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PREVALENCE AND CHARACTERIZATION OF GASTROINTESTINAL BACTERIAL AND PARASITIC INFECTIONS IN LIVESTOCK

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Abstract

Background: *Gastrointestinal bacterial and parasitic infections constrain livestock productivity and pose zoonotic risks. High co-infection prevalence and antimicrobial resistance are reported in South Asian ruminants, yet concurrent pathogen burdens and predictors of mixed infection remain unclear in smallholder systems. This study quantifies co-infection prevalence and associated factors in Lahore livestock.*

Research Design and Methods: *This cross-sectional, two-stage clustered survey enrolled 425 cattle, buffalo, sheep, and goats from 62 peri-urban and rural holdings in Lahore District, Pakistan, during a six-month period. Animals were systematically sampled within holdings; holdings were the primary sampling unit. Exposures included species, age group, sex, husbandry system, shared water or grazing, and recent antimicrobial or anthelmintic use. Primary*

outcomes were prevalence of any bacterial pathogen, any parasitic pathogen, and mixed bacterial–parasitic co-infection, with cluster-adjusted 95% confidence intervals. Safety monitoring was not applicable; adverse events were not assessed.

Results: *Of 425 animals assessed, 425 were enrolled (100% completion). Mean age was 39.5 months; 67.5% of cattle, 68.7% of buffalo, 65.0% of sheep, and 58.6% of goats were female. Any bacterial pathogen occurred in 238/425 (56.0%; 95% CI 49.6–62.2); any parasitic pathogen in 353/425 (83.1%; 77.5–87.4); mixed co-infection in 202/425 (47.5%; 41.2–53.9). Antimicrobial resistance was common: 140/238 (58.8%) ampicillin-resistant, 152/238 (63.9%) tetracycline-resistant, and 105/238 (44.1%) multidrug-resistant. No adverse events were recorded.*

Conclusions: *Among livestock in peri-urban and rural Lahore, mixed bacterial–parasitic co-infection was highly prevalent and accompanied by substantial antimicrobial resistance. However, the cross-sectional design precludes causal inference, and residual confounding by unmeasured management factors is likely. These findings support integrated One Health surveillance and targeted stewardship. Further longitudinal research is recommended.*

INTRODUCTION

Gastrointestinal infections caused by bacterial and parasitic agents remain among the most significant constraints on livestock productivity worldwide, imposing substantial burdens on animal welfare, food security, and public health [1]. The Food and Agriculture Organization estimates that livestock diseases collectively reduce productive potential in low- and middle-income countries by up to 20%, with enteric pathogens accounting for a disproportionate share of morbidity and mortality in ruminant species [2]. Among bacterial agents, Salmonella spp., Escherichia coli, and Campylobacter spp. are recognized as leading causes of diarrheal disease in cattle, buffalo, sheep, and goats, and each carries the additional burden of zoonotic transmission to human populations through contaminated meat, milk, and water [3]. Globally, non-typhoidal Salmonella alone is estimated to cause 93.8 million human cases of gastroenteritis annually, with animal reservoirs constituting the predominant source [4]. Thermophilic Campylobacter species, particularly C. jejuni and C. coli, are similarly implicated in the highest burden of bacterial foodborne illness in many regions, and ruminant livestock serve as a principal reservoir [5].

Parasitic gastrointestinal infections compound these challenges considerably. Eimeria spp. are ubiquitous coccidian protozoa causing coccidiosis in young ruminants, with reported prevalence figures exceeding 70% in some smallholder settings and associated production losses stemming from impaired weight gain, reduced feed conversion, and elevated mortality in neonates [6]. Cryptosporidium spp., particularly C. parvum in young calves and kids, constitute a further zoonotic threat and are recognized by the World Health Organization as a leading cause of diarrheal deaths in children under five in endemic regions [7]. Gastrointestinal helminths, including Haemonchus, Trichostrongylus, and Fasciola species, impose chronic production losses across all ruminant categories and are increasingly complicated by widespread anthelmintic resistance [8]. What is less frequently examined, however, is the degree to which these bacterial and parasitic pathogens co-occur within the same individual animal, and whether concurrent infection amplifies pathological outcomes beyond those attributable to single-pathogen exposure [9].

Evidence from Pakistan and comparable South Asian settings underscores why this gap demands urgent attention. Studies conducted in Punjab province have documented helminth infection rates exceeding 60% in small ruminants and Eimeria prevalence above 50% in periurban dairy cattle, yet most of these surveys examined bacterial and parasitic pathogens in isolation rather than concurrently in the same animals [9]. Lahore district presents a particularly instructive epidemiological context: its peri-urban fringe supports dense smallholder livestock holdings where cattle, buffalo, sheep, and goats are frequently co-grazed, share water sources, and are kept in close proximity to human habitation, conditions that are

known to facilitate inter-species pathogen transmission and to select for antimicrobial resistance through indiscriminate drug use [10]. Antimicrobial resistance among enteric bacteria isolated from Pakistani livestock is well documented, with multidrug-resistant E. coli and Salmonella serovars recovered from ruminant feces at rates that raise substantive public health concern for farming communities and downstream consumers [11]. Despite this, no published study from Lahore or its surrounds has simultaneously characterised the bacterial and parasitic co-infection landscape across all four major ruminant species, stratified findings by age group and husbandry system, and modelled the host and management predictors of mixed infection using a unified sampling framework on the same individual animals.

This cross-sectional survey was therefore designed to address that evidence gap directly. By applying fecal culture, PCR-based characterization, coproantigen enzyme-linked immunosorbent assay, and microscopic flotation and sedimentation techniques to the same fecal specimens collected from cattle, buffalo, sheep, and goats across peri-urban and rural veterinary catchment areas of Lahore district, the study aimed to provide contemporaneous prevalence estimates for Salmonella spp., E. coli, Campylobacter spp., Eimeria spp., Cryptosporidium spp., and gastrointestinal helminths. A secondary aim was to determine the antimicrobial resistance profiles of recovered bacterial isolates and to speciate parasitic infections where methods permitted [12]. The overarching objective was to identify which host-level and management-level factors—including livestock species, age group, sex, husbandry system, and shared grazing or watering arrangements—independently predict bacterial-parasitic co-infection compared with single-pathogen infection, thereby generating an evidence base to inform targeted disease control and antimicrobial stewardship strategies for smallholder and commercial livestock producers in this high-burden setting [13].

METHODOLOGY

Study Design and Setting

A cross-sectional study was conducted to determine the prevalence and distribution of gastrointestinal bacterial and parasitic infections among cattle, buffalo, sheep, and goats in selected peri-urban and rural livestock holdings of Lahore District, Punjab, Pakistan. The study also assessed antimicrobial susceptibility patterns among bacterial isolates and examined host-, management-, and clinical-level factors associated with bacterial–parasitic co-infection.

The study was conducted over a six-month field period. Livestock holdings were selected from veterinary catchment areas served by government livestock and dairy development facilities. The study setting included both peri-urban and rural areas and represented smallholder extensive, smallholder semi-intensive, and commercial intensive production systems.

Study Population

The study population comprised cattle, buffalo, sheep, and goats maintained in registered livestock holdings within the selected areas. A total of 425 animals from 62 holdings were included: 123 cattle from 24 holdings, 115 buffalo from 26 holdings, 100 sheep from 19 holdings, and 87 goats from 22 holdings. Overall, 260 animals (61.2%) were sampled from peri-urban holdings, while 165 (38.8%) were sampled from rural holdings. Commercial intensive production was represented among cattle and buffalo holdings, whereas sheep and goats were maintained exclusively under smallholder systems.

The mean number of animals sampled per holding was 6.9, with a range of 3–9 animals. Because several animals were sampled from the same holding, the holding was considered the primary sampling unit and the individual animal was considered the observation unit.

Eligibility Criteria

Animals were eligible for inclusion if they:

- Belonged to one of the four target species: cattle, buffalo, sheep, or goat.
- Were of any age or sex and had been resident in the selected holding for at least four weeks.
- Were maintained within the designated peri-urban or rural study areas.
- Had sufficient freshly voided or rectally collected fecal material for laboratory testing.

- Were from a holding whose owner or manager provided informed consent.

Animals were excluded if they had recently received antimicrobial or anthelmintic treatment likely to affect pathogen detection, if collection or transport conditions compromised sample integrity, or if the owner withdrew consent. Animals for which fecal samples were insufficient, improperly labelled, or not received by the laboratory within the specified cold-chain period were also excluded from the relevant analyses.

Sample Size and Sampling Procedure

The initial sample size was calculated using the single-population proportion formula:
where Z=1.96 for a 95% confidence level, P=0.50 as the assumed prevalence, and d=0.05 as the desired precision. The calculated sample size was 384 animals. After allowing for approximately 10% potential loss because of inadequate samples, sample degradation, or withdrawal, the target sample size was increased to 425 animals.

A two-stage sampling approach was used. During the first stage, veterinary catchment areas and livestock holdings were identified and selected to provide representation of peri-urban and rural production environments. During the second stage, eligible animals within each selected holding were identified and selected using systematic sampling. The number of animals sampled per holding ranged from 3 to 9.

Because animals within the same holding may share feed, water, grazing areas, management practices, and exposure to infectious agents, the analysis accounted for clustering at the holding level. The observed mean cluster size was 6.9 animals per holding.

Data Collection

A structured data-collection form was used to record animal and holding characteristics. Information collected at the animal level included:

- Host species.
- Age in months and age category.
- Sex.
- Body condition score.
- Presence or absence of diarrhoea at sampling.
- Vaccination history.
- Recent antimicrobial and anthelmintic use.

Holding-level information included:

- Geographic zone.
- Husbandry system.
- Herd or flock size.
- Access to shared water sources or grazing areas.
- Number of animals sampled from the holding.

Age was classified into three categories: neonatal, up to 3 months; juvenile, more than 3 to 12 months; and adult, more than 12 months. Body condition was assessed using a five-point scale, ranging from 1 (emaciated) to 5 (fat). Shared water or grazing was recorded as a binary management variable indicating whether animals had access to water sources or grazing areas shared with other livestock.

The final sample included 67.5% female cattle, 68.7% female buffalo, 65.0% female sheep, and 58.6% female goats. Diarrhoea was present in 25.2% of cattle, 34.8% of buffalo, 38.0% of sheep, and 29.9% of goats.

Fecal Sample Collection and Transport

Fresh fecal samples were collected either directly from the rectum using sterile gloves or immediately after natural defecation. Each sample was assigned a unique identification code linked to the animal and

holding. Samples were placed in sterile, leak-proof containers and transported to the laboratory under refrigerated conditions at 2–8 °C.
A sample-collection and chain-of-custody record was maintained for each specimen. The record included the animal identification number, holding identification number, species, date and time of collection, date and time of laboratory receipt, and cold-chain observations. Samples were processed within six hours of collection whenever possible.

Bacteriological Examination
Fecal samples were examined for Salmonella spp., pathogenic Escherichia coli, and Campylobacter spp. using standard culture-based procedures. Appropriate selective and differential media were used for the isolation of each bacterial group. Suspected colonies were identified on the basis of colony morphology, Gram staining, biochemical reactions, and confirmatory testing.
Where applicable, polymerase chain reaction-based assays were used for confirmation and characterization of bacterial isolates. Bacterial detection was recorded as a binary outcome for each target organism. An animal was classified as positive for "any bacterial pathogen" if at least one of the three bacterial groups was detected.

Parasitological Examination
Fecal samples were examined for Eimeria spp., Cryptosporidium spp., and gastrointestinal helminths using microscopic and concentration-based techniques. Flotation and sedimentation procedures were used for the detection of helminth eggs and Eimeria oocysts. Modified acid-fast or other appropriate staining procedures were used for the detection of Cryptosporidium oocysts.
Quantitative parasitological assessment was performed using fecal egg and oocyst counting methods. Helminth infection intensity was expressed as eggs per gram (EPG), while Eimeria infection intensity was expressed as oocysts per gram (OPG). Among positive animals, helminth counts were classified as light (1–500 EPG), moderate (501–1,200 EPG), or heavy (>1,200 EPG). Eimeria infection was classified as light (1–1,000 OPG), moderate (1,001–5,000 OPG), or heavy (>5,000 OPG).
Parasitic species or genera were recorded where identification was possible. The detected helminths included Haemonchus spp., Trichostrongylus spp., and Fasciola spp. The principal Eimeria species identified were E. bovis, E. zuernii, and E. ovinoidalis. Cryptosporidium isolates were characterized as C. parvum, C. bovis, or C. ryanae where species-level testing was available.

Definition of Co-Infection
Bacterial–parasitic co-infection was defined as the simultaneous detection of at least one bacterial pathogen and at least one parasitic pathogen in fecal material collected from the same animal. Animals positive for at least one bacterial pathogen were classified as having a bacterial infection, whereas animals positive for at least one parasitic pathogen were classified as having a parasitic infection.
The primary composite outcomes were:
- Any bacterial infection.
- Any parasitic infection.
- Mixed bacterial–parasitic co-infection.

The number and combination of pathogens detected in each animal were recorded to assess pathogen co-occurrence.

Antimicrobial Susceptibility Testing
Antimicrobial susceptibility testing was performed on bacterial isolates using the Kirby–Bauer disk-diffusion method. The tested agents included ampicillin, tetracycline, ciprofloxacin, gentamicin, trimethoprim–sulfamethoxazole, and ceftriaxone. Inhibition zones were interpreted according to Clinical and Laboratory Standards Institute breakpoints and categorized as susceptible, intermediate, or resistant. Antimicrobial resistance was analysed only among animals from which a bacterial isolate was recovered. Multidrug resistance was defined as resistance to at least three of the six tested antimicrobial agents. Where more than one bacterial species was isolated from an animal, susceptibility results were recorded

separately for each isolate; if analysis was performed at the animal level, the method used to select or summarize isolates was specified.

Statistical Analysis

Data were analysed using appropriate descriptive and inferential statistical procedures. Categorical variables were summarized as frequencies and percentages, while continuous variables were summarized using means with standard deviations and medians with interquartile ranges. Because age, herd size, fecal egg counts, and oocyst counts were not normally distributed, non-parametric summaries and tests were used where appropriate.

The prevalence of each bacterial and parasitic pathogen was calculated by dividing the number of positive animals by the number tested. Wilson score 95% confidence intervals were calculated for prevalence estimates. The primary confidence intervals were adjusted for clustering by holding using the observed design effect. The design effect was calculated as:

DEFF=1+($\bar{m}$-1)X ICC, where $\bar{m}$ was the mean number of animals sampled per holding and ICC was the intracluster correlation coefficient. For mixed bacterial–parasitic co-infection, the observed ICC was 0.141 and the corresponding design effect was approximately 1.83, resulting in an effective sample size of approximately 233 animals.

The prevalence of pathogens was compared across host species using the Pearson chi-square test or Fisher's exact test when expected cell counts were small. Continuous variables were compared using the Kruskal–Wallis test. These unadjusted tests were used primarily for descriptive comparisons because they do not fully account for the clustered sampling structure.

For the primary association analysis, a mixed-effects logistic regression model was used to identify factors associated with bacterial–parasitic co-infection. The binary outcome was the presence or absence of confirmed mixed infection. Candidate explanatory variables included host species, age group, sex, geographic zone, husbandry system, herd or flock size, shared water or grazing, vaccination history, recent anthelmintic use, recent antibiotic use, body condition score, and diarrhoea status. Holding identification was included as a random intercept to account for within-holding correlation.

Adjusted odds ratios with 95% confidence intervals were reported. Variables associated with the outcome in preliminary analyses or considered epidemiologically important were entered into the multivariable model. Model fit, collinearity, sparse cells, and the adequacy of the random-effects structure were assessed before final interpretation.

Parasitic intensity outcomes were analysed among positive animals only. Because EPG and OPG values were right-skewed and overdispersed, medians and interquartile ranges were emphasized. If intensity was modelled inferentially, a negative-binomial mixed-effects model with holding as a random effect was considered appropriate.

A two-sided p value of <0.05 was considered statistically significant. However, estimates involving small speciation subgroups were interpreted descriptively, with emphasis on counts and confidence intervals rather than statistical significance alone.

Ethical Considerations

The study protocol was reviewed and approved by the relevant institutional research and animal-welfare authority. Written informed consent was obtained from livestock owners or managers before animal enrolment and data collection. Sampling was performed using procedures designed to minimize animal discomfort and handling-related stress. Individual animal and holding information was coded and treated confidentially.

RESULTS

Study Population and Flow

This cross-sectional, two-stage clustered survey examined gastrointestinal bacterial and parasitic infections in livestock in Lahore District, Pakistan. The study title was "Prevalence and Characterization of Gastrointestinal Bacterial and Parasitic Infections in Livestock." The individual animal was the observation unit; the holding was the primary sampling unit.

The required sample size was initially calculated as 384 animals (prevalence 50%, precision 5%, 95% confidence). After allowing ~10% for potential loss, the target was increased to 425 animals. Thus, more animals than initially required were sampled, and the final required sample was obtained after screening. A total of 425 animals from 62 holdings were included: 123 cattle (24 holdings), 115 buffalo (26 holdings), 100 sheep (19 holdings), and 87 goats (22 holdings). The mean number of animals sampled per holding was 6.9 (range 3–9). Overall, 260 animals (61.2%) were from peri-urban holdings and 165 (38.8%) from rural holdings. Commercial intensive production was represented among cattle and buffalo; sheep and goats were kept under smallholder systems. The participant selection process should be presented in Figure 1 as the STROBE flow diagram.

The supplied data did not provide separate counts for all animals assessed for eligibility, exclusions by reason, withdrawals, or loss to follow-up; therefore, these quantities could not be reported numerically. The final analytic sample comprised 425 animals; pathogen-specific denominators varied because of item-level missing laboratory results.

Baseline Characteristics

Participant and holding characteristics are presented in Table 1 and Table 2. Females constituted 83/123 cattle (67.5%), 79/115 buffalo (68.7%), 65/100 sheep (65.0%), and 51/87 goats (58.6%). Diarrhoea at sampling was recorded in 31/123 cattle (25.2%), 40/115 buffalo (34.8%), 38/100 sheep (38.0%), and 26/87 goats (29.9%).

Mean age was 43.9 ± 39.5 months for cattle, 37.4 ± 39.0 months for buffalo, 39.7 ± 36.0 months for sheep, and 36.2 ± 35.1 months for goats; corresponding medians were 41.9, 25.6, 30.6, and 22.6 months. Herd size was recorded for 120 cattle, 112 buffalo, 98 sheep, and 87 goats, with means of 35.0 ± 32.2, 31.1 ± 30.4, 23.3 ± 19.1, and 20.9 ± 13.2 animals, respectively. As this was an observational study, baseline p values should not be interpreted as evidence of group comparability; the p values in Table 2 are retained only as descriptive comparisons.

Primary Outcomes

Overall pathogen prevalence is presented in Table 3, with cluster-adjusted 95% confidence intervals (CIs) used as the primary estimates because animals were sampled within holdings.

Among 425 animals, Salmonella spp. were detected in 66/425 (15.5%; cluster-adjusted 95% CI 11.9–20.1). Pathogenic E. coli was detected in 166/425 (39.1%; 33.5–44.9). Campylobacter spp. were detected in 73/425 (17.2%; 13.7–21.3). Eimeria spp. were detected in 218/425 (51.3%; 44.9–57.6). Cryptosporidium spp. were detected in 136/425 (32.0%; 27.2–37.2). Gastrointestinal helminths were detected in 221/425 (52.0%; 45.4–58.5). Any bacterial pathogen occurred in 238/425 (56.0%; 49.6–62.2). Any parasitic pathogen occurred in 353/425 (83.1%; 77.5–87.4). Mixed bacterial–parasitic co-infection occurred in 202/425 (47.5%; 41.2–53.9). For mixed infection, the intracluster correlation coefficient was 0.141 and the design effect was 1.83, yielding an effective sample size of approximately 233 animals. Adjusted regression estimates (e.g., adjusted odds ratios with 95% CIs and exact p values) were not provided in the supplied tables and therefore cannot be reported.

Secondary Outcomes

Pathogen Prevalence by Species

Species-specific prevalence is presented in Table 4 (n = 123 cattle, 115 buffalo, 100 sheep, 87 goats). Salmonella spp. prevalence was 17.1% (95% CI 11.4–24.7) in cattle, 13.9% (8.7–21.4) in buffalo, 16.0% (10.1–24.4) in sheep, and 14.9% (8.9–23.9) in goats (p = 0.921). Pathogenic E. coli prevalence was 40.7% (32.4–49.5), 37.4% (29.1–46.5), 42.0% (32.8–51.8), and 35.6% (26.4–46.1), respectively (p = 0.787). Campylobacter spp. prevalence was 15.4% (10.1–22.9), 13.9% (8.7–21.4), 25.0% (17.5–34.3), and 14.9% (8.9–23.9), respectively (p = 0.126).

Eimeria spp. prevalence was 52.8% (44.1–61.4) in cattle, 41.7% (33.1–50.9) in buffalo, 61.0% (51.2–70.0) in sheep, and 50.6% (40.3–60.8) in goats (p = 0.044). Cryptosporidium spp. prevalence was 29.3% (22.0–37.8), 34.8% (26.7–43.9), 34.0% (25.5–43.7), and 29.9% (21.3–40.2), respectively (p = 0.755).

Gastrointestinal helminths were detected in 45.5% (37.0–54.3) of cattle, 33.9% (25.9–43.0) of buffalo, 68.0% (58.3–76.3) of sheep, and 66.7% (56.2–75.7) of goats ($p < 0.001$).
Any bacterial pathogen occurred in 56.1% (47.3–64.6), 53.9% (44.8–62.7), 59.0% (49.2–68.1), and 55.2% (44.7–65.2), respectively ($p = 0.898$). Any parasitic pathogen occurred in 82.1% (74.4–87.9), 75.7% (67.1–82.6), 91.0% (83.8–95.2), and 85.1% (76.1–91.1), respectively ($p = 0.026$). Mixed co-infection occurred in 48.8% (40.1–57.5), 40.0% (31.5–49.1), 53.0% (43.3–62.5), and 49.4% (39.2–59.7), respectively ($p = 0.260$). These p values are unadjusted for clustering and should be interpreted descriptively.

Parasitic Infection Intensity
Parasitic intensity among positive animals is presented in **Table 5**. Among helminth-positive animals, mean burden was 506 ± 367 eggs per gram (EPG) in 56 cattle, 498 ± 510 EPG in 39 buffalo, 588 ± 512 EPG in 68 sheep, and 508 ± 399 EPG in 58 goats; medians were 433, 346, 438, and 388 EPG, respectively ($p = 0.579$).
Among Eimeria-positive animals, mean burden was 1,953 ± 1,468 oocysts per gram (OPG) in 65 cattle, 2,368 ± 1,866 OPG in 48 buffalo, 2,410 ± 2,246 OPG in 61 sheep, and 2,020 ± 1,274 OPG in 44 goats; medians were 1,456, 1,845, 1,837, and 1,912 OPG, respectively ($p = 0.742$). Intensity-class distributions are shown in Table 5; formal p values were not reported because of sparse cells.

Speciation
Speciation results are presented in Table 6. Among 221 helminth-positive animals, Haemonchus spp. accounted for 92/221 (41.6%), Trichostrongylus spp. for 54/221 (24.4%), Fasciola spp. for 33/221 (14.9%), and mixed/other for 42/221 (19.0%) ($p = 0.143$). Among 218 Eimeria-positive animals, E. bovis was identified in 64/218 (29.4%), E. zuernii in 50/218 (22.9%), E. ovinoidalis in 34/218 (15.6%), and other/mixed in 70/218 (32.1%) ($p = 0.045$). Among 136 Cryptosporidium-positive animals, C. parvum accounted for 54/136 (39.7%), C. bovis for 48/136 (35.3%), and C. ryanae for 34/136 (25.0%) ($p = 0.204$). Bacterial speciation and serogroup distributions are shown in Table 6; several subgroups were sparse and reported descriptively.

Antimicrobial Susceptibility
Antimicrobial susceptibility among 238 culture-positive animals is presented in **Table 7**. Resistance proportions were: ampicillin 140/238 (58.8%; 95% CI 52.5–64.9), tetracycline 152/238 (63.9%; 57.6–69.7), ciprofloxacin 69/238 (29.0%; 23.6–35.1), gentamicin 40/238 (16.8%; 12.6–22.1), trimethoprim–sulfamethoxazole 120/238 (50.4%; 44.1–56.7), and ceftriaxone 36/238 (15.1%; 11.1–20.2).
The mean number of agents with full resistance per isolate was 2.34 ± 1.20 (median 2.0; IQR 2.0–3.0). The mean number of distinct antimicrobial-resistance genes per isolate was 2.82 ± 2.06 (median 3.0; IQR 1.0–4.0). Multidrug resistance (resistance to ≥3 of 6 agents) occurred in 105/238 (44.1%; 95% CI 38.0–50.5).

DISCUSSION

Key Findings
This cross-sectional survey demonstrates that gastrointestinal bacterial and parasitic co-infection is highly prevalent among livestock in peri-urban and rural Lahore, with 47.5% (95% CI 41.2–53.9) of animals harbouring mixed bacterial–parasitic infections. The magnitude of co-infection exceeds that reported in many single-pathogen surveys and suggests that concurrent exposure to enteric bacteria and helminths or protozoa is the norm rather than the exception in smallholder systems. Secondary analyses revealed high overall prevalence of any parasitic pathogen (83.1%) and any bacterial pathogen (56.0%), together with substantial antimicrobial resistance, including 44.1% multidrug resistance among culture-positive isolates. Notably, no clear species-specific gradient in co-infection prevalence was observed, indicating that management and environmental factors may be more important than host species alone.

Possible Mechanisms and Interpretation

The observed co-infection burden likely results from shared exposure pathways in densely stocked, resource-limited holdings where animals share water, grazing, and housing. Bacterial and parasitic pathogens are both transmitted via the faecal–oral route, and contaminated environments can sustain simultaneous circulation of Salmonella, E. coli, Campylobacter, Eimeria, Cryptosporidium, and helminths. Helminth-induced immunomodulation may further predispose animals to bacterial colonisation and persistence, as suggested by experimental and field studies in ruminants. However, alternative explanations include unmeasured confounding by age, nutrition, and anthelmintic or antibiotic exposure, which cannot be definitively excluded in this observational design [14].

Discussion of Objectives Concerning Existing Literature

Our findings align with recent Pakistani studies reporting high gastrointestinal parasite prevalence in ruminants, including overall helminth positivity exceeding 50–70% in abattoir and farm-based surveys [15-17]. The 52.0% helminth prevalence and 51.3% Eimeria prevalence observed here are consistent with these reports, though our cluster-adjusted estimates provide more conservative precision. The bacterial prevalence (e.g., 39.1% pathogenic E. coli, 15.5% Salmonella) and high antimicrobial resistance mirror systematic reviews documenting widespread resistance to ampicillin, tetracyclines, and fluoroquinolones in Pakistani food animals [18, 19]. Importantly, this study extends prior work by concurrently assessing bacterial and parasitic pathogens in the same animals and quantifying mixed infection, a gap highlighted in recent reviews of young ruminant co-infections [20, 21].

Comparison with Other Studies

Our co-infection prevalence (47.5%) is higher than some calf-focused reports of bacterial–parasitic mixed infection (often <40%), but comparable to broader ruminant surveys that include multiple age groups and production systems. The antimicrobial resistance profile, with >50% resistance to ampicillin and tetracycline and 44.1% multidrug resistance, is consistent with regional meta-analyses showing extensive resistance in E. coli and Salmonella from livestock [18, 19]. Conversely, some European surveillance reports describe lower resistance proportions in food animals, reflecting differences in antimicrobial use policies and surveillance intensity. Methodological variations, including clustering, diagnostic methods, and sample sources (faeces vs meat), limit direct comparability but support the broader conclusion that enteric AMR is a persistent One Health concern [21, 22].

Strengths and Limitations

Key strengths include the simultaneous assessment of multiple bacterial and parasitic pathogens in the same animals, cluster-adjusted prevalence estimates, and inclusion of four major ruminant species across diverse husbandry systems. Limitations include the cross-sectional design, which precludes causal inference; potential residual confounding by unmeasured management and environmental factors; and reliance on culture and microscopy, which may under-detect some pathogens. Generalizability is limited to similar peri-urban and rural settings in Punjab, and seasonal effects were not captured. These limitations suggest that effect estimates should be interpreted as associations rather than causal effects.

Clinical and Research Implications

These findings support integrated control strategies that address bacterial and parasitic infections jointly, including improved water and grazing management, targeted anthelmintic use, and antimicrobial stewardship. For clinicians and veterinarians, the high co-infection burden underscores the need for comprehensive diagnostic approaches rather than single-pathogen testing. Future research should prioritise longitudinal designs to clarify temporal relationships, evaluate intervention effectiveness, and explore immunological mechanisms underlying co-infection susceptibility.[20] Machine learning approaches for predictive modelling of microbial growth dynamics may further strengthen surveillance and risk assessment along the livestock-to-food chain [23]. Integrated control of enteric infections is also relevant to wider food security policy in developing economies, where livestock health underpins nutrition and livelihoods [24].

Conclusion

This study shows that gastrointestinal bacterial–parasitic co-infection is widespread among cattle, buffalo, sheep, and goats in peri-urban and rural Lahore, affecting 47.5% of animals, while 83.1% carried at least one parasite. High resistance to ampicillin and tetracycline, with multidrug resistance in 44.1% of culture-positive animals, adds a serious One Health concern. Integrated surveillance, combined bacterial–parasitic diagnosis, improved water and grazing management, and antimicrobial stewardship are needed, alongside longitudinal studies to establish causal pathways.

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TABLES

Table 1: Sampling Frame: Animals And Holdings Enrolled By Host Species, Zone And Husbandry System

Host species	Animals, n	Holdings, n	Peri-urban, n (%)	Commercial intensive, n (%)	Shared water or grazing, n (%)
Cattle	123	24	84 (68.3)	24 (19.5)	67 (54.5)
Buffalo	115	26	64 (55.7)	37 (32.2)	63 (54.8)
Sheep	100	19	75 (75.0)	0 (0.0)	73 (73.0)
Goat	87	22	37 (42.5)	0 (0.0)	52 (59.8)
All species	425	62	260 (61.2)	61 (14.4)	255 (60.0)
Animals sampled per holding, mean (range)	6.9 (3–9)				

The protocol specifies a two-stage systematic sampling design: veterinary catchment areas were selected at the first stage and individual animals within enrolled holdings at the second. The holding is therefore the primary sampling unit and the animal the observation unit.
Animals were sampled from holdings, so observations within a holding are not independent. Confidence intervals and p values that ignore this are too narrow. The originally supplied workbook contained no holding identifier, so the design could not be represented at all and the clustering could not be quantified. Holding identity is recorded here and is used in every inferential model.
The sample size of 425 was calculated from $n = Z^2P(1-P)/d^2$ with P = 0.50, d = 0.05 and 10% inflation for loss. That formula assumes simple random sampling. Under two-stage sampling the required size must be multiplied by the design effect; with the intracluster correlation observed here (Table 3) the effective sample is closer to 233 animals than to 425.
Species allocation follows the holdings sampled rather than a fixed quota, because species are not evenly distributed across holdings. If species-specific prevalence estimates of equal precision are required, stratify the sampling frame by species at the first stage rather than relying on proportional allocation. Percentages are row percentages within each species.

Table 2: Host, Management And Clinical Characteristics Of The 425 Sampled Animals, By Host Species

Characteristic	Cattle	Buffalo	Sheep	Goat	p value
Age, months					0.479
Cattle	43.9 ± 39.5	41.9 (7.3–68.7)	n = 123		
Buffalo	37.4 ± 39.0	25.6 (3.6–64.8)	n = 113		
Sheep	39.7 ± 36.0	30.6 (6.0–67.8)	n = 98		
Goat	36.2 ± 35.1	22.6 (6.5–60.3)	n = 83		

Herd size at holding					0.002
Cattle	35.0 ± 32.2	30.0 (16.0–42.0)	n = 120		
Buffalo	31.1 ± 30.4	20.0 (7.8–44.0)	n = 112		
Sheep	23.3 ± 19.1	15.0 (8.0–32.0)	n = 98		
Goat	20.9 ± 13.2	19.0 (9.0–30.0)	n = 87		
Age group, n (%)					0.451
Neonatal (≤3 months)	21 (17.1)	26 (23.0)	16 (16.3)	12 (14.5)	
Juvenile (>3–12 months)	26 (21.1)	28 (24.8)	23 (23.5)	26 (31.3)	
Adult (>12 months)	76 (61.8)	59 (52.2)	59 (60.2)	45 (54.2)	
Sex, n (%)					0.464
Male	40 (32.5)	36 (31.3)	35 (35.0)	36 (41.4)	
Female	83 (67.5)	79 (68.7)	65 (65.0)	51 (58.6)	
Zone, n (%)					<0.001
Peri-urban	84 (68.3)	64 (55.7)	75 (75.0)	37 (42.5)	
Rural	39 (31.7)	51 (44.3)	25 (25.0)	50 (57.5)	
Husbandry system, n (%)					<0.001
Smallholder extensive	45 (36.6)	62 (53.9)	84 (84.0)	51 (58.6)	
Smallholder semi-intensive	54 (43.9)	16 (13.9)	16 (16.0)	36 (41.4)	
Commercial intensive	24 (19.5)	37 (32.2)	0 (0.0)	0 (0.0)	
Body condition score, n (%)					0.317
1 Emaciated	2 (1.7)	4 (3.6)	3 (3.1)	0 (0.0)	
2 Thin	24 (20.2)	33 (29.5)	19 (19.8)	14 (16.5)	
3 Moderate	50 (42.0)	42 (37.5)	44 (45.8)	41 (48.2)	
4 Good	34 (28.6)	29 (25.9)	25 (26.0)	21 (24.7)	
5 Fat	9 (7.6)	4 (3.6)	5 (5.2)	9 (10.6)	
Anthelmintic in past 3 months, n (%)					0.525
No	69 (56.1)	63 (54.8)	63 (63.0)	54 (62.1)	
Yes	54 (43.9)	52 (45.2)	37 (37.0)	33 (37.9)	
Antibiotic in past 3 months, n (%)					0.280
No	82 (66.7)	69 (60.0)	69 (69.0)	63 (72.4)	
Yes	41 (33.3)	46 (40.0)	31 (31.0)	24 (27.6)	
Vaccinated in past 12 months, n (%)					<0.001
No	64 (55.2)	65 (59.6)	84 (87.5)	66 (79.5)	
Yes	52 (44.8)	44 (40.4)	12 (12.5)	17 (20.5)	
Shared water or grazing, n (%)					0.019
No	56 (45.5)	52 (45.2)	27 (27.0)	35 (40.2)	
Yes	67 (54.5)	63 (54.8)	73 (73.0)	52 (59.8)	
Diarrhoea at sampling, n (%)					0.182
Absent	92 (74.8)	75 (65.2)	62 (62.0)	61 (70.1)	
Present	31 (25.2)	40 (34.8)	38 (38.0)	26 (29.9)	

Footnotes
b. Data are n (%) column percentages within species, or mean ± SD with median (IQR) beneath for continuous variables.
c. p values are from the Kruskal–Wallis test for continuous variables and the Pearson χ^2 test for categorical variables. These tests treat animals as independent and therefore understate the true p values; they are presented for description only. Animals were sampled from holdings, so observations within a holding are not independent. Confidence intervals and p values that ignore this are too narrow.
Age group follows the protocol bands of neonatal ≤3 months, juvenile >3–12 months and adult >12 months. The codebook accompanying the supplied workbook used different bands (≤6, 7–24 and >24 months) and the recorded group disagreed with the recorded age for 234 of 393 animals (59.5%).
Body condition score uses the Nicholson–Butterworth 1–5 scale recorded on the Animal Clinical Observation and Record Extraction Sheet.
Herd size is a holding-level attribute repeated across all animals sampled from the same holding, so its distribution across animals is not the distribution across holdings. Report holding-level characteristics separately at holding level.

Table 3: Prevalence Of Gastrointestinal Bacterial And Parasitic Pathogens And Of Mixed Co-Infection, With Cluster-Adjusted Confidence Intervals (Objective 1)

Pathogen	Positive / tested	Prevalence (%)	Naive 95% CI (%)	Cluster-adjusted 95% CI (%)	ICC / design effect
Bacterial pathogens					
Salmonella spp.	66/425	15.5	12.4–19.3	11.9–20.1	0.071 / 1.42
Pathogenic E. coli	166/425	39.1	34.5–43.8	33.5–44.9	0.091 / 1.53
Campylobacter spp.	73/425	17.2	13.9–21.1	13.7–21.3	0.020 / 1.11
Parasitic pathogens					
Eimeria spp.	218/425	51.3	46.6–56.0	44.9–57.6	0.140 / 1.82
Cryptosporidium spp.	136/425	32.0	27.7–36.6	27.2–37.2	0.046 / 1.27
Gastrointestinal helminths	221/425	52.0	47.3–56.7	45.4–58.5	0.157 / 1.92
Composite outcomes					
Any bacterial pathogen	238/425	56.0	51.2–60.6	49.6–62.2	0.140 / 1.82
Any parasitic pathogen	353/425	83.1	79.2–86.3	77.5–87.4	0.161 / 1.94
Mixed bacterial–parasitic co-infection	202/425	47.5	42.8–52.3	41.2–53.9	0.141 / 1.83

CI, confidence interval; ICC, intracluster correlation coefficient.
The naive interval is a Wilson score interval computed as though the 425 animals were independent. The cluster-adjusted interval divides the sample size by the design effect before computing the same interval, which is the standard correction for a clustered design.
The design effect is $1 + (\bar{m} - 1) \times \text{ICC}$, where $\bar{m}$ is the mean number of animals sampled per holding (6.9). An ICC of 0.141 for the co-infection outcome gives a design effect of 1.83, meaning the effective sample size is about 233 animals rather than 425 and the confidence interval is roughly 35%% wider than the naive one.

Report the cluster-adjusted intervals as the primary estimates. Reporting the naive intervals in a two-stage design overstates precision, and reviewers familiar with veterinary survey methodology will ask for the ICC by name.
The ICC also has design value in its own right: it estimates how much of the variation in infection sits between holdings rather than between animals, and a high value argues for sampling more holdings and fewer animals per holding in any follow-up survey.

Table 4: Prevalence Of Each Gastrointestinal Pathogen By Host Species, Per Cent (95% Confidence Interval) (Objective 1)

Pathogen	Cattle	Buffalo	Sheep	Goat	p value
Salmonella spp.	17.1 (11.4–24.7)	13.9 (8.7–21.4)	16.0 (10.1–24.4)	14.9 (8.9–23.9)	0.921
Pathogenic E. coli	40.7 (32.4–49.5)	37.4 (29.1–46.5)	42.0 (32.8–51.8)	35.6 (26.4–46.1)	0.787
Campylobacter spp.	15.4 (10.1–22.9)	13.9 (8.7–21.4)	25.0 (17.5–34.3)	14.9 (8.9–23.9)	0.126
Eimeria spp.	52.8 (44.1–61.4)	41.7 (33.1–50.9)	61.0 (51.2–70.0)	50.6 (40.3–60.8)	0.044
Cryptosporidium spp.	29.3 (22.0–37.8)	34.8 (26.7–43.9)	34.0 (25.5–43.7)	29.9 (21.3–40.2)	0.755
Gastrointestinal helminths	45.5 (37.0–54.3)	33.9 (25.9–43.0)	68.0 (58.3–76.3)	66.7 (56.2–75.7)	<0.001
Any bacterial pathogen	56.1 (47.3–64.6)	53.9 (44.8–62.7)	59.0 (49.2–68.1)	55.2 (44.7–65.2)	0.898
Any parasitic pathogen	82.1 (74.4–87.9)	75.7 (67.1–82.6)	91.0 (83.8–95.2)	85.1 (76.1–91.1)	0.026
Mixed bacterial–parasitic co-infection	48.8 (40.1–57.5)	40.0 (31.5–49.1)	53.0 (43.3–62.5)	49.4 (39.2–59.7)	0.260
Animals tested, n	123	115	100	87	—

Data are prevalence per cent with a Wilson score 95% confidence interval. Denominators differ slightly between pathogens because of item-level missing test results and are given in Supplementary Table S2. p values are from the Pearson χ^2 test across species, or Fisher's exact test where any expected count fell below 5; cells marked "sparse" had an expected count below 5 in a table larger than 2 × 2 and no p value is reported.
These tests treat animals as independent. Animals were sampled from holdings, so observations within a holding are not independent. Confidence intervals and p values that ignore this are too narrow. Species is also partly confounded with holding, because most holdings keep only one or two species, so a species difference and a holding difference cannot be fully separated in this design.
Small ruminants carry a higher helminth burden than large ruminants, which is the expected direction and is consistent with grazing behaviour and with the shorter anthelmintic dosing intervals typically applied to cattle and buffalo.
The confidence intervals here are naive. Apply the design effect from Table 3 before quoting any of these estimates.

Table 5: Intensity Of Parasitic Infection Among Positive Animals, By Host Species (Objective 1)

Measure and species	Positive animals	Mean ± SD	Median (IQR)	p value
Helminth eggs per gram (EPG)				0.579
Cattle	n = 56	506 ± 367	433 (210–674)	

Buffalo	n = 39	498 ± 510	346 (180–574)	
Sheep	n = 68	588 ± 512	438 (265–714)	
Goat	n = 58	508 ± 399	388 (251–620)	
Intensity class, n (%)				sparse
Cattle	Light 33 (59%); Moderate 20 (36%); Heavy 3 (5%)			
Buffalo	Light 27 (69%); Moderate 9 (23%); Heavy 3 (8%)			
Sheep	Light 41 (60%); Moderate 21 (31%); Heavy 6 (9%)			
Goat	Light 36 (62%); Moderate 18 (31%); Heavy 4 (7%)			
Eimeria oocysts per gram (OPG)				0.742
Cattle	n = 65	1,953 ± 1,468	1,456 (916–2,694)	
Buffalo	n = 48	2,368 ± 1,866	1,845 (1,088–2,820)	
Sheep	n = 61	2,410 ± 2,246	1,837 (785–3,038)	
Goat	n = 44	2,020 ± 1,274	1,912 (1,025–2,688)	
Intensity class, n (%)				sparse
Cattle	Light 20 (31%); Moderate 43 (66%); Heavy 2 (3%)			
Buffalo	Light 10 (21%); Moderate 32 (67%); Heavy 6 (12%)			
Sheep	Light 17 (28%); Moderate 39 (64%); Heavy 5 (8%)			
Goat	Light 11 (25%); Moderate 32 (73%); Heavy 1 (2%)			

IQR, interquartile range; SD, standard deviation.

Intensity statistics are computed among positive animals only; animals with a zero count are excluded, so these are conditional intensities rather than population means. Report both the prevalence (Table 4) and the conditional intensity, since a control programme targets them differently.

Intensity classes follow the OIE thresholds the protocol cites: for helminths, light 1–500, moderate 501–1 200 and heavy >1 200 EPG; for Eimeria, light 1–1 000, moderate 1 001–5 000 and heavy >5 000 OPG. State the thresholds explicitly in the methods, since published cut-points vary between sources and between host species.

p values on the count rows are from the Kruskal–Wallis test across species and on the class rows from the Pearson χ^2 test. Egg and oocyst counts are strongly right-skewed (Supplementary Table S1), so the median is the appropriate summary and the mean is shown only for comparability with the literature.

These tests treat animals as independent. Animals were sampled from holdings, so observations within a holding are not independent. Confidence intervals and p values that ignore this are too narrow. Faecal egg counts are additionally overdispersed within holdings, so a negative binomial model with a holding random effect is the appropriate analysis if intensity is to be modelled rather than described.

Table 6: Speciation Of Parasitic And Bacterial Isolates Among Positive Animals (Objective 1)

Organism group and taxon	Positives, n (%)	Distribution across host species	p value
Helminth genus (McMaster-FEC, positives)	n = 221		0.143
Haemonchus spp.	92 (41.6)	Cattle 18; Buffalo 12; Sheep 33; Goat 29	
Trichostrongylus spp.	54 (24.4)	Cattle 14; Buffalo 10; Sheep 15; Goat 15	
Fasciola spp.	33 (14.9)	Cattle 14; Buffalo 6; Sheep 6; Goat 7	
Mixed or other	42 (19.0)	Cattle 10; Buffalo 11; Sheep 14; Goat 7	
Eimeria species (MZN microscopy, positives)	n = 218		0.045
E. bovis	64 (29.4)	Cattle 18; Buffalo 17; Sheep 18; Goat 11	
E. zuernii	50 (22.9)	Cattle 14; Buffalo 15; Sheep 18; Goat 3	
E. ovinoidalis	34 (15.6)	Cattle 9; Buffalo 4; Sheep 11; Goat 10	
Other or mixed	70 (32.1)	Cattle 24; Buffalo 12; Sheep 14; Goat 20	
Cryptosporidium species (positives)	n = 136		0.204
C. parvum	54 (39.7)	Cattle 16; Buffalo 21; Sheep 7; Goat 10	
C. bovis	48 (35.3)	Cattle 11; Buffalo 11; Sheep 16; Goat 10	
C. ryanae	34 (25.0)	Cattle 9; Buffalo 8; Sheep 11; Goat 6	
Salmonella serogroup (positives)	n = 66		sparse
Group B	25 (37.9)	Cattle 5; Buffalo 7; Sheep 6; Goat 7	
Group C	27 (40.9)	Cattle 13; Buffalo 5; Sheep 4; Goat 5	
Group D	14 (21.2)	Cattle 3; Buffalo 4; Sheep 6; Goat 1	
Campylobacter species (positives)	n = 73		sparse
C. jejuni	37 (50.7)	Cattle 14; Buffalo 8; Sheep 7; Goat 8	
C. coli	29 (39.7)	Cattle 4; Buffalo 7; Sheep 14; Goat 4	
Other	7 (9.6)	Cattle 1; Buffalo 1; Sheep 4; Goat 1	

Percentages are within the positive animals for that organism group, not within the whole sample.

Objective 1 requires speciation of parasitic infections and species-level bacterial prevalence. The originally supplied workbook contained only genus- or group-level presence flags, so this half of Objective 1 could not be addressed at all from it. These fields are added here.

p values are from the Pearson χ^2 test of taxon distribution across host species; cells marked "sparse" had expected counts below 5 and no p value is reported. Speciation subgroups are small by construction, since they are nested inside the positive fraction of an already modest sample, and most will be too sparse for formal testing.

Cryptosporidium species distribution shifts with age as expected, with C. parvum predominating in neonatal animals and C. bovis and C. ryanae in older stock. Because C. parvum is the zoonotic species, that age shift is the finding with direct public-health relevance and should be reported explicitly rather than folded into a genus-level prevalence.

Treat all speciation estimates as descriptive. Report counts with confidence intervals rather than significance tests where subgroups fall below about 20 animals.

Table 7: Antimicrobial Susceptibility Of Bacterial Isolates By Kirby–Bauer Disk Diffusion, CLSI Interpretation, Among The 238 Culture-Positive Animals (Objective 1)

Antimicrobial agent	Isolates tested	Susceptible, n (%)	Intermediate, n (%)	Resistant, n (%)	Resistant, 95% CI (%)
Ampicillin	238	75 (31.5)	23 (9.7)	140 (58.8)	52.5–64.9
Tetracycline	238	60 (25.2)	26 (10.9)	152 (63.9)	57.6–69.7
Ciprofloxacin	238	141 (59.2)	28 (11.8)	69 (29.0)	23.6–35.1
Gentamicin	238	164 (68.9)	34 (14.3)	40 (16.8)	12.6–22.1
Trimethoprim–sulfamethoxazole	238	99 (41.6)	19 (8.0)	120 (50.4)	44.1–56.7
Ceftriaxone	238	183 (76.9)	19 (8.0)	36 (15.1)	11.1–20.2
Agents with full resistance per isolate, mean ± SD	2.34 ± 1.20			2.0 (2.0–3.0)	
Distinct AMR genes per isolate (mPCR), mean ± SD	2.82 ± 2.06			3.0 (1.0–4.0)	
Multidrug resistance (≥3 agents), n (%)	105/238			44.1	38.0–50.5

CI, confidence interval; CLSI, Clinical and Laboratory Standards Institute; mPCR, multiplex polymerase chain reaction; SD, standard deviation. Susceptibility was determined by Kirby–Bauer disk diffusion and interpreted against CLSI breakpoints, as the protocol specifies. Percentages are row percentages within each agent and sum to 100 across the susceptible, intermediate and resistant columns.

The originally supplied workbook contained no per-antibiotic susceptibility results at all, only a count of AMR genes, and reported that count for 148 of 181 animals in which no bacterial pathogen had been detected. Antimicrobial resistance is a property of an isolate; an animal with no isolate cannot have one. All AMR fields here are confined to culture-positive animals.

Multidrug resistance is defined as full resistance to at least three of the six agents tested. The conventional definition is non-susceptibility to at least one agent in three or more antimicrobial classes; state which definition is used, and note that the six agents here span five classes, so an agent-count definition and a class-count definition will not always agree.

Where an animal carried more than one bacterial species, the susceptibility result recorded is that of the predominant isolate. In the real study, report susceptibility per isolate rather than per animal, and state how animals with multiple isolates were handled.