ORIGINAL ARTICLE

Cattle Shed Sanitation Associated with High Fly Density and Transmission of *Cryptosporidium spp.*: A One Health Perspective

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ABSTRACT

Introduction: *Cryptosporidium spp*. is responsible for more than 50% of diarrheal cases in cattle and poses a zoonotic threat to human health through environmental contamination and mechanical transmission by houseflies. Oocysts may adhere to the exoskeleton or be present in the digestive tract, regurgitation, and feces of flies, facilitating transmission between livestock and human environments. This study aimed to determine the association between cattle shed sanitation, fly density, and the transmission of *Cryptosporidium spp*. within a One Health framework. **Methods:** A cross-sectional study was conducted in 40 cattle sheds in Pakusari District, Jember Regency, Indonesia. Fly density was assessed using fly traps, and oocyst detection was performed using a modified Ziehl-Neelsen staining technique. Shed sanitation factors observed included shed distance from the farmer's house, ventilation, shed orientation, manure handling, and construction materials. Chi-square and logistic regression analyses were applied. **Results:** High fly density was observed in 77.5% of sheds, and *Cryptosporidium spp*. oocysts were detected in 10%. Bamboo wall construction and ground-level manure piling increased the odds of high fly density by 29.657 and 13.650 times, respectively. Unventilated sheds demonstrated a 22.856-fold higher risk of oocyst carriage by houseflies. **Conclusion:** These findings highlight the importance of improving shed sanitation and fly control as part of an integrated One Health approach to prevent *Cryptosporidium spp*. transmission at the human–animal–environment interface.
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INTRODUCTION

Shed hygiene plays a crucial role in protecting the health of both livestock and dairy farmers. Farmers who work in poorly maintained environments are reported to be 3.59 times more likely to experience diarrhea (1). Poor shed sanitation also increases the risk of health problems such as diarrhea, nausea, vomiting, and itching by 36.2% (2). Cryptosporidium parvum is responsible for more than 50% of diarrhea cases in cattle (3,4). A study conducted in Malaysia reported that 15.9% of cattle were positive for Cryptosporidium oocyst (3) while research in Ghana found a 26.5% infection rate among cattle kept in poorly sanitized stables (5). Infected cattle can excrete up to 1x10ºº oocysts per day (6). *Cryptosporidium spp*. is a zoonotic pathogen capable of transmission from animals to humans (7) and direct contact with infected cattle increases the risk of human infection by 8.3-fold. Clinical symptoms in humans typically include watery diarrhea and dehydration, which may be fatal in severe cases. Currently, no vaccine is available to prevent Cryptosporidium infection in either cattle or humans, and the only approved treatment, nitazoxanide, has limited efficacy in immunocompromised individuals (8). Given the substantial burden of HIV/AIDS in Jember Regency, individuals with compromised immunity are at heightened risk of severe and chronic cryptosporidiosis, making environmental transmission from livestock through vectors such as houseflies a critical public health concern. Cattle waste generates foul odors that attract flies (9). Houseflies are the most common species found around households and barns and can act as mechanical vectors for intestinal parasites in both humans and animals, including *Cryptosporidium spp*. (10). Cryptosporidium oocysts may be present on the exoskeleton, within the digestive tract, and in the regurgitation and feces of houseflies, with concentrations reaching an average of 10^8 oocysts/cm . Jember Regency was the fourth-highest Regency for HIV/AIDS cases after Surabaya City, Pasuruan Regency, and Malang Regency. The number of people with HIV/AIDS in Jember Regency from 2004 to April 2018 was 3,786 cases, while the number of AIDS cases was 943 cases (11). In this context, the high prevalence of HIV/AIDS is clinically significant when linked to environmental health risks such as *Cryptosporidium spp*. infection, which is known to cause severe and prolonged diarrhea in immunocompromised individuals (12). The rise of cryptosporidiosis, especially among vulnerable populations such as young children, older adults, and individuals with HIV/AIDS, has become a serious health concern in areas with limited healthcare resources. Despite this growing issue, there is still a lack of research in Indonesia and other Southeast Asian countries examining how poor cattle shed hygiene(13) and high fly density may facilitate the spread of Cryptosporidium from animals to humans through mechanical transmission. This study aimed to determine the correlation between barn sanitation, fly density, and transmission of *Cryptosporidium spp*. in cattle, to contribute epidemiological evidence using a One Health approach to guide preventive actions relevant to human health protection.

MATERIALS AND METHODS

Samples

This cross-sectional study was conducted in the Pakusari District, Jember Regency, East Java, Indonesia, where the environmental conditions were well suited to the study objectives. Fly density was assessed by collecting houseflies from 40 cattle sheds. The sample size was determined based on previous studies conducted in comparable rural settings, which reported transmission rates of *Cryptosporidium spp*. via houseflies ranging from 10% to 30%. (14). The goal was to have a 95% confidence level and a 15% error margin. This size of 40 meets the smallest number of samples usually needed for cross-sectional studies that use statistical methods such as chi-square and logistic regression to examine risk factors. The study also aimed for at least 80% statistical power to detect real connections between cleanliness of the shed and the number of flies. Choosing 40 sheds also made sense due to practical reasons, such as how easy it was to reach the sites, time limits, and the need to do thorough checks on each fly sample. A purposeful sampling method was used, focusing on sheds that were active, had regular manure, and showed signs of fly activity, to make sure the study reflected real conditions in Pakusari District. Fly capture was conducted by opening the trap for 30 seconds, and the procedure was repeated ten times (10 x 30 seconds). The mean of the five highest counts was used to calculate the final fly density. Fly density was categorized according to the Indonesian Ministry of Health guidelines, where ≤2 flies per trap were classified as low density, and >2 flies per trap were classified as high density. In addition, fly traps were placed in each cattle shed for 24 hours to collect samples for oocyst detection. Shed hygiene factors evaluated as potential determinants of fly density included shed distance from the house, ventilation, orientation relative to sunlight exposure, manure handling practices, disinfection procedures, as well as roof, wall, and floor materials.

Detection of *Cryptosporidium spp.* oocysts on fly samples

Fly samples were processed at the Parasitology Laboratory, Faculty of Medicine, University of Jember. The procedure included: (1) placing each fly in a 15 mL centrifuge tube containing 2 mL of 0.9% NaCl solution for washing; (2) shaking the tube for 2 minutes; (3) removing the fly from the tube; (4) centrifuging the suspension at 3,000 rpm for 5 minutes; and (5) discarding the supernatant and transferring 100 µL of sediment onto a glass slide using a pipette. A modified Ziehl-Neelsen (MZN) staining method was performed to detect *Cryptosporidium spp*. oocysts, which were observed under a light microscope at 1000x magnification using oil immersion. Oocyst size was measured using Image Raster 3.0 software and compared to the World Health Organization (WHO) reference standards.

Statistical Analysis

Data were analyzed using SPSS software (version 24). Chi-square and logistic regression tests were applied to assess the associations between variables. A p-value of <0.05 was considered statistically significant.

Ethical Approval

All procedures conducted in this study adhered to ethical standards and were approved by the Ethics Committee of the Faculty of Medicine, University of Jember (Approval No. 1.567/H25.1.11/KE/2022).

RESULTS

Shed sanitation and high fly density

Among the 40 cattle sheds assessed, 77.5% exhibited high fly density, while only 22.5% showed low fly density. High fly density was predominantly observed in sheds located less than 10 meters from the farmer's residence, oriented in a north–south direction, and constructed with tile roofing (80.6%, 67.7%, and 80.6%, respectively). Additionally, sheds with adequate ventilation, bamboo flooring or walls, and manure managed by ground piling demonstrated elevated fly density (77.4%, 96.8%, and 71%, respectively). These findings highlight the substantial influence of shed proximity, structural design, and construction materials on fly density levels in cattle-rearing environments.

The chi-square test indicated that shed wall material and manure handling practices had a significant association with fly density. Shed wall material demonstrated an odds ratio (OR) of 15.00 (p = 0.030; 95% CI: 1.325–169.870), while cattle manure handling showed an OR of 8.556 (p = 0.018; 95% CI: 1.483–49.353). These findings, which align with the logistic regression analysis presented in Table I, suggest important implications for livestock management. Specifically, optimizing shed construction and improving manure handling practices may effectively reduce fly density, thereby supporting better animal health and productivity.

Table I. Analysis of chi-square and logistic regression tests of shed sanitation and fly density

Variables	Fly Density				X^2		Logistic regression		
	Low		High						
	n	%	n	%	*p*	OR [95% CI]	B	*p*	OR [95% CI]
Shed distance from the house									
<10 meters	5	55.6	25	80.6	0.190	3.333 [0.681–16.317]	-	-	-
≥10 meters	4	44.4	6	19.4					
Air ventilation									
Unventilated	3	33.3	7	22.6	0.665	0.583 [0.115–2.952]	-	-	-
Ventilated	6	66.7	24	77.4					
She direction									
Facing to the west/east	2	22.2	10	32.3	0.697	1.667 [0.292–9.520]	-	-	-
Facing to the north/south	7	77.8	21	67.7					
Roof material									
Asbestos	1	11.1	6	19.4	1.000	1.920 [0.200–18.432]	-	-	-
Tile	8	88.9	25	80.6					
Wall material									
Bamboo	6	66.7	30	96.8	0.030*	15.000 [1.325–169.870]	3.390	0.029*	29.657 [1.411-623.354]
Brick	3	33.3	1	3.2					
Floor material									
Bamboo	7	77.8	30	96.8	0.121	8.571 [0.678–108.414]	-	-	-
Cement	2	22.2	1	3.2					
Manure handling									
Piled on the ground	2	22.2	22	71					
Stored in the sacks	7	77.8	9	29	0.018*	8.556 [1.483–49.353]	2.614	0.023*	13.650 [1.445-128.902]
Constant							-2.836	0.077*	0.059 [-]

**Significant*
B=Beta coefficient, OR=*Odds Ratio*, CI=*Confidence Interval*

Logistic regression further revealed that wall material and manure handling exhibited a strong influence on fly density. Manure piling directly on the floor without cover increased the likelihood of high fly density by 8.556 times, making it the most influential factor. Bamboo wall construction also had a significant effect, with a B coefficient of 3.390, indicating a positive correlation. Likewise, manure piling showed a B coefficient of 2.614, reinforcing its substantial role in increasing fly density. These findings emphasize the multifactorial nature of fly infestation in cattle sheds and highlight the need for targeted environmental modifications.

Shed sanitation and transmission of *Cryptosporidium spp.*

We detected *Cryptosporidium spp.* oocysts in 4 (10%) of the 40 cattle sheds examined. This detection rate is comparable to findings reported in Malaysia, where a 15.9% prevalence was observed among dairy cattle (3), and to a study in Ghana, where infection rates reached 26.5% in cattle housed in poorly sanitized stables (5). Although regional differences in environmental conditions, vector abundance, and husbandry practices may influence infection prevalence, these findings collectively reinforce the link between inadequate sanitation and an increased risk of Cryptosporidium transmission in livestock environments.

The presence of oocysts in our samples highlights the potential zoonotic threat posed by contaminated cattle sheds, particularly in areas with close human-animal interaction. The morphology of *Cryptosporidium spp.* oocysts identified in this study is shown in Figure 1.

The detection of *Cryptosporidium spp.* oocysts in sheds located less than 10 meters from the farmers' homes and constructed with tile roofs represents a noteworthy finding. Approximately 75% of these infected sheds lacked adequate ventilation and were oriented either east or west. Additionally, 50% featured bamboo wall or floor materials, and manure was managed by direct piling on the ground. These environmental and structural factors may contribute to parasite persistence and increased transmission risk. The chi-square and logistic regression results presented in Table 2 further emphasize the potential influence of shed sanitation on the transmission of *Cryptosporidium spp.*, highlighting the need for additional research to better understand environmental determinants of infection risk.

The chi-square analysis showed no significant association (p > 0.05) between shed sanitation and shed orientation

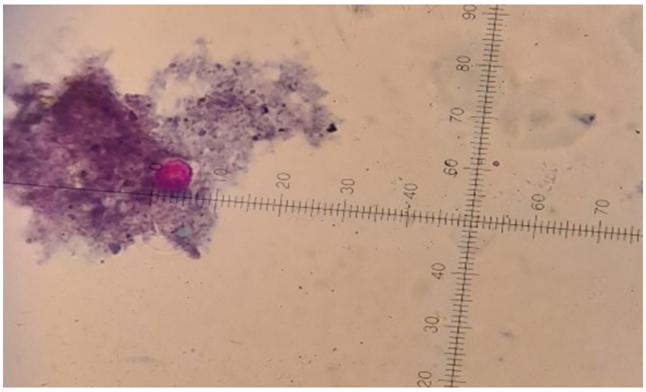

Figure 1: The morphology of Cr. *Cryptosporidium spp*. oocyst from a housefly sample stained using the modified Ziehl-Neelsen (MZN) method and observed under 1000x magnification. The oocyst appears as a bright red, round structure measuring approximately 4–6 µm

or manure handling practices (p = 0.073 and p = 1.000, respectively). However, significant correlations were observed with ventilation (p = 0.042), wall material (p = 0.043), and floor material (p = 0.022). Sheds lacking ventilation were 12.429 times more likely to be infested with *Cryptosporidium spp*. compared to ventilated sheds (95% CI: 1.117–138.238). Conversely, sheds with bamboo walls and bamboo flooring demonstrated lower odds of infestation, with odds ratios of 0.059 (95% CI: 0.005–0.664) and 0.029 (95% CI: 0.002–0.466), respectively. These findings indicate that certain structural materials may act as protective factors against Cryptosporidium contamination. Stronger, more durable shed construction materials may minimize fly harboring and reduce the likelihood of mechanical transmission of *Cryptosporidium spp*.

Logistic regression analysis identified ventilation as the most influential factor associated with *Cryptosporidium spp*. transmission to houseflies. Unventilated sheds demonstrated a significantly higher likelihood of *Cryptosporidium spp*. infestation, with a regression coefficient (B) of 3.129 and an odds ratio (OR) of 22.856 (95% CI: 1.255–416.170). This indicates that the absence of ventilation markedly increases the likelihood of oocyst presence on houseflies. The negative constant value reflects the inverse baseline probability when other variables are held constant, underscoring the complex interaction between structural conditions and parasite transmission dynamics.

DISCUSSION

Cattle shed sanitation is not merely a routine activity, but a critical component of maintaining a healthy and hygienic livestock environment, with direct implications for both animal health and environmental quality (15). According to the Indonesian Ministry of Agriculture Regulation No. 46/2015, key components of shed sanitation include shed location, ventilation, lighting/orientation, manure management, and the materials used for the roof, walls, and floor.

In this study, 75% of sheds were located within 10 meters of the farmers' homes. Such close proximity increases the risk of zoonotic disease transmission from cattle to humans (12). A minimum distance of at least 10 meters is recommended to reduce this risk. Although proximity may facilitate easier livestock monitoring and reduce theft concerns, inadequate separation from residential areas may compromise public health.

Good ventilation was observed in 30 sheds, and sufficient lighting was present in 28. Adequate ventilation supports animal well-being by reducing heat stress, enhancing air circulation, and minimizing foul odors associated with high humidity levels (16). Similarly, proper lighting, particularly in sheds oriented north–south, helps create a more comfortable environment for cattle, reduces stress, and supports overall health and productivity (2).

Cattle shed construction should prioritize strength, safety, durability, and ease of cleaning. In this study, 33 sheds were equipped with tile roofs, which tend to be more durable and easier to maintain compared to asbestos or straw roofing materials. Approximately 90% of shed walls were constructed from bamboo, which, although not as durable as brick, is widely used due to its lower cost and local availability. Similarly, most sheds featured bamboo flooring. While bamboo provides adequate structural support, its porous characteristics make it more difficult to clean than cement flooring, as it can retain dirt and moisture.

Fly control was generally implemented through the use of fly traps placed in areas of high fly activity, such as near manure piles or feed storage zones. This method is considered more environmentally sustainable than insecticide application, which may contribute to insect resistance and pose health risks to humans and other animals. Bamboo was the most commonly used material for fly traps; however, it is prone to rapid contamination and can be difficult to clean, potentially reducing its long-term effectiveness.

This study did not find a significant association between ventilation and fly density, which contrasts with previous findings. Nonetheless, implementing ventilation-based control measures remains advisable due to the overall high fly density observed in the sheds (15). One recommended intervention is the addition of wire mesh to ventilation openings to limit fly entry while maintaining airflow (17). This guidance provides a clear path forward, even in the face of unexpected results. In this study, 60% of the sheds managed manure by piling it directly on the ground, a practice associated with several health risks. Exposed manure can emit strong odors that attract flies (18) and serves as a breeding site for pathogenic microorganisms that may contaminate surrounding water and the environment (19). Prior studies have shown that exposed manure increases the likelihood of high fly density by 3.81 times compared to properly managed manure (10) and poor manure handling has been associated with a 4.7-fold higher risk of elevated fly density (15). Therefore, manure should be stored under waterproof coverings or placed in sealed containers or bags to reduce fly proliferation (20). Additionally, proximity to human dwellings has been linked to higher fly density, further emphasizing the importance of appropriate shed placement (21).

The roof, as the primary surface exposed to solar radiation, plays a critical role in regulating temperature and humidity within livestock housing. In this study, sheds constructed with clay tile roofs, which are known for their heat-dissipating properties, provided a more favorable internal microclimate compared to those with asbestos roofing (22). Roof material, along with the shed's orientation relative to sunlight, are key factors influencing internal temperature and humidity. Although humidity creates suitable conditions for fly breeding, it does not directly affect adult fly activity (21,23,24).

Musca domestica (house flies) were the predominant species observed in the cattle sheds, frequently found on animal bodies, manure, and waste materials on the shed floor. Ventilation emerged as the most influential factor in the transmission of *Cryptosporidium spp.* via houseflies. Poor ventilation reduces air circulation and promotes elevated humidity, which may enhance the survival of oocysts. *Cryptosporidium spp.* oocysts can survive for up to two weeks at 30°C but are inactivated at temperatures above 50–60°C or below –20°C (25). Their survival is further reduced under low humidity and high-temperature conditions (26).

Cryptosporidium spp. is a protozoan parasite that infects the epithelial lining of the digestive tract in both animals and humans via the fecal–oral route (27). Infection occurs when oocysts—the infective form—are transmitted either through direct contact with contaminated feces or indirectly through ingestion of contaminated food or water (28). Water is a critical vehicle for transmission, as oocysts are highly resistant to environmental degradation and conventional water treatment methods. Mechanical vectors such as houseflies can facilitate contamination of food with *Cryptosporidium spp.* oocysts, contributing to indirect transmission (29). Additionally, close proximity of cattle sheds to residential areas (e.g., less than 10 meters) may increase the risk of groundwater contamination through oocyst leaching, particularly in areas with inadequate waste management systems (30). Placing cages less than 10 meters from houses can contaminate water sources by leaching oocysts through the surrounding groundwater (31). Effective prevention strategies include maintaining proper food hygiene and implementing fly control measures such as environmental sanitation, use of fly traps, and insecticide application when appropriate (32). This emphasis on water contamination serves to make the audience feel cautious and vigilant, understanding the potential risks and the need for preventive measures. These findings reflect the One Health perspective, which emphasizes the interconnectedness of human, animal, and environmental health. Cattle are important reservoirs of *Cryptosporidium spp.*, and if manure is not appropriately managed near homes, it becomes easier for the disease to spread from animals to people. Houseflies serve as mechanical vectors, facilitating the transfer of oocysts from contaminated animal environments to food, water sources, and residential areas. This kind of spread is hazardous for more vulnerable people, such as young children and people with weak immune systems, like those living with HIV/AIDS. Therefore, it is important to use different strategies together to reduce the burden of cryptosporidiosis, such as better sanitation, controlling flies, properly getting rid of waste, and community-based health education.

CONCLUSION

This study contributes evidence for early environmental surveillance to prevent cryptosporidiosis outbreaks in communities near livestock production areas. The crucial role of shed wall material, manure handling, and ventilation influences fly density and *Cryptosporidium spp.* transmission to humans as an ectoparasite. Houseflies in and around the sheds can act as mechanical vectors of *Cryptosporidium spp.*, potentially contaminating food and causing foodborne illness. Therefore, it is essential to take precautions to prevent *Cryptosporidium spp.* infection. Prevention strategies include managing flies, improving shed sanitation, and ensuring food and water purity. Notably, practicing good personal hygiene is a powerful tool in this prevention arsenal, empowering individuals to protect themselves from *Cryptosporidium spp.* infection.

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