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Original Article

Fecal ingestion rate based on worker activity patterns during stool handling in a ruminant farm

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Abstract

Farm workers who handle livestock stools face an increased risk of infection by pathogenic bacteria, such as *Escherichia coli* O157 and *Salmonella spp*., leading to millions of severe health issues and thousands of fatalities annually. The aim of this study was to assess the impact of these pathogens by measuring their concentrations, determining rates of unintentional fecal consumption, and conducting a quantitative assessment of microbial risk. An integrated farm in Sukabumi City, Indonesia, was examined for *E. coli* O157 and *Salmonella spp*. in livestock stools. Additionally, the study monitored the rate of incidental fecal ingestion among farm workers. Stool samples were collected (n= 40) from ruminants and analyzed following ISO 16649-1:2018, ISO 9308-1:2014, and ISO 6579-1:2017/Amd.1:2020. The study tracked worker's behavior daily to determine the contact time. The fecal ingestion rate was calculated by multiplying the estimated stool weight ingested by the contact time workers spent cleaning livestock stools in the barn each day. Microbial analysis revealed that the highest concentration of *E. coli* O157 in beef cattle stools was 2.49 $\log_{10}$ CFU/g. The study determined mean fecal ingestion rates during the dry season (8.64 mg/day) and rainy season (6.84 mg/day). Results from the quantitative microbial risk assessment showed that stool from beef cattle posed a higher risk of *E. coli* O157 infection compared to other ruminants, with an estimated disease burden of 9.8×10^{-3} pppy. This study represents the first comprehensive quantitative evaluation of fecal ingestion by farm workers during animal husbandry. The findings underscore the need for improved worker safety measures, such as enhanced sanitation practices and protective equipment, to mitigate the risks of handling livestock stools.

Keywords: Disease burden, estimated stool weight, farm workers, fecal handling, hand-to-mouth frequency

Introduction

Farmers and farm workers are regularly exposed to livestock feces through direct and indirect contact. Activities such as barn maintenance and prolonged handling of manure or natural fertilizer in agriculture can potentially increase fecal contamination of hands [1]. Frequent contact may increase the likelihood of encountering harmful bacteria. Two common pathogens found in agricultural environments are *Escherichia coli* O157 and *Salmonella spp*., both of which pose a risk of gastroenteritis to farmers and farm workers, potentially causing diarrhea and

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abdominal cramps [2,3]. *E. coli* O157 is responsible for 2.8 million severe cases of illness annually, with a prevalence of approximately 43 instances per 100,000 individuals worldwide [4]. *Salmonella spp*. infections result in 150 million cases and 60,000 fatalities globally each year. In Southeast Asia, the reported incidence rate stands at 21–22 instances per 100,000 individuals. [5].

Tropical and developing countries provide favorable conditions for the growth of these pathogens [6,7]. A Vietnamese investigation determined that farmers face a risk of 0.28 diarrheal disease episodes per person yearly [8]. In Indonesia, which has 13 million farming households [9], vulnerability to these bacteria is significant. Livestock stool contains *E. coli* O157 and *Salmonella spp*. [10], and these bacteria are present in farm environments [11] and slaughterhouses [12]. In stool samples collected from central cattle farms across West Java Province, including the areas of Bandung, Cianjur, Sukabumi, and Depok, the proportion of *E. coli* O157 to total *E. coli* was found to be 0.75 (94/126) [13]. In a cattle farming facility located in Subang, West Java Province, *Salmonella spp*. was detected in 10.8% (8/74) of the analyzed stool samples [14].

Livestock fecal samples were collected directly from the rectum or fresh feces on the barn floor. After sampling, *E. coli* and *Salmonella spp*. were subjected to microbial culture by enriching them in selective agar media. This culture method is the conventional technique for identifying bacteria based on their morphological characteristics [15]. If bacterial morphology is consistent, researchers can proceed with further analyses.

Farm workers may become infected by *E. coli* O157 and *Salmonella spp*. present in animal feces [16] through fecal-oral, inhalation, and fomite routes [17], and fecal-oral transmission is particularly significant [18,19]. While studies on fecal ingestion rate among farm workers handling livestock stools are limited, some studies have examined the excreta ingestion rate among farmers in Vietnam [20], with most studies focusing on soil ingestion rates among adult populations in the agricultural sector [21–24], as well as other occupations [25–27].

The ingestion rate is essential, as it, combined with pathogen concentrations, serves as an input for quantitative microbial risk assessment (QMRA) [28,29]. The QMRA model employs a bottom-up methodology to calculate the likelihood of infection and associated disease burden [30]. This study focused on quantifying the fecal ingestion rate of farm workers handling ruminant stool. Incorporating empirical data on pathogen concentrations and fecal ingestion rates into QMRA enhances the accuracy and reliability of risk assessments. The aim of this study was to quantify *E. coli* O157 and *Salmonella spp*. concentrations at an integrated farm in Indonesia, to determine the rate of inadvertent fecal ingestion and to perform a QMRA analysis to evaluate the disease burden experienced by farm workers.

Fecal ingestion rate was determined using various methods. Methods known for their reliability include the tracer method, observation and recording method, chemical marker method, DNA analysis, and site-specific information [31–35]. The observation and recording method used in this study involved monitoring and recording worker behavior according to daily activity patterns. The fecal ingestion rate was estimated by multiplying the weight of the feces by the contact time workers spent handling livestock feces cleaning in the barn each day. Therefore, the accuracy of the number of samples in the laboratory and in the field, the weight of the feces by gravimetry, and statistical data analysis, along with its sensitivity, are required.

Once QMRA is calculated, the risk of disease occurrence among workers can be controlled. A range of preventive strategies can be employed to mitigate the risk of infection with *E. coli* O157 and *Salmonella spp*. These approaches include elimination and substitution, implementation of engineering and administrative controls, and the utilization of standard safety gear. By adopting these measures, the incidence of diseases associated with these pathogens can be reduced effectively.

Methods

Study area and participants

This study analyzed *E. coli* and *Salmonella spp*. in livestock stools (n=40) and observed incidental fecal ingestion rates of workers (n=4) at an integrated teaching factory-based farm housing 50

ruminants in Sukabumi City, Indonesia. Sample collection occurred during both the rainy and dry seasons, spanning from 2023 to the middle of 2024. A QMRA framework incorporating pathogen concentration, fecal ingestion rate, and distribution parameters (**Table 1**) was used to estimate the disease burden.

Table 1. Input parameters with distributions and statistics in QMRA calculations

Model parameter	Unit	Probability distribution function and parameter statistics		Reference
		Distribution	Value	
Fecal manure ingestion rate ($Fecal_{IR}$)				
$Fecal_{IR}$	mg/day	-	-	This research
Estimation of weight on stool at worker's face (EWS)	mg	-	-	This research
Frequency of workers working with ruminant stool (FWS)	times/hour	Weibull	-	This research
Duration of working with stool as recorded by camcorder (DCC)	hour/day	Weibull	-	This research
Concentration of pathogens				
Pathogenic fraction to EHEC O157	-	Point, ratio *E. coli* O157 to *E. coli* in Asiatic region	0.0455	Median of studies from Indonesia: Suardana *et al.* (2017) and Ferasyi *et al.* (2019)
Concentration of *Salmonella spp.* in stool	MPN/g	Log-normal	-	This research
Beta-poisson dose-response				
Parameter *E. coli* O157 (α, N_{50})	-	Point	α= 0.4, N_{50}= 207	Haas *et al.* 2014
Parameter *Salmonella spp.* (α, N_{50})	-	Point	α= 0.31, N_{50}= 23,600	Haas *et al.* 2014
Days of exposure	n days	Point, based on the calculation of farmers working days in Indonesia	275	Liem *et al.* 2021
Risk characterization				
Pill|inf	-	Point, illness to infection	*E. coli* O157 = 0.4 *Salmonella spp.* = 0.2	USEPA 2015
Severity weight for gastroenteritis	-	Point, consist of: mild, moderate, severe, fatal	0.07, 0.39, 0.39, 1	Haas *et al.* 2009, Katukiza *et al.* 2013
Frequency	-	Point, consist of: mild, moderate, severe, fatal	*E. coli* O157 = 0.94, 0.05, 0.01, 0.0002 *Salmonella spp.* = 0.94, 0.06, 0.009, 0.0001	Haas *et al.* 2009, Katukiza *et al.* 2013
Duration of illness	years	Point, consist of: mild, moderate, severe, fatal	*E. coli* O157 = 0.015, 0.029, 0.044, 54 *Salmonella spp.* = 0.015, 0.029, 0.044, 52	Haas *et al.* 2009, Katukiza *et al.* 2013

Procedures for the collection and analysis of microbial data from samples

Microbial sample collection

Stool samples were collected from animal housing facilities following the animal research: Reporting of in vivo experiments (ARRIVE) guidelines [36]. The sample size was determined using a proportion calculator based on the prevalence of *Salmonella spp*. (8.01%) [37] and *E. coli* (60%) [38] in ruminant stools in Asia. A French study achieved a 90% confidence interval (CI) with 10% precision [7]. A total of 40 samples were collected for *E. coli* and *Salmonella spp*. analyses, with specific quantities for each livestock type, are presented in **Table 2**. The samples were aseptically collected in sterile vials (JVLAB, Hong Kong) between November and December 2023, transported under cold conditions, and refrigerated for processing within 24 hours.

Table 2. Specific quantities for each animal type for sampling

Sample source	Quantity of sampling	Details
Stool of dairy cattle	10	Cattle stools representing their respective ages were taken during sampling for each species: • 3–8 months (n=5) • 18–24 months (n=5)
Stool of beef cattle	10	
Stool of goat	10	Small ruminant stools representing their respective ages were taken during sampling for each species: • 0–3 months (n=2) • 3–7 months (n=2) • 7–12 months (n=2) • 12–60 months (n=2) • Above five years (n=2)
Stool of sheep	10	
Total	40	

Isolation and detection of Escherichia coli and Salmonella spp.

E. coli was isolated and detected from samples using an adapted version of the ISO 16649-1:2018 (Microbiology of the food chain — Horizontal method for the enumeration of beta-glucuronidase-positive *E. coli*) and ISO 9308-1:2014 (Water quality — Enumeration of *E. coli* and coliform bacteria). Fecal samples were cultured on selective MacConkey agar and incubated. Presumptive *E. coli* colonies were confirmed by Kovács indole reagents (Merck, Germany).

Salmonella spp. isolation and identification followed ISO 6579-1:2017/Amd.1:2020 (Microbiology of the food chain — Horizontal method for the detection, enumeration, and serotyping of Salmonella — Part 1: Detection of *Salmonella spp*.) [42], with modifications. Samples were first enriched with Buffered Peptone Water (Merck, Germany) and then incubated at 37°C for a period of 18–24 hours. Subsequently, they were further enriched in Rappaport Vassiliadis Soya broth and Tetrathionate broth (Merck, Germany) at 42°C for 24 hours. The samples were cultured on Bismuth Sulfite Agar and Xylose Lysine Deoxycholate agar (HiMedia, India). Samples with *Salmonella* characteristic morphologies were quantified using the most probable number (MPN). Biochemical confirmation involved incubation in Lysine Iron agar (Merck, Germany) and Triple Sugar Iron agar (Merck, Germany) at 37°C for 24 hours.

Procedure for collecting and analyzing data on incidental fecal ingestion rate

Estimated weight of stool on the worker's face

This study employed methods from Vietnam [20] to estimate stool weight transferred to the mouth of workers. Laboratory and field simulations measured stool residue on the hands of workers post-contact and estimated stool transfer to the facial area. The data collection and analysis steps used to estimate the incidental fecal ingestion rates are presented in **Figure 1**.

Environmental factors, including temperature and rainfall, affect the moisture content of livestock feces and worker behavior during the measurement of fecal ingestion rate in two seasons. In the rainy season, the moisture content in ruminant feces increases to 80% or more, while in the dry season, the moisture content in ruminant feces ranges from 70–75% [43]. Adjustments were made to the weight of the flour to resemble the weight of livestock feces in both seasons using a moisture analyzer (Halogen JS110-1T, Starpack Indonesia).

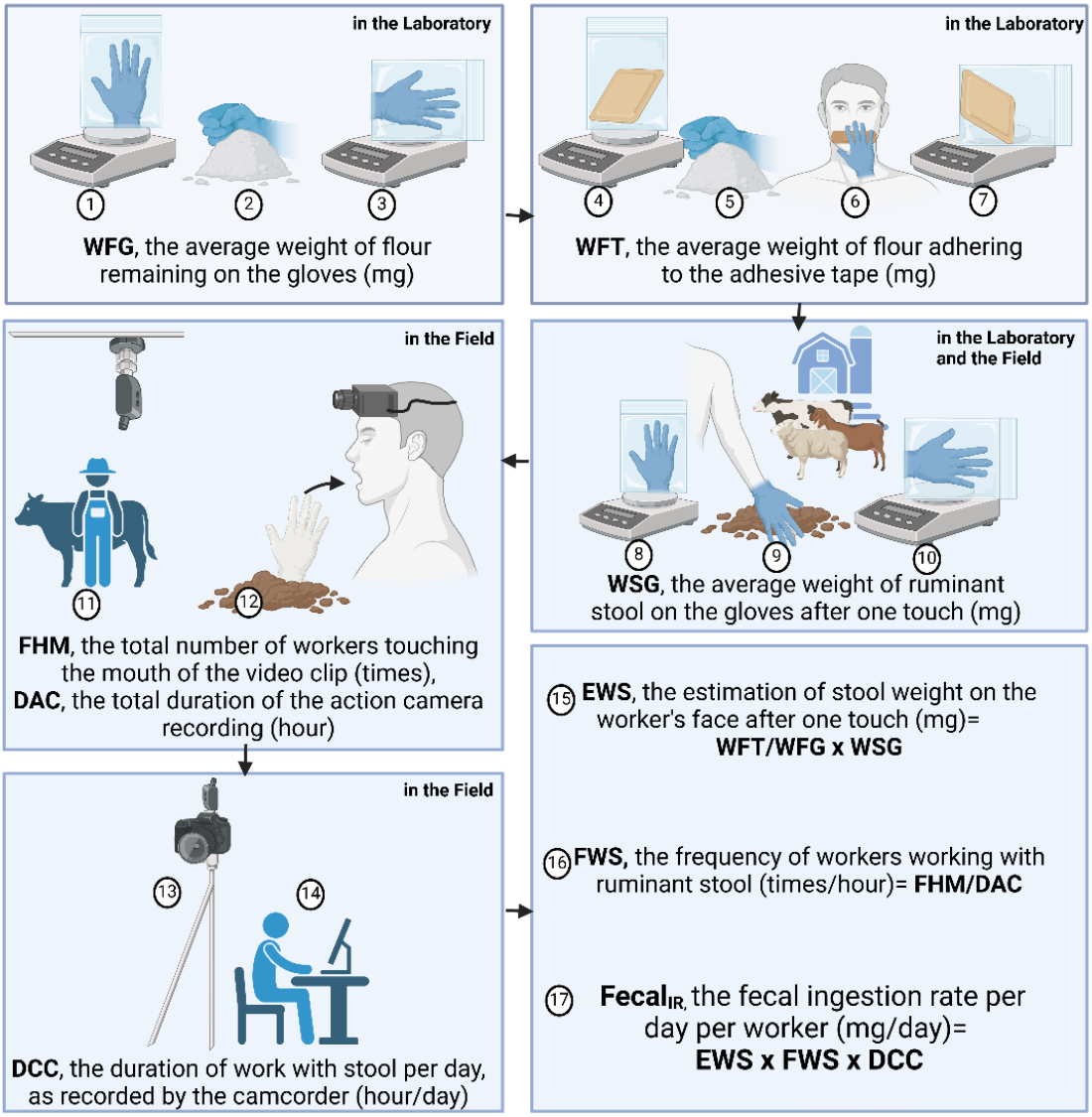

Figure 1. Steps for data collection and analysis of incidental fecal ingestion rates. The bold text represents the variables calculated to obtain $Fecal_{IR}$ values. Created in BioRender.

Laboratory and field simulations were performed to measure the stool residue on the hands after contact. The study site comprised four workers, all of whom, along with two volunteers, were recruited for the field study. In the laboratory setting, six volunteers were enlisted to correspond with the number of participants in the field study. In the laboratory, latex gloves were weighed using a regularly calibrated mass balance (PAJ1003CN OHAUS 1000 mg precision, OHAUS Corp. USA) after being placed in Ziplock® bags (Bagus, Indonesia). Participants wore latex gloves in contact with moistened flour (Segitiga Biru Bogasari Flour Mills, Indonesia) to simulate ruminant stools and gently clapped their hands to remove excess flour. The gloves were weighed to determine flour residue (WFG). In the field, participants wore pre-weighed gloves, contacted ruminant stool, and dried the gloves at room temperature (25°C) for two weeks before re-weighing to measure stool residue (WSG). For precision, the gravimetric method was applied as a reference, and all the weight measurements were repeated three times.

The weight of ruminant stool transferred to the worker's mouth (WFT) was simulated by applying flour to the face. Participants wore gloves, touched flour, and wore their mouths. Flour was removed using Nexcare® dermal adhesive tape (20 × 10 cm) and weighed to measure the adhered flour. Estimation of weight on stool at worker's face (EWS) may be overestimated, assuming that all flours are fully converted into ingested livestock stool.

The formula for estimating stool weight transferred to a worker's mouth after a single contact is as follows (Eq.1) [20]: $EWS\ (mg) = \left(\frac{WFT\ (mg)}{WFG\ (mg)}\right)\ x\ WSG(mg)$ (Eq.1), where EWS represents the estimated stool weight on the face of the worker after one contact (mg), WFT is the average flour weight on the adhesive tape (mg), WFG is the average flour weight on the gloves (mg), and WSG is the mean weight of ruminant stool on the gloves after a single contact (mg).

Calculation of the frequency and duration of fecal touching by workers

All four workers provided informed consent for confidential video recording for research purposes. All four workers or participants were instructed to perform the tasks normally. The researchers recorded the standard safety gear and frequency of direct hand-to-mouth (FHM) contact after touching ruminant stools, excluding actions such as mask use, elbow touches, or shirt wiping. The micro activity videography method based on previous studies [44,45] used 24-hour CCTVs (PTZ 5, Dahua Corp.) in ruminant areas to analyze worker activities over five working days during both the rainy and dry seasons. Each worker was recorded for 3–4 hours/day with a head-mounted action camera (DJI Osmo Action 4, DJI Corp.), focusing on cleaning cattle and small ruminant barns. Footage was synchronized using a timecode generator (TC-1, Deity Microphones). Two researchers monitored the action camera (DAC) and used a camcorder (FDR-AX700, Sony Corp.) to quantify the daily contact with ruminant stool (DCC). The duration of each activity was calculated manually and averaged daily.

The worker stool handling frequency was calculated using Eq. 2 [20]: $FWS\ \left(\frac{times}{hour}\right) = \frac{FHM(times)}{DAC\ (hour)}$ (Eq.2), where frequency of workers working with ruminant stool FWS represents the frequency of workers handling ruminant stool (times/hour), FHM denotes the total instances of workers touching their mouth in video clips (times), and DAC is the total duration of action camera recordings (hours).

The daily fecal ingestion rate of each worker (mg/day) was calculated using Eq.3 [20]: $Fecal_{IR}\left(\frac{mg}{day}\right) = EWS(mg)\ x\ FWS\ \left(\frac{times}{hour}\right) x\ DCC\ \left(\frac{hour}{day}\right)$ (Eq.3), where $Fecal_{IR}$ represents the fecal ingestion rate per worker (mg/day), determined by the approximated mass of fecal matter in the worker's facial area following a single contact (EWS, mg), frequency of contact with ruminant stool (FWS, times/hour), and duration of stool-related work per day recorded by the camcorder (DCC, hour/day).

QMRA framework

The QMRA *model*, modified from Haas *et al.* [28], assessed the risk of *E. coli* O157 and *Salmonella spp.* infection in workers exposed to gastrointestinal pathogens while handling livestock stools. Haas *et al.* (2014) [28] presented a dose–response model, while Sano *et al.* (2019) [29] provided risk characterization. A Monte Carlo simulation comprising 10,000 iterations was used to assess the exposure and risk [46]. The maximum permissible and average calculated disease burdens per person per year (pppy) were established in accordance with World Health Organization (WHO) guidelines [14,16]. The assessment of disease burden utilized disability-adjusted life-years (DALYs), which consist of the sum of Years of Life Lost (YLL) and Years Lived with Disability (YLD) [47].

Statistic and sensitivity analysis

Statistical analyses were conducted using GraphPad Prism software version 10.0.0 (GraphPad Software, LLC, CA, USA). One-way analysis of variance (ANOVA) (p<0.05) was used to assess variations in pathogen levels across fecal samples from different ruminant livestock species. The bootstrap technique [20,21], replicated 1,000 times, was used to calculate the mean WFT and EWS weights and their corresponding 95% CI. To compare WSG and WFG sample weights, student's t-test (p<0.05) was conducted. A two-tailed independent parametric t-test (p<0.05) was applied to examine seasonal differences in the $Fecal_{IR}$ values between the rainy and dry seasons. The sensitivity of the ingestion rate equation was evaluated using Spearman's correlation coefficient, which identifies significant variables [17]. Oracle Crystal Ball v.11.1.4716 (Oracle Corp., Texas, USA) was used to generate probability density functions, conduct a sensitivity

analysis of the $Fecal_{IR}$ equation, and perform Monte Carlo simulations of the QMRA parameter distribution.

Results

Prevalence of *E. coli* O157 and *Salmonella spp.* in stool samples

E. coli and *Salmonella spp.* were identified using culture methods, namely, the streak plate method for isolating pure colonies, followed by the pour plate and spread plate methods to count viable bacteria. Additionally, the liquid culture technique was used to store both bacteria for further analysis. The concentration of *E. coli* O157 was estimated to be 4.55% [48,49] of the total *E. coli* concentration after the enumeration process was completed.

According to **Figure 2A**, *E. coli* O157 levels differed among the various types of livestock stool samples. The concentrations in stool samples from dairy and beef cattle varied from 1.61–3.07 log_{10} CFU/g. In goat stool samples, concentrations ranged from 1.61 to 2.21 log_{10} CFU/g, while in sheep stool samples, they varied from 1.61 to 3.01 log_{10} CFU/g. Beef cattle stool exhibited the highest mean *E. coli* O157 concentration (2.49; 95%CI: 2.28–2.7 log_{10} CFU/g), whereas goat feces showed the lowest (0.43; 95%CI: 0.01–0.85 log_{10} CFU/g). Statistical evaluation revealed substantial variations in stool samples among the different livestock species. In particular, the results indicated significant disparities between goats and dairy and beef cattle, with *p*-values less than 0.001 for each comparison. *Salmonella spp.* was detected only in goat stool samples, with concentrations ranging from 1.15–2.08 log_{10} MPN/g (**Figure 2B**).

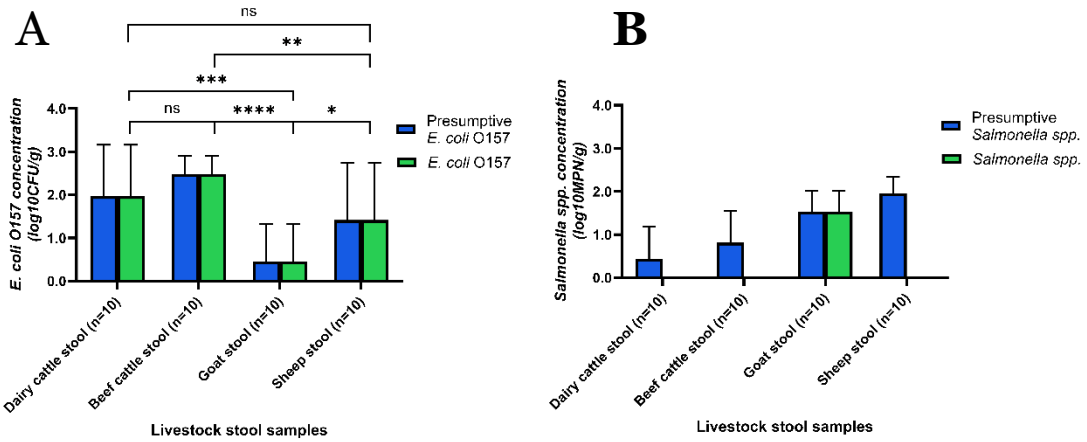

Figure 2. *E. coli O157* and *Salmonella spp.* concentrations in livestock stools. A) *E. coli* O157 concentration in each ruminant species based on rainy season sampling results. The graph displays the data for 40 ruminant fecal samples along the horizontal axis, with the vertical axis indicating *E. coli* O157 concentrations in log_{10} CFU/g. Two distinct colors were utilized to differentiate between the bacterial levels: blue represents the presumptive *E. coli* O157 concentration, and green denotes the confirmed *E. coli* O157 concentrations. Significant differences were observed among goat-sheep stool, beef cattle-sheep stool (one-way ANOVA; */** $p<0.05$), dairy cattle-goat stool, and beef cattle-goat stool (one-way ANOVA; ***/**** $p<0.001$), whereas other comparisons were not significant (ns). B) *Salmonella spp.* was detected solely in goat stools during the rainy season. The horizontal axis of the graph displays the 40 stool samples, with blue indicating presumptive *Salmonella spp.* levels and green showing confirmed *Salmonella spp.* concentrations. The vertical axis represents the log_{10} MPN/g concentration.

$Fecal_{IR}$ estimation

Fecal ingestion rates ($Fecal_{IR}$) were estimated by combining observational data, empirical measurements, and mathematical modeling. This process involves mimicking the remaining stool in the mouth by weighing the flour after a single touch, followed by recording behaviors, such as hand-to-mouth or hand-to-face contact, which results in fecal ingestion. Behaviors were recorded using various approaches, such as comprehensive interviews and analysis of recorded footage (from CCTV, camcorders, and head-mounted action cameras). Workers exhibited varied

activity patterns (**Figure 3**). However, on average, barn cleaning constituted the largest proportion (34.5%), followed by feed preparation (21.75%). Livestock care accounted for 18% of the activities, whilst feed distribution and environmental cleaning comprised 14.5% and 8.25%, respectively. The weight of stool remaining in the facial area of the workers during these activities was measured (Eq. 1), and the frequency and duration of these contacts were calculated (Eq. 2). The ingestion rate was determined by multiplying the stool weight in the facial area of the workers by the exposure frequency and duration adjusted for seasonal variations (Eq. 3).

The mean mass of wheat flour on gloves after contact with wheat flour/WFG (**Table 3**) was 9.41 mg (95%CI: 9.39; 9.43) in the rainy season and 0.44 mg (95%CI: 0.36; 0.52) in the dry season. After a single contact, the amount of wheat flour remaining on the face/WFT measured 0.29 mg (95%CI: 0.29; 0.3) during the rainy season and 0.05 mg (95%CI: 0.04; 0.05) in the dry season. Cattle stool on worker gloves/WSG was quantified for both seasons: 12.76 mg (95%CI: 12.55; 12.97) in the rainy season and 0.53 mg (95%CI: 0.47; 0.59) in the dry season. After contact, EWS was 0.31 mg (95%CI: 0.3; 0.32) in the rainy season and 0.10 mg (95%CI: 0.07; 0.13) in the dry season.

Table 3. Estimation of stool weight on workers' faces in the ruminant area during two seasons

Material	n	Parameter probability density functions	Mean (mg) (95% CI)	SD	Min	Max	*p*-value
Rainy season							
WFG[a]	24	Beta (0; 18.86; 100; 100)[1]	9.41 (9.39; 9.43)	0.64	7.55	11.24	$p<0.05$[3]
WSG[a]	24	Beta (3.37; 29.82; 0.84; 1.52)[1]	12.76 (12.55; 12.97)	6.89	3.37	29.69	
WFT[a]	18	Beta (0; 0.58; 100; 100)[1]	0.29 (0.29; 0.3)	**0.02**	0.22	0.36	$p<0.05$[2]
EWS[a]	24		0.31 (0.3; 0.32)	0.2	0.08	0.96	$p<0.05$[2]
Dry season							
WFG[a]	36	Beta (0.07; 1.51; 0.3; 0.91)[1]	0.44 (0.36; 0.52)	0.43	0.07	1.5	$p<0.05$[3]
WSG[a]	36	Beta (0.05; 1.3; 0.49; 0.78)[1]	0.53 (0.47; 0.59)	0.4	0.05	1.3	
WFT[a]	18	Beta (0.02; 0.11; 1.13; 2.55)[1]	0.05 (0.04, 0.05)	0.02	0.02	0.11	$p<0.05$[2]
EWS[a]	36		0.10 (0.07; 0.13)	0.21	0	1.89	$p<0.05$[2]

[1]Continuous Beta distribution. The numbers in parentheses represent the four parameters: min., max., α value, and β value
[2]95% confidence interval after the mean weight was calculated based on 1,000 bootstrap samples
[3]Statistical analysis using t-test (student t-test) to compare WSG and WFT
[a]WFG is flour in gloves, WSG is stools in hand, WFT is flour in face, and EWS is estimation of stool in face

Furthermore, video clips recorded from the ruminant area showed that the mean duration of incidental hand-to-mouth and face-area contact per day during the rainy season was 1 hour 49 minutes (95%CI: 1 hour 46 minutes to 1 hour 51 minutes), representing approximately 26.71% of the total working time (**Table 3**). During the dry season, the mean duration of contact increased to 3 hours 3 minutes (95%CI: 2 hours 59 minutes to 3 hours 7 minutes), or approximately 44.85% of the total working time. Individuals touched their mouth and face an average of 12.4 and 28.2 times following incidental fecal contact during the rainy and dry seasons, respectively. Thus, the ingestion rate value in the ruminant area based on Eq.3 was 6.84 mg/day (95%CI: 6.52; 7.16) during the rainy season and 8.64 mg/day (95%CI: 7.52; 9.76) during the dry season, respectively.

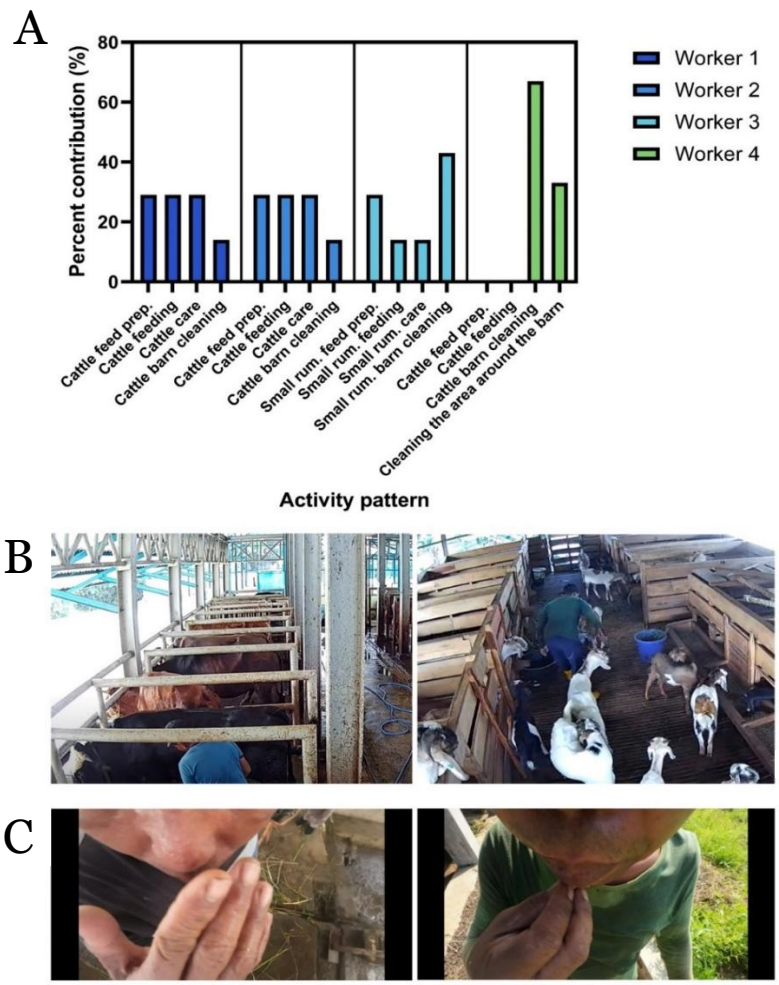

Figure 3. Worker activity patterns in the ruminant farm. A) Percentage contribution (%) of daily tasks performed by the workers. Each color represents the different activities of each worker. The ruminant farm employs four workers with specific responsibilities in each barn: Workers 1 and 2 are tasked with cattle husbandry, worker 3 is responsible for goat and sheep husbandry, and worker 4 helps the other three workers in the removal of ruminant manure. Workers had an average work duration of 6.8 hours per day, 28 days a month, and 11 months a year. B) Images captured by closed-circuit television (CCTV) cameras in the cattle barn (left image) and goat barn (right image). C) Action camera images depicting a worker in the cattle barn accidentally touching their mouth with their hands (left image), followed by a worker in the goat barn accidentally touching their mouth with their hands (right image).

$Fecal_{IR}$ between two seasons and sensitivity analysis of $Fecal_{IR}$

A comparison of $Fecal_{IR}$ values revealed no significant differences between the dry and rainy seasons (**Table 4**). The sensitivity of each parameter that constructs the ingestion rate equation in the ruminant area during the two seasons is presented in **Figure 4**. Sensitivity analysis revealed that in both seasons, $Fecal_{IR}$ in stools discovered on worker gloves (WSG) had a substantial negative impact (-0.63). In contrast, the incidence of hand-to-mouth contact after stool handling (FHM) (0.39) and the duration of hand-to-mouth contact (DCC) (0.37) had a lesser effect on $Fecal_{IR}$.

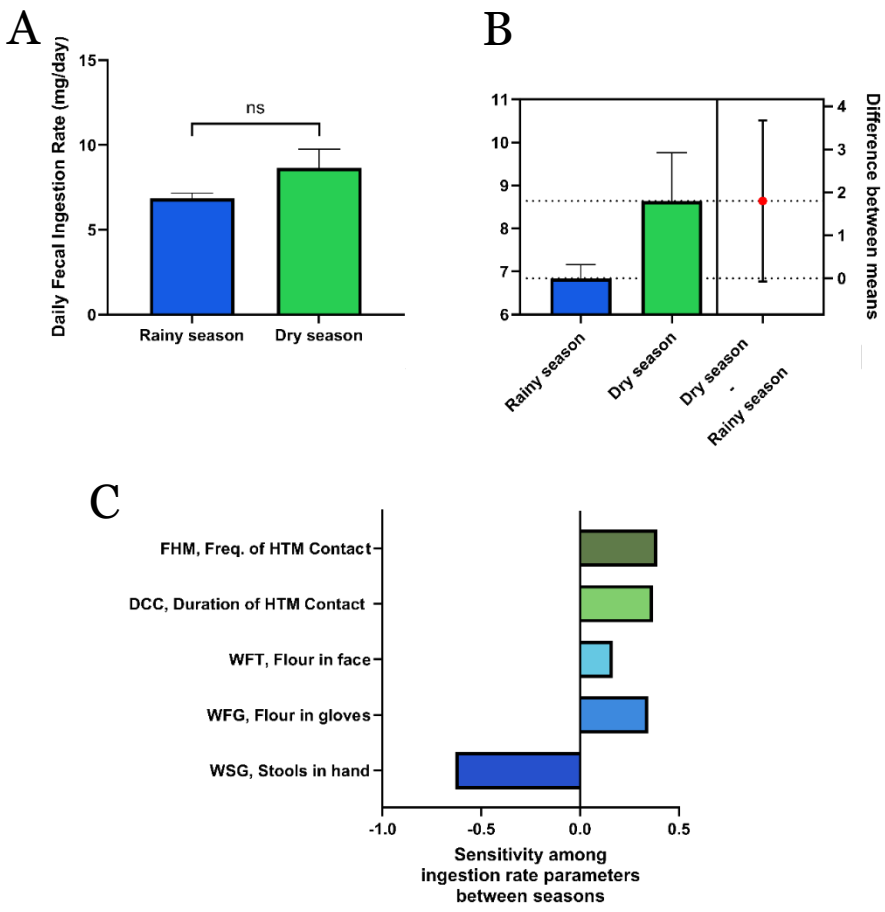

Figure 4. Fecal ingestion rate and its sensitivity test. A) The fecal ingestion rate between the rainy (indicated by blue) and dry seasons (indicated by green) did not differ significantly (not significant, ns) based on the unpaired t-test. B) There were no statistically significant differences in the means of $Fecal_{IR}$ between the rainy (blue) and dry (green) seasons. C) Sensitivity of fecal ingestion rate between the dry and rainy seasons, consisting of frequency of HTM Contact/FHM (0.39), duration of HTM Contact/DCC (0.37), flour in face/WFT (0.16), flour in gloves/WFG (0.34), and stools in hand/WSG (-0.63). Each color distinguishes between variables.

Table 4. Duration and frequency of hand-to-mouth contact after handling stool, and daily ingestion rate in the ruminant area for 6.8 working hours

Parameter	Distribution function (Location, Scale, Shape)	Mean (95% CI), after bootstrap 1,000 times	Standard Deviation (SD)	Min	Max
Rainy season					
DCC, (hours per day)*	Weibull (-1; 3; 3.67)	1.81 (1,77; 1,85)	0.73	0.12	3.6
FHM, (times/day)*	Weibull (-12; 63; 3.46)	12.4 (12.13; 12.67)	5.2	4	29
$Fecal_{IR}$, (mg/day)*		6.84 (6.52; 7.16)	6.22	1.26	42.6
Dry season					
DCC, (hours per day)*	Weibull (0; 4; 2.45)	3.05 (2.98; 3.12)	1.44	0.52	7.8
FHM, (times/day)*	Weibull (-13; 124; 3.1)	28.2 (27.5; 28.9)	13.5	0	100.4

Parameter	Distribution function (Location, Scale, Shape)	Mean (95% CI), after bootstrap 1,000 times	Standard Deviation (SD)	Min	Max
$Fecal_{IR}$, (mg/day)*		8.64 (7.52; 9.76)	21.53	0.23	233.2
$Fecal_{IR}$ difference between seasons**	ns				

*DCC: duration of hand-to-mouth contact (the daily duration of ruminant workers touching livestock stool is 4 hours of data collection divided into 2 sessions); $Fecal_{IR}$: daily fecal ingestion rate; FHM: the rate at which hands encounter the mouth after handling stool.
**Two-tailed independent parametric t-test ($p>0.05$, ns is considered as not significant)

DALYs of *E. coli* O157 and *Salmonella spp.* to livestock workers

According to the assessment of the yearly *E. coli* O157 infection risk (**Figure 5A**), stool from beef cattle exhibited the most significant hazard, with an annual infection likelihood of 2.41%. Workers exhibited a 2.17% risk of *Salmonella spp.* infection from goat stools (**Figure 5B**), which was approximately equivalent to the annual risk of *E. coli* O157 infection. The median DALYs value for *E. coli* O157 and *Salmonella spp* is presented in **Figures 5C & 5D**. The concentrations of *E. coli* O157 in livestock stools collected during the rainy season and $Fecal_{IR}$ measurements revealed varying levels of DALYs in the different livestock. The median DALYs values for *E. coli* O157, arranged from highest to lowest, were as follows: 1) beef cattle stool (9.8×10^{-3} pppy), 2) dairy cattle stool (5.9×10^{-3} pppy), 3) sheep stool (3.9×10^{-3} pppy), and 4) goat stool (7.1×10^{-4} pppy). For *Salmonella spp.*, the median DALYs value was calculated only from goat stool, with a value of 5.6×10^{-5} pppy.

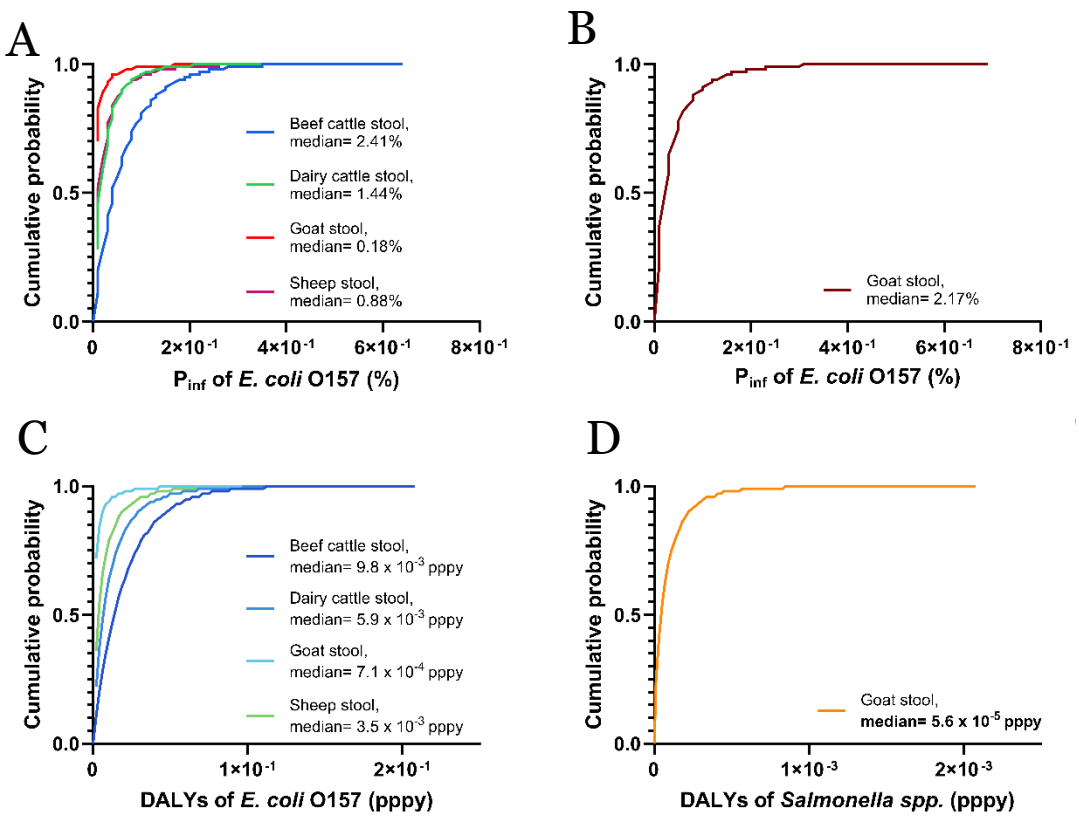

Figure 5. The risk of infection from *E. coli* O157 and *Salmonella spp.* pathogens in livestock stools, along with the associated Disability-Adjusted Life Years (DALYs). A) Infection risk values of *E. coli* O157 from different ruminant stools are presented. The x-axis represents the infection risk values in percentage (%), whereas the y-axis shows the cumulative probability (0–1) of *E. coli* O157 from each ruminant species. The graph uses colors to differentiate between species, with

the median infection risk values for each species indicated. B) The graph displays the infection risk percentages of *Salmonella spp*. in various ruminant stools. The x-axis indicates the infection risk in percentage, whereas the y-axis represents the cumulative probability (0–1) of the presence of *Salmonella* spp. in each ruminant species. C) The DALYs values for *E. coli* O157 in various ruminant stools. The horizontal axis shows DALYs in pppy, whereas the vertical axis depicts the cumulative probability (0–1) of *E. coli* O157 presence across different ruminant species. Colors were used to distinguish between species, with the median DALY values highlighted. D) The *Salmonella spp*. DALYs in goat stools, where the horizontal axis represents DALYs in pppy, and the vertical axis shows the cumulative probability from 0 to 1. Orange was used to indicate the DALYs values, and the median value was clearly marked on the graph.

Risk reduction interventions for *E. coli* O157 and *Salmonella spp*. exposure among farm workers can involve integrating occupational health safety and Water, Sanitation, and Hygiene (WASH) measures according to control measures (**Figure 6**), such as elimination, substitution, engineering controls and sanitation, administrative controls, the use of safety equipment gear, and hygiene practices. Compliance with occupational health and safety standards on the farm should also be enforced by the head of the barn, including monitoring the use of safety equipment.

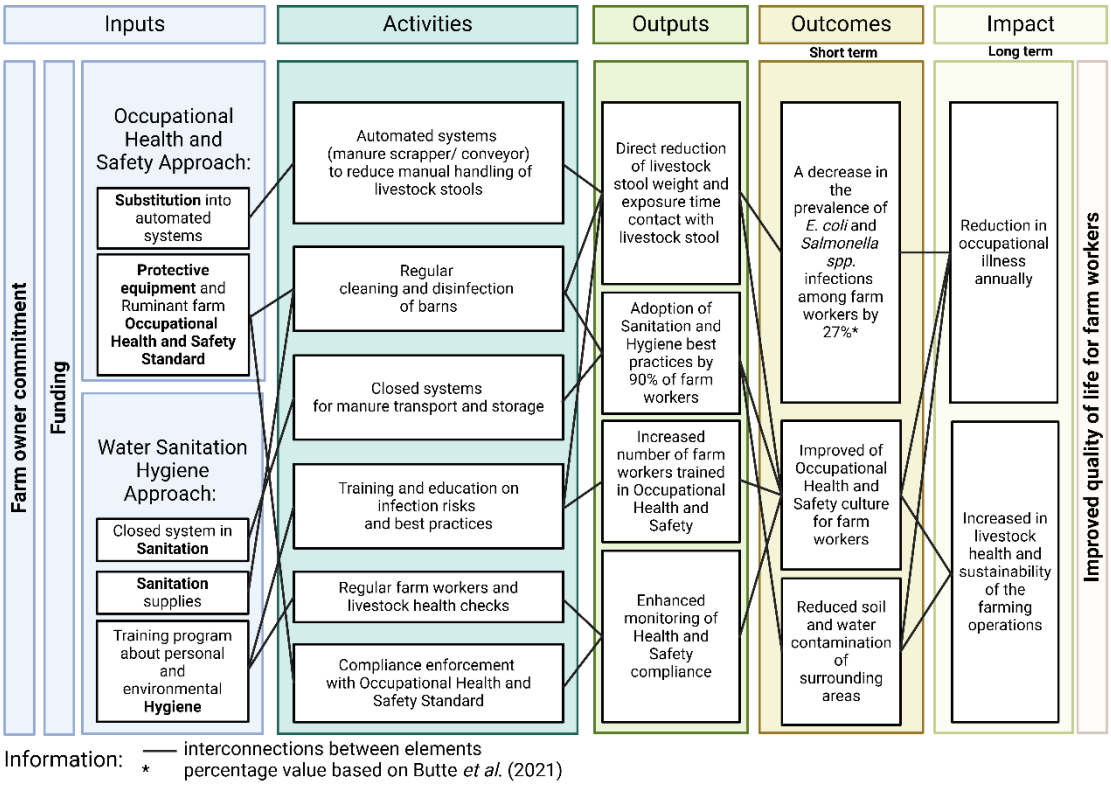

Figure 6. Proposed intervention strategy that integrates occupational health and safety (OHS) protocols with Water, Sanitation, and Hygiene (WASH) initiatives. This plan seeks to attenuate the incidence of pathogenic infections caused by *E. coli* and *Salmonella spp*. among farm workers. This comprehensive approach addresses the potential health risks associated with bacterial exposure in farming environments, with both short- and long-term impacts. Different colors were used to distinguish inputs, activities, outputs, outcomes, and impacts, whereas black lines connected the elements.

Discussion

In this study, it was found that *E. coli* O157 made up 4.55% of the total *E. coli* population in the stool samples collected from livestock on an integrated farm. Among the different animal groups studied, fecal samples from beef cattle showed the highest levels of *E. coli*. Cattle typically have higher concentrations of *E. coli* compared to other livestock [50], which can be attributed to several factors. Cattle raised in intensive farming systems are commonly fed high-grain concentrates and agricultural byproducts. These concentrates are rich in energy and nutrients, promoting faster cattle fattening. Concentrates are more efficient than forage, requiring less feed

for the same weight gain. Additionally, readily available and cost-effective agricultural by-products can lower production costs and optimize resource utilization. Cattle have rumens that efficiently digest high-fiber forage [51]. However, high-grain concentrates can alter the gut environment, making it more conducive to *E. coli* growth. Specific conditions in the cattle gut, such as a low pH of approximately 5.5 [52] and changes in microbial composition, can also enhance the growth of *E. coli*, particularly at the recto-anal junction [53], where *E. coli* O157 colonizes its host. High-grain concentrates and agricultural by-products can increase the population of starch-fermenting bacteria, such as *Streptococcus bovis* and *Lactobacillus spp*. [52], both of which produce lactic acid and lower the gut pH. Conversely, the population of fiber-digesting bacteria, such as *Ruminococcus spp*. and *Fibrobacter succinogenes* [52], tends to decrease. Other microbiota, such as protozoa and archaea, are also disrupted because of their sensitivity to pH changes [43,54].

In this study, *Salmonella spp*. was detected only in goat stool samples. Even when intensively reared, goats tend to consume everything because of their more active behavior [55], unlike other ruminant livestock. Goats also differ from other ruminants in their intestines because they produce higher levels of ammonia gas, especially when the feed provided is high in grain, and the surrounding environmental conditions are inadequate for growth [56]. Elevated levels of ammonia gas can affect microbial community composition and decrease the generation of volatile fatty acids (VFA). As the main energy source for ruminants, VFA are essential and play vital roles in numerous metabolic functions [57]. Disrupted microbes include *Ruminobacter amylophilus, Prevotella ruminicola, Selenomonas ruminantium, Butyrivibrio fibrisolvens,* and *Fibrobacter succinogenes* [58,59]. These microbes play a role in protein degradation, cellulose fermentation, fiber fermentation, and VFA production. The behavior of goats and the presence of higher ammonia concentrations trigger their exposure to *Salmonella spp*. Moreover, *Salmonella spp*. comprises various serovars; for instance, *S. dublin* predominantly infects cattle, *S. abortusovis* primarily infects sheep, *S. bareilly* commonly infects goats, and *S. typhimurium* can infect mammals, including humans [60]. In the present study, the serovars infecting goats in the integrated farming area might have been distinct from those infecting cattle and sheep. Therefore, further studies involving serotyping are required.

The statistical analysis of fecal ingestion rates employed several methods: 1) parameter probability density functions, including a beta distribution for estimating stool on the mouth, a Weibull distribution for the duration of hand-to-mouth contact, and the frequency of hand-to-mouth contact after handling stool; 2) the bootstrap method to calculate mean weights of flour on adhesive tape and estimated stool on the mouth with 95% CI through repeated resampling; 3) a Student's t-test ($p<0.05$) to compare the average weights of flour and ruminant stool on gloves after one touch sample to determine significant differences between the two groups; and 4) a two-tailed independent parametric t-test ($p<0.05$) to examine seasonal differences in $Fecal_{IR}$ values between rainy and dry seasons, assessing differences in both directions.

Spearman's correlation coefficient assessed the sensitivity of the ingestion rate equation and identified significant variables, evaluating the association's strength and direction between two ranked variables. The negative value for the average weight of ruminant stool on gloves after one touch (-0.63) suggests that as manure on hands increases, the fecal ingestion rate decreases, possibly due to workers exercising greater caution or washing their hands more frequently. Other variables, such as the frequency and duration of contact, also influenced fecal ingestion rates, albeit less significantly. This study accurately estimated fecal ingestion rates and identified the most influential factors by combining observational data with rigorous statistical and sensitivity analyses.

The QMRA in this study considered both the hazard from *E. coli* and *Salmonella spp*. concentrations in ruminant stools and the level of fecal ingestion by workers. Sensitivity analysis showed that the quantity of manure adhering to the hands of workers had a more significant impact on fecal ingestion than the frequency or length of hand-to-mouth contact. This is because ruminant manure is dense and moist, making it more likely to stick to hands and harder to clean. Both fresh and old cattle manure, present particular challenges. This high moisture level makes manure fluid and sticky, so it easily adheres to surfaces, such as hands, tools, and equipment. Sticky manure forms a thin layer that clings to the surface, making it difficult to remove. Cleaning

wet manure is more demanding than cleaning dry manure and often requires water and cleaning agents, which can be time-consuming and labor-intensive.

This study represents the first comparative analysis of incidental $Fecal_{IR}$ values derived from livestock activity across different commodities. Previous research has predominantly focused on soil ingestion rates among farmers in the United States [22,24], adults in Canada [26], adults in the United States [25], and human excreta ingestion rates among workers in Northern Vietnam [20]. Both prior studies and the US EPA (2017) standards have demonstrated varying ingestion rates in comparison with the present investigation. The ingestion rate of livestock fecal material is also higher than that of other materials such as human excreta [20] and soil [26]. Ruminants have a digestive system that allows them to process large amounts of fibrous plant material, resulting in significant stool quantities. The multi-chambered stomachs of ruminants facilitate the breakdown of plant material through microbial fermentation, ultimately producing large amounts of stool. A single cow can produce approximately 60 kg of stool per day [61], whereas an average adult human produces approximately 400–500 grams of stool per day [62]. This means that more material can be ingested when handling ruminant livestock stools through contaminated hands than when handling other materials.

At all data points, *the E. coli* O157 DALYs exceeded the WHO ingestion standard (10^{-6} pppy) [47] by at least two factors. According to this study, beef cattle feces exhibited the maximum median DALYs attributed to *E. coli* O157 (9.8×10^{-3} pppy), which was lower than that reported in excreta in Ghana (31 pppy) [35]. The DALYs of *Salmonella spp*. in this study also exceeded WHO standards by 10^{-6} pppy [47]. Few studies have focused on the DALYs for *Salmonella spp*. in livestock stools affecting agricultural workers, necessitating the use of references from other sources, such as sewage sludge studies, which may not accurately reflect livestock conditions. Sadeghi *et al*. found that the median DALYs for *Salmonella spp*. in sewage sludge in Iran were significantly lower than the WHO standard, with an infection risk of 4.7×10^{-7} [34]. Conversely, a study by Kryzanowski *et al*. found a higher median risk of infection (2.4×10^{-2}) [63] associated with sewage sludge.

Elevated DALY values for *E. coli* O157 and *Salmonella spp*., surpassing WHO standards, pose substantial ramifications for workforce health. These consequences include reduced labor efficiency due to illness-related absences, heightened medical costs for agricultural operations, potential dissemination of pathogens within work environments, compromised quality of life stemming from acute infections, and possible chronic complications. Strategies for risk reduction may concentrate on minimization of incidental fecal ingestion among workers. Minimizing incidental fecal ingestion is a practical strategy to mitigate health risks from pathogens such as *E. coli* O157 and *Salmonella spp*. Implementing behavioral modifications, improved sanitation, and rigorous hygiene practices can be swiftly executed, directly reducing exposure and providing sustainable, long-term benefits.

In planning interventions, measures can be implemented to reduce the level of $Fecal_{IR}$, as the estimated weight of stools on the faces of workers is a more significant variable than the others. These measures included: 1) minimizing direct contact with livestock stools through substitution into automated systems; 2) providing protective equipment and ruminant farm Occupational Health and Safety Standards; 3) using closed systems for manure transport and storage; 4) providing sanitation supplies; and 5) conducting training program about personal and environmental hygiene. The proposed sanitation interventions comprised items 1, 3, and 4. Implementing these activities leads to less direct contact with animal stools, better hygiene practices, more knowledgeable workers, and improved monitoring of health and safety. These immediate results help reduce *E. coli* O157 and *Salmonella spp*. infections among workers, improve their health and safety and decrease environmental contamination. For example, Mara *et al*. found that a 1% reduction in *E. coli* O157 at all exposure points can lower the annual infection risk by up to 25% [64]. Butte *et al*. reported that these measures can reduce the likelihood of *E. coli* O157 infection by up to 27%, and using standard safety gear can decrease ingestion rates by up to 99% [35]. In the long term, these efforts create a safer working environment for farm workers, improve public health, and enhance the productivity and sustainability of farming operations.

This study was conducted on a farm in Sukabumi, West Java, which has a tropical climate. This climate affects the health of livestock and the behavior of farm workers. Intensive ruminant farming was performed on this farm involved feeding livestock with rice straw and feed concentrates. Veterinarians can address any livestock health issues. However, the practices observed on this farm may not be the same in other regions, limiting the generalizability of the findings. Furthermore, the study involved analysis of 40 stool samples and included six participants. While this study provides valuable insights, its findings may not be broadly applicable. In addition to *E. coli* and *Salmonella spp*., ruminant farms and their surrounding environments harbor other pathogens that can cause gastrointestinal diseases. These include *Brucella spp., Shigella spp.*, and *Clostridium spp.*, which are frequently found in the fecal matter of livestock [65,66]. Future research should include larger sample sizes, investigation of additional pathogens, and studies conducted in various locations to confirm and enhance the reliability of the results.

Conclusion

This study demonstrated that the incidental fecal ingestion rate and associated health risks among workers engaged in livestock management vary according to the species of livestock, the types of activities involved, and seasonal factors. Barn cleaning represented the most significant portion of the activity patterns recorded on the livestock farm. This activity has the potential to enhance fecal ingestion rates. In this study, the greatest median burden of disease (quantified in DALYs) was caused by beef cattle stool samples for *E. coli* O157, whereas that for *Salmonella spp*. was significantly lower in goat stool samples. This result emphasizes the necessity for targeted interventions to mitigate health impacts, specifically by decreasing livestock stool weight on the hands and faces of workers. Effective measures include improved sanitation through implementing automated systems, utilizing closed systems for manure transport and storage, and ensuring the availability of sanitation supplies. To bolster the reliability of the findings, forthcoming studies ought to employ larger cohorts, explore a wider array of pathogens, and be undertaken across diverse geographical settings.

Ethics approval

Ethical approval for conducting experiments and interviews with individuals engaged in livestock-related work was granted by the Health Studies Ethics Committee at Universitas Indonesia's Medical School and Cipto Mangunkusumo Hospital (Decision Letter No. KET-1254/UN2/F1/ETIK/PPM.00.02/2023). Furthermore, the collection of fecal samples from ruminants was sanctioned by IPB University's Faculty of Veterinary Medicine and Biomedical Sciences (decision letter no. 106/KEH/SKE/IX/2023).

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Competing interests

The authors declare that they have no conflicts of interest.

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Underlying data

Data will be provided upon request to the corresponding author.

Declaration of artificial intelligence use

xxx.

How to cite

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