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Original Article

Impact of Livestock Shed Sanitation on Zoonotic Parasite Exposure in Stunted Children from Farming Communities

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Abstract

Background: Poor livestock shed sanitation may facilitate transmission of intestinal protozoa and contribute to child undernutrition, particularly stunting. However, evidence on zoonotic risks in smallholder farming communities remains limited.

Methods: A cross-sectional study was conducted among 79 stunted children (<5 years) and 79 cattle in Jember Regency, Indonesia. Anthropometric status was assessed using the WHO criteria. Shed sanitation was evaluated through structured observation (location, orientation, construction, waste handling, cleanliness). Stool samples from children and cattle were examined for *Cryptosporidium* spp. using modified Ziehl Neelsen staining and for *Entamoeba* spp. by direct microscopy. Data were analyzed with Chi-square tests and multivariable logistic regression adjusting for household and socioeconomic factors.

Results: *Cryptosporidium* spp. was detected in 13.5% of children and 32.9% of cattle. *Entamoeba* spp. was found in 11.4% of children. Shed orientation without sunlight exposure was associated with *Cryptosporidium* spp. infection (OR = 14.3; 95% CI: 1.39–149.2; $P = 0.021$). Shed location within 10 m of the home was associated with *Entamoeba* spp. infection (adjusted OR = 17.8; 95% CI: 1.13–279.6; $P = 0.041$). Other sanitation variables showed non-significant but plausible associations.

Conclusion: Poor shed sanitation, particularly proximity to homes, and lack of sunlight exposure, was associated with intestinal protozoa among stunted children. While causality cannot be inferred due to diagnostic and design limitations, the findings highlight the importance of livestock management in child health. Further studies with molecular diagnostics and larger representative samples are recommended.

Introduction

Intestinal protozoal infections remain a major public health concern in developing countries, especially among vulnerable groups such as children under five years old

(1). Key contributing factors include poor sanitation, inadequate access to clean water, and low health awareness, all supporting continued transmission (2). Among the most



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concerning zoonotic protozoa are *Cryptosporidium* spp. and *Entamoeba* spp., which are significantly associated with malnutrition and stunting in children (3). These infections are particularly dangerous because they can lead to iron deficiency anemia, physical growth delays, and other chronic health issues (4). *Cryptosporidium* spp. is a protozoan parasite capable of infecting humans and livestock, mainly through contaminated water or direct contact with infected animals or their feces. Despite frequent underdiagnosis in low-resource areas, cryptosporidiosis ranks the second leading cause of diarrheal illness and mortality in children under five, following rotavirus (5,6). Globally, over 200,000 child deaths have been linked to this infection in South Asia alone and annually, approximately 8500 cases of cryptosporidiosis are reported in the United States (7). In Indonesia, prevalence rates range from 4% to 11%, particularly affecting rural children living near livestock (8).

Similarly, *Entamoeba* spp., the pathogen responsible for amoebiasis, presents serious health threats in regions with poor sanitation. It is among the top seven pathogens responsible for severe diarrhea in young children, as the Global Enteric Multicenter Study (GEMS) reported (9). Chronic infection can cause long-term effects such as malnutrition and impaired nutrient absorption, contributing to stunting (10). This relationship is bi-directional: malnourished children are more susceptible to infection, and the infection worsens nutritional deficiencies by limiting nutrient uptake and increasing metabolic demands (11).

Stunting continues to be a national concern in Indonesia. The 2022 Indonesian Nutrition Status Survey (SSGI) reported a national stunting rate of 21.6%. However, in Jember Regency, East Java, the prevalence is far higher at 34.9%, the highest in the province (12). Within Jember, Panti District has one of the most alarming stunting rates, exceeding national and the WHO thresholds. The area's

dense dairy cattle population, inadequate livestock management, and poor environmental sanitation raises questions about the potential zoonotic transmission of protozoa from animal sheds (13,14). Poorly maintained animal sheds can act as reservoirs for zoonotic parasites. Location, sunlight exposure, waste disposal, ventilation, and hygiene significantly influence infection risk. Lack of sunlight and poor airflow contribute to damp, pathogen-friendly conditions that increase disease transmission to animals and nearby humans, especially young children (15). Previous studies in Jember, (16, 17), have primarily focused on farmers, neglecting the vulnerable child population. Despite growing concern, there is limited research focusing on the link between livestock shed sanitation and protozoal infections in stunted children.

This study addressed that gap by exploring how specific shed sanitation factors, contribute to the risk of *Cryptosporidium* and *Entamoeba* spp. infections in stunted toddlers in Panti District.

Materials and Methods

Study Design and Setting

This study employed an analytical observational approach with a cross-sectional design, conducted from September to December 2023 in farming communities with a high prevalence of child stunting in Panti Sub-District, Jember Regency, East Java, Indonesia.

Study Population and Sampling

Purposive sampling was used to recruit 79 stunted toddlers and 79 cattle fecal samples from the same households. The sample was selected by purposive sampling technique, and the minimum sample size was computed using the formula of Lemeshow. The inclusion criteria required children to have a confirmed diagnosis of stunting (Height-for-Age Z-score < -2 SD), determined based on existing diagnostic records at the community health service in accordance with the WHO standards, in-

cluding the use of calibrated equipment and duplicate measures, as part of routine child growth monitoring, and provided parental informed consent (<https://pubmed.ncbi.nlm.nih.gov/16817681/>). Participants were excluded if fecal samples could not be obtained from either the child or the household's cattle.

Ethical approval for the study was obtained from the Ethics Committee of the Faculty of Medicine, University of Jember (Approval No. 5103/UN25.1.10.2/KE/2023).

Assessment of Shed Sanitation

Livestock shed sanitation was evaluated by direct observation using a structured checklist adapted from the Indonesian Minister of Agriculture Regulation No. 46/2015. Five components were assessed: shed location (distance from home), shed orientation (related to sunlight exposure), shed building construction (ventilation, stability), waste handling frequency, and shed cleanliness (presence of accumulated waste, stagnant water). The reliability of the questionnaire checklist was confirmed with a Cronbach's alpha coefficient of 0.81.

Stool Sample Collection and Laboratory Examination

Child and cattle stool samples were collected in sterile containers, transported within 2 hours, and examined at the Parasitology Laboratory, Faculty of Medicine, University of Jember. *Cryptosporidium* spp. detected using modified Ziehl–Neelsen (MZN) staining. While commonly used in low-resource settings, this method has limited sensitivity and specificity, which is acknowledged as a study limitation. *Entamoeba* spp. identified using direct smear microscopy at $\times 1000$ magnification. Because light microscopy cannot differentiate *E. histolytica* from *E. dispar* or *E. moshkovskii*, all findings are reported as *Entamoeba* spp. throughout this manuscript.

Data Analysis

Data was analyzed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA).

Descriptive statistics summarized sociodemographic, environmental, and laboratory findings. Bivariate analysis was performed using Chi-square or Fisher's exact tests to explore associations between shed sanitation factors and infection status. Multivariable logistic regression was conducted to adjust for potential confounders. Odds Ratios (OR) with 95% Confidence Intervals (CI) were calculated. A P -value < 0.05 was considered statistically significant. The regression models included key variables, but the modest sample size and cross-sectional nature limit causal inference and the ability to adjust for all potential confounders.

Results

The majority of the children were male (52.4%) and most were between 12–23 months of age (31%), representing the early toddler period when vulnerability to infections and nutritional deficiencies is relatively high. Regarding maternal characteristics, most of the mothers were housewives (90.5%), with half of them aged between 26–35 years (50%), which reflects the typical reproductive age group in this community. The majority of parents had a low level of formal education and a low family income (92.9%), indicating a socioeconomic background that may influence both childcare practices and access to health services. These descriptive findings provide contextual information about the study population but were not subjected to further inferential analysis.

Microscopic examination of livestock feces revealed that 32.91% were contaminated with *Cryptosporidium* spp. oocysts, suggesting a possible zoonotic risk on the farm, as presented in Table 1. Representative microscopic findings of *Cryptosporidium* spp. oocysts identified in both stunted toddlers and cattle are shown in Fig. 1. The evaluation of livestock shed sanitation revealed several environmental concerns. Almost one-quarter (24.05%) of sheds

were located less than 10 meters from the household which could increase environmental contamination, while 25.32% lacked adequate sunlight exposure due to their orientation and structural barriers. Nearly half (48.10%) were poorly constructed with limited

ventilation, and waste management practices were often inadequate: 34.18% of farmers reported irregular manure disposal, and 43.04% of sheds displayed poor cleanliness characterized by manure accumulation, stagnant water, and residual feed.

Table 1: Statistical analysis of shed sanitation with *Cryptosporidium* spp. Infection

Shed Sanitations	%	<i>Cryptosporidium</i> spp. infection in children				P-value	OR	95% CI
		Positive		Negative				
		No.	%	No.	%			
Shed location								
Close to home, ranged < 10 m	24.05	7	8.85	12	15.19	0.08	6.50	0.88, 47.89
Far from home, ranged ≥ 10 m	73.42	4	6.33	56	70.89			
Shed direction								
Without sun-exposed	25.32	6	7.59	14	17.72	0.02*	14.29	1.39,149.21
Sun-exposed	74.68	5	6.33	54	68.35			
Shed building								
Unventilated, unstable, and close to other buildings	48.10	9	11.39	29	36.71	0.18	5.14	0.51, 51.29
Ventilated, stable, and far than other buildings	51.90	2	2.53	39	49.37			
Waste handling								
Aperiodic	34.18	7	8.86	20	25.32	0.32	3.30	0.47, 22.94
Daily	65.82	4	5.06	48	60.76			
Shed cleanliness								
Scattered feed, animal waste with stagnant water	43.04	8	10.13	26	32.91	0.16	5.85	0.58, 58.43
Without scattered feed, animal waste and stagnant water	56.96	3	3.80	42	53.16			
Cattle Feces								
Positive	32.91	7	8.86	19	24.05	0.64	1.93	0.28, 13.16
Negative	67.09	4	5.06	49	62.03			

*Shows significant association at P-value < 0.05
OR: Odds Ratio, CI: Confidence Interval

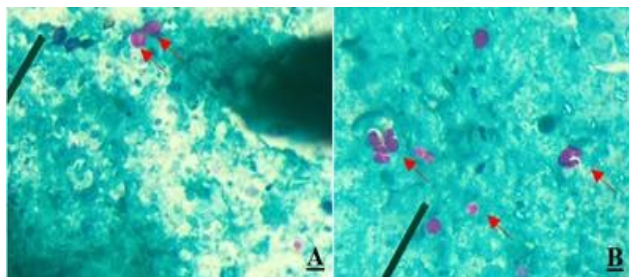


Fig. 1: *Cryptosporidium* spp. oocysts in children and livestock

A= Oocysts in stunted toddlers (red arrow);
 B=Oocysts in cattle (red arrow)

Table 2: Multivariate analysis of shed sanitation for *Cryptosporidium* spp. Infection

Shed Sanitations	B	P-value	OR	95% CI
Shed location	1.320	0.25	4.74	0.39-35.33
Shed direction	2.659	0.03*	14.29	1.39-149.21
Shed building	1.364	0.28	3.91	0.34-45.75
Shed cleanliness	0.878	0.52	2.41	0.16-35.92

*Shows significant association at P-value < 0.05
 OR: Odds Ratio, CI: Confidence Interval

Multivariate logistic regression analysis was conducted to assess the association between shed sanitation factors and *Cryptosporidium* spp. infection in livestock. Among the variables analyzed, shed direction was the only factor showing a statistically significant association with infection (P=0.03). Sheds with poor orientation had 14.29 times higher odds of being associated with infected livestock compared to those with adequate direction.

Shed location near the household was not significantly associated with infection (OR:

When analyzed in relation to *Cryptosporidium* spp. infection among children, shed orientation emerged as a significant factor. Children living near sheds without adequate sunlight exposure had markedly higher odds of infection (P = 0.021). Other sanitation variables showed no significant associations but remained biologically plausible contributors to exposure as shown in Table 2.

4.74; 95% CI: 0.39-35.33; P=0.25). Similarly, no significant associations were found for shed building construction (OR: 3.91; 95% CI: 0.34-45.75; P=0.28) or shed cleanliness (OR: 2.41; 95% CI: 0.16-35.92; P=0.52).

Microscopy findings were reported as *Entamoeba* spp., acknowledging the inability to differentiate *E. histolytica* from non-pathogenic species. The association between the sanitation conditions of cattle sheds and the prevalence of *Entamoeba* spp. infection in stunted toddlers are summarized in Table 3. A microscopic stool examination revealed that 11.39% of stunted children had an *Entamoeba* spp. infection, a finding that underscores the urgent need for public health interventions.

Bivariate analysis was conducted to examine the association between livestock shed sanitation components and *Entamoeba* spp. infection in children. Shed location showed a significant association with infection (P=0.008). Among children living in households with sheds located less than 10 meters from the home, 7.59% were positive for *Entamoeba* spp., compared to 3.80% of children with sheds located further away.

Table 3: Bivariate Analysis of Livestock Shed Sanitation for *Entamoeba* spp. infection

Livestock Sheds Sanitation Components	<i>Entamoeba</i> spp. infection in children							
	Total %	Positive No.	Positive %	Negative No.	Negative %	P-value	OR	95% CI
Shed location								
Close to home, ranged < 10m	25.32	6	7.59	14	17.72	0.008*	20.67	1.95-218.71
Far from home, ranged ≥ 10m	74.68	3	3.80	56	70.89			
Shed direction								
Without sun-exposed	34.18	7	8.86	20	25.32	0.018*	14.50	1.41-148.57
Sun-exposed	65.82	2	2.53	50	63.29			
Shed building								
Unventilated, unstable, and close to other buildings	58.23	8	10.13	38	48.10	0.36	3.79	0.39-37.20
Ventilated, stable, and higher than other buildings	41.77	1	1.27	32	40.51			
Waste handling								
Aperiodic	34.18	7	8.86	20	25.32	0.13	4.67	0.67-32.49
Daily	65.82	2	2.53	50	63.29			
Shed Cleanliness								
Scattered feed, animal waste with stagnant water	54.43	8	10.13	35	44.30	0.34	4.71	0.48-46.23
Without scattered feed, animal waste and stagnant water	45.57	1	1.27	35	44.30			
Cattle Feces								
Positive	17.72	2	2.53	12	15.19	0.64	1.97	0.29-13.21
Negative	82.28	7	8.86	58	73.42			
Total		9	11.39	70	88.61			

*Shows significant association at P -value < 0.05

OR: Odds Ratio, CI: Confidence Interval

Shed direction was also significantly associated with infection ($P=0.018$). Infection was present in 8.86% of children from households with sheds lacking sun exposure, compared to 2.53% of children from households with sun-exposed sheds.

No significant associations were found between *Entamoeba* spp. infection in children and shed building construction ($P=0.36$), waste handling practices ($P=0.13$), shed cleanliness ($P=0.34$), or the presence of *Entamoeba* spp. in cattle feces ($P=0.64$).

Following bivariate analysis, multivariable logistic regression was performed to adjust for socioeconomic and hygiene-related factors. In the adjusted model, shed location remained independently associated with *Entamoeba* spp. infection in children ($P=0.041$), as presented in Table 4. Shed direction was not statistically significant in the multivariate model ($P = 0.06$). Shed cleanliness was also not significantly associated with infection ($P = 0.18$). The constant of the model was statistically significant ($P = 0.004$).

Table 4: Multivariate analysis of shed sanitation for *Entamoeba* spp. Infection

Shed Sanitation	B	P-value	OR	95% CI
Shed location	2.879	0.04*	17.8	1.13-279.57
Shed direction	1.874	0.18	6.52	0.85-257.55
Shed cleanliness	5.596	0.004	0.004	0.43- 99.14
Constanta				-

*: Shows significant association at $P\text{-value} < 0.05$

OR: Odds Ratio, CI: Confidence Interval

The bivariate analysis showed that shed proximity to the house and lack of sunlight exposure were significantly associated with *Entamoeba* spp. infection in children, whereas shed construction, waste handling, cleanliness, and cattle fecal status were not significantly

Discussion

This study investigated the associations between livestock management, environmental sanitation, and the prevalence of *Entamoeba* spp. and *Cryptosporidium* spp. infections in stunted children living in a rural farming community. Associations with shed proximity and lack of sunlight exposure suggest that environmental sanitation around animal enclosures contributes to transmission risk. Most affected children were male toddlers aged 12–23 months, a particularly

The orientation of livestock enclosures was also found to influence the risk of infection. In Panti district, 65.82% of sheds have an orientation that allows exposure to sunlight. Sunlight, a natural disinfectant, is crucial in reducing shed humidity and inhibiting the growth of microorganisms, including protozoa. *Entamoeba* spp. cyst can survive in humid (Fig. 2), cold environments but becomes inactive at temperatures above 40 °C. Therefore, good shed orientation that allows ample sunlight can significantly minimize the risk of infection, offering a hopeful prospect for disease control.

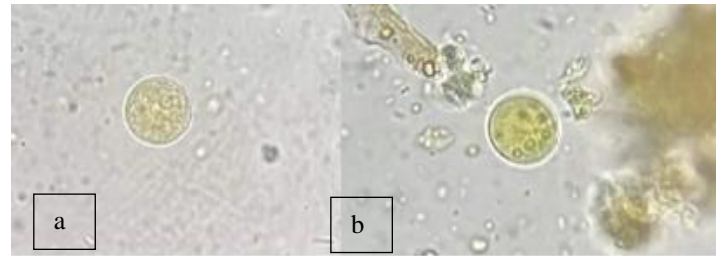


Fig. 2: *Entamoeba* spp. cysts (a).in a stool sample from a toddler. (b). in a cattle stool sample

associated. In the multivariate analysis, after adjustment for other variables, only shed location remained independently associated with infection, while shed direction and shed cleanliness were no longer statistically significant.

vulnerable group due to their nutritional needs and frequent contact with contaminated environments. Contributing factors included low maternal education, limited access to health information, and low family income, which may hinder awareness and sanitation practices. The prevalence of *Entamoeba* spp. infection in this population (11.39%) was slightly higher than that in Ethiopia (8.6%) (19) and slightly lower than Egypt (12.7%) (20).

A significant association was observed between the location and orientation of livestock sheds and the incidence of *Entamoeba*

spp. infection. Sheds situated less than 10 meters from homes increased the risk of infection by nearly 18 times. Sheds that lacked sunlight exposure also posed higher risks, as *Entamoeba* spp. thrives in cool and moist conditions but becomes inactive at higher temperatures. Although shed construction materials, cleanliness, and waste disposal methods did not show a statistically significant link to infection, poor waste management practices, such as dumping manure into rivers, and children not wearing footwear in contaminated areas remained public health concerns. Importantly, this study emphasized that *Entamoeba* spp. transmission is not limited to direct contact with animals (21). However, direct attribution to zoonotic spread from cattle remains speculative, as cattle are not established hosts for *E. histolytica*. Indirect exposure through contaminated soil, water, food, or vectors (like flies) also plays a critical role. While good hygiene practices among respondents, such as regular handwashing and bathing, likely reduced infection risk, educational campaigns promoting environmental hygiene and livestock sanitation are urgently needed to break the cycle of transmission.

Cryptosporidium spp. was detected in both children and cattle, consistent with its role as a zoonotic pathogen in low-resource settings. The association between shed orientation and child infection suggests that limited sunlight exposure may create damp conditions that favor oocyst survival. Sunlight reduces humidity, inhibits oocyst survival, and deters flies, mechanical vectors that can carry *Cryptosporidium* oocysts. Although other aspects of shed sanitation (e.g., construction material or distance from homes) were not statistically significant, they remain relevant. Bamboo structures, for example, retain moisture more than cement, while unregulated waste disposal increases environmental contamination risk. Interestingly, although direct transmission between livestock and

children via *Cryptosporidium* spp. was not statistically confirmed, the high prevalence in animals suggests that livestock may act as zoonotic reservoirs (22). Flies and other vectors were not directly studied, but their potential role is supported by previous research (17). Proper ventilation and sunlight in sheds are therefore essential not only for animal health but also to reduce pathogen survival. This aligns with prior studies indicating that ultraviolet light and high temperatures reduce *Cryptosporidium* spp. viability.

The broader public health implications of these findings are significant. Repeated exposure to enteric pathogens may contribute to subclinical intestinal conditions, sometimes referred to as Environmental Enteric Dysfunction (EED), which is hypothesized to impair nutrient absorption and contribute to growth faltering (23,24). While this study did not measure EED biomarkers, the association between poor shed sanitation and protozoan infections suggests a potential pathway through which environmental contamination could affect child nutritional status in these communities. Observational data from Bangladesh, Ethiopia, and Zimbabwe further support this link (25). EED often results from poor hygiene, exposure to animal feces, and inadequate livestock management, all conditions prevalent in rural farming households.

In light of these findings, the study advocates for cross-sectoral, One Health interventions that integrate human health, veterinary practices, and environmental management. Interventions should include better livestock enclosure design, improved waste disposal systems, hand hygiene promotion, and public education, especially among farming communities (26). Educating farmers about the significance of shed orientation, safe distances from homes, and consistent hygiene practices can significantly reduce the risk of zoonotic infections in children (27). Ultimately, a unified One Health

approach is essential to reduce zoonotic transmission and improve child nutrition and health outcomes. Integrating environmental, human, and animal health efforts will strengthen prevention strategies and address the root causes of stunting in ecologically complex rural settings (28).

This study has several limitations that should be considered when interpreting the findings. The diagnostic methods relied on microscopy, which has limited sensitivity and cannot reliably distinguish between morphologically similar protozoan species. The cross-sectional design also restricts the ability to establish causal relationships. In addition, the relatively small sample size and purposive recruitment of stunted children may limit the generalizability of the results.

Nevertheless, the study provides valuable insights into the role of livestock management and environmental sanitation in rural settings. Practical improvements such as optimizing shed orientation, ensuring regular waste handling, and strengthening hygiene education have the potential to reduce protozoan exposure among children. Future studies employing molecular diagnostics, larger and more representative samples, and longitudinal designs will be important to confirm these associations and clarify causal pathways.

Conclusion

This study found significant associations between poor cattle shed sanitation, particularly shed orientation and proximity to homes, and a higher prevalence of *Cryptosporidium* spp. and *Entamoeba* spp. infections in stunted children. Sheds located close to houses and those lacking sunlight exposure were linked to damp conditions that may support the survival of infectious stages. It is important to interpret these findings considering the study's limitations, including the cross-sectional design, which precludes causal inferences, the use of microscopic identification for *Entamoeba* spe-

cies, and the relatively small sample size which resulted in imprecise effect estimates. Despite these limitations, the results underscore the potential importance of environmental sanitation. Improving livestock housing design, particularly ensuring adequate sunlight exposure and maintaining a safe distance from living quarters, coupled with enhanced hygiene education, may be valuable components of strategies aimed at reducing parasitic infections and protecting the health of vulnerable children in rural farming communities.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article

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