers in Veterinary Science*, 8, 648500. <https://doi.org/10.3389/fvets.2021.648500>
- Vercruysse, J. (1982). The coccidia of sheep and goats in Senegal. *Veterinary Parasitology*, 10(4), 297–306. [https://doi.org/10.1016/0304-4017\(82\)90080-2](https://doi.org/10.1016/0304-4017(82)90080-2)
- Wang, C. R., Xiao, J. Y., Chen, A. H., Chen, J., Wang, Y., Gao, J. F., & Zhu, X. Q. (2010). Prevalence of coccidial infection in sheep and goats in northeastern China. *Veterinary Parasitology*, 174(3–4), 213–217. <https://doi.org/10.1016/j.vetpar.2010.08.026>