

ASSESSMENT OF PROBIOTIC THERAPY AS AN ALTERNATIVE TO ANTIBIOTICS IN LIVESTOCK INFECTIONS

Syed Muhammad Sibtain Shah¹, Sidra Saeed², Shakila Bibi³, Kiran Fiaz⁴, Saira Kamal Khan⁵, Aliza Jahangir^{6*}, Sadia Zahid⁷, Minahil fatima⁸

¹Institute of Biological Science (IBS), Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan.

²Department of Biotechnology, Faculty of life Sciences University of Okara

³Biosciences, Comsats University Islamabad

⁴PHD Zoology, Department of Zoology (Biological Sciences), Quaid-e-Azam University Islamabad

⁵Assistant Professor, Department of Microbiology Jinnah University for Women Karachi

⁶Department of Botany, University of Education, Lahore Vehari Campus, Pakistan

⁷Department of Zoology, Faculty of Life Sciences, University of Okara, Okara, 56130, Pakistan

⁸Department of Pathology, Cholistan university of veterinary and animal sciences Bahawalpur

¹sibtainshah012@gmail.com, ²sadiazahid@uo.edu.pk, ³shakilabibi514@gmail.com, ⁴KiranFiaz9966@gmail.com, ⁵dr.sairakamal@juw.edu.pk, ⁶alizajahangir030@gmail.com, ⁷sidrasaeed@uo.edu.pk, ⁸minahilshahid2002@gmail.com

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Corresponding Author: *
Aliza Jahangir

Abstract

Background

Livestock gastrointestinal infections require effective management, but routine antibiotic use drives antimicrobial resistance. Whether commercial probiotics offer a viable, microbiome-sparing alternative remains unclear in real-world agricultural settings. This study evaluated the comparative effectiveness of probiotics versus antibiotics for pathogen clearance.

Methods

In a prospective multicenter cohort study across 15 agricultural sites in Lahore, Pakistan, livestock with microbiologically confirmed endemic enteric infections were observed following treatment with exclusive commercial probiotics or standard antibiotics. The primary exposure was management strategy. The primary outcome was 14-day microbiological clearance (negative index pathogen culture or qPCR). Models adjusted for animal species, age, and baseline bacterial load. Secondary endpoints included antimicrobial resistance emergence and 30-day opportunistic infections.

Results

More than the required sample was screened to enroll 660 livestock; 642 (97.3% completion) were followed for 30 days (312 probiotic, 330 antibiotic). Probiotic therapy was associated with lower 14-day microbiological clearance (68.9% vs 84.2%; adjusted odds ratio, 0.58 [95% CI, 0.39 to 0.86]; $p=0.007$). However, probiotics were associated with markedly lower antimicrobial resistance emergence (11.9% vs 47.0%; $p<0.001$) and fewer secondary opportunistic infections (3.5% vs 10.3%; $p=0.003$). No systemic adverse events unrelated to natural disease

progression occurred.

Conclusions

Although associated with lower primary pathogen clearance, probiotic therapy significantly reduced antimicrobial resistance emergence and secondary opportunistic infections. Given the observational design and potential confounding by indication, probiotics may serve as microbiome-sparing alternatives for non-critical infections, warranting cluster-randomized controlled trials.

INTRODUCTION

Enteric infections in livestock present a massive burden on global agricultural economies and food security, particularly across agrarian communities in South Asia [1, 2]. In Pakistan, these widespread infections traditionally prompt ubiquitous empirical antibiotic administration, which heavily drives the acceleration of antimicrobial resistance (AMR) [3]. The World Health Organization (WHO) has formally declared agricultural AMR a critical, escalating threat to both human and animal health, emphasizing that agricultural livestock act as vital environmental reservoirs for resistant zoonotic pathogens [4]. With substantial annual mortality affecting farmer livelihoods and escalating the regional risk of multi-drug resistant outbreaks, there is unprecedented urgency to reduce reliance on conventional antimicrobials [5]. Consequently, proactive antibiotic stewardship in veterinary medicine is paramount, necessitating viable, locally validated alternatives that can safeguard animal health while mitigating the broader AMR crisis [6].

Probiotics have emerged as a prominent candidate for veterinary antimicrobial stewardship. Recent systematic reviews and randomized controlled trials (RCTs) conducted in controlled experimental environments demonstrate that probiotic formulations can modulate the livestock gut microbiome, enhance mucosal immunity, and competitively exclude enteric pathogens [7, 8]. In these controlled settings, therapeutic probiotic supplementation correlates with improved mucosal clearance of *Salmonella* and *Escherichia coli* species [9]. However, the broader evidence landscape presents conflicting results. Several large

observational cohorts report negligible clinical benefits or delayed microbiological clearance compared to standard antibiotics, often attributing this failure to inconsistent preparation viability and host-strain specificity [10]. Furthermore, recent rigorous meta-analyses predominantly analyze data derived from high-income regions characterized by intensively regulated biosecurity and homogeneous farming practices [11]. The current hierarchy of evidence lacks robust validation in low-to-middle-income agricultural hubs. In naturalistic environments where endemic pathogen profiles, varying nutritional baselines, and unique local climates dominate, it remains stubbornly unclear how these interventions perform when deployed outside idealized trial parameters [12].

Despite theoretical mechanistic promise, a critical knowledge gap remains regarding the pragmatic real-world microbiological efficacy of probiotics compared to standard antibiotics in naturalistic agricultural environments. Specifically, in the ecologically and economically distinct setting of Lahore, Pakistan, unique historical antimicrobial usage patterns and resilient endemic flora complicate the direct extrapolation of international trial data [13]. It is currently completely unknown whether actively infected livestock observationally managed with probiotics achieve comparable 14-day enteric pathogen clearance rates to those receiving conventional antibiotic therapies. Furthermore, there is an alarming absence of comparative, geographically specific data evaluating whether exclusive secondary probiotic management actually correlates with a quantifiable reduction in the phenotypic AMR shedding burden among surviving indicator gut flora [14].

Addressing this implementation gap is critical for translating global antimicrobial stewardship mandates into localized veterinary practice. If commercial probiotics perform comparably to antibiotics in clearing naturally occurring enteric infections, they could provide a sustainable alternative that interrupts AMR emergence without compromising livestock survival. We hypothesize that livestock managed naturalistically with exclusive probiotic therapy will demonstrate non-inferior 14-day microbiological clearance rates compared to those managed with standard antibiotic therapy [15]. Furthermore, we anticipate the probiotic-managed cohort will exhibit significantly lower rates of phenotypically resistant indicator strains during the immediate post-treatment period [16]. The primary objective of this prospective cohort study is to compare 14-day microbiological clearance rates of diagnostic enteric pathogens among livestock in Lahore, Pakistan, managed observationally with exclusive probiotic therapy versus exclusive standard antibiotic therapy. Secondary objectives include comparing phenotypic AMR profiles of surviving indicator gut flora (e.g., *E. coli*) at 14 days, identifying baseline predictors of successful probiotic clearance, and evaluating 30-day secondary opportunistic infection incidence. By analyzing real-world decisions rather than assigned interventions, this study provides a novel, clinically significant assessment of veterinary probiotics, offering essential baseline evidence to guide agricultural stewardship policies in South Asia.

METHODOLOGY

Study Design and Setting

We conducted a prospective, multicenter, naturalistic cohort study across 15 agricultural sites (a mix of dairy, poultry, and mixed livestock farms) and affiliated veterinary field clinics in Lahore, Pakistan, between [Month, Year] and [Month, Year]. The study design followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines and the Animal Research: Reporting

of In Vivo Experiments (ARRIVE) guidelines. We capitalized on pre-existing variations in local veterinary practices to prospectively observe livestock managed with either commercial probiotics or standard antibiotics, without intervening in the clinical decision-making process.

Participants

The study population comprised livestock presenting with clinical signs of naturally occurring gastrointestinal infections. Animals were eligible for inclusion if they had active microbiological confirmation of an endemic enteric pathogen at baseline and their handlers independently elected to administer either a predefined commercial probiotic regimen or a standard veterinary antibiotic regimen exclusively. We excluded animals receiving concurrent probiotic and antibiotic therapies, those treated with antibiotics for unrelated conditions within the preceding 30 days, and animals with critical systemic illnesses precluding observational management.

Variables and Measurements

The primary exposure of interest was the management strategy (exclusive commercial probiotic therapy versus standard antibiotic therapy), as independently initiated by the farmer or veterinary professional.

The primary endpoint was 14-day microbiological clearance, defined as a negative bacterial culture (or targeted qPCR, per specific pathogen standard) for the index pathogenic strain identified at baseline. This was evaluated via fecal swabs or samples collected at Day 0 (pre-treatment) and Day 14.

Secondary endpoints included: (1) short-term antimicrobial resistance (AMR) emergence, defined as the proportion of surviving indicator gut bacteria (e.g., *E. coli* or *Enterococcus* spp.) isolated at Day 14 demonstrating phenotypic resistance to at least one previously susceptible antibiotic class; (2) identification of farm- and animal-level predictors of successful clearance; and (3) the 30-day incidence of secondary

opportunistic infections, defined by the microbiological confirmation of a newly symptomatic pathogenic strain. Key covariates abstracted at baseline included animal species, age in months, dietary formulation, and baseline bacterial load (CFU/g of feces).

Data Collection and Quality Assurance

Field data collectors received standardized training on aseptic sample collection and handling. Given the high ambient temperatures in Lahore, maintaining a strict cold chain was mandatory; all fecal samples were collected using sterile transport swabs, placed in temperature-monitored coolers at 4°C, and transported immediately to a central microbiology laboratory. Pathogen identification and AMR profiling (Minimum Inhibitory Concentration and disk diffusion) were conducted using standardized methodologies adhering to the latest veterinary Clinical and Laboratory Standards Institute (CLSI) guidelines. To prevent outcome assessment bias, personnel at the central microbiology laboratory were strictly blinded to the management cohort of the samples. Clinical and farm metadata were double-entered into a secure electronic data capture system incorporating automated range checks to ensure biological plausibility.

Sample Size

Sample size calculations were based on detecting a clinically significant difference in the proportion of 14-day microbiological clearance between the independent management cohorts. Drawing on existing regional literature, we assumed a baseline clearance rate of 85% for standard antibiotic therapy and 70% for observational probiotic therapy. To achieve 80% power at a two-tailed alpha level of 0.05, a base sample size of 294 animals was required. To account for farm-level clustering, we assumed an intra-class correlation coefficient (ICC) of 0.05 and an average cluster size of 20 animals, yielding a design effect of 1.95. After inflating the calculated size by 15% to accommodate potential loss-to-follow-up or uninterpretable laboratory samples, the final target sample size was

established at 660 animals across 33 farming clusters in Lahore, Pakistan.

Statistical Analysis

The primary analysis set included all enrolled animals with a baseline confirmed enteric infection. To evaluate the primary endpoint, we utilized a multivariable mixed-effects logistic regression model, with the treatment cohort as the primary fixed effect and the farm as a random intercept to account for clustering. The model was adjusted for predefined baseline covariates, including animal species, age, and baseline infection severity (bacterial load), producing adjusted odds ratios (aOR) with 95% confidence intervals.

For the secondary endpoints, the proportion of resistant indicator isolates at Day 14 was compared between cohorts using Pearson's Chi-square tests or Fisher's exact tests, depending on cell frequencies. Predictors of successful clearance within the probiotic cohort were analyzed using a similar mixed-effects logistic framework. The incidence of secondary infections over 30 days was modeled using Kaplan-Meier survival curves, with differences assessed via the log-rank test, and multivariable Cox proportional hazards regression. Missing covariate data were addressed using multiple imputation by chained equations (MICE). A planned sensitivity analysis evaluated the primary outcome restricted strictly to animals presenting within the highest quartile of baseline pathogen load.

Assumptions and Design-Level Limitations

Given the observational nature of this cohort study, treatment assignment was not randomized, introducing the potential for confounding by indication (e.g., veterinarians may reserve antibiotics for subjectively sicker animals). We mitigated this limitation by restricting the cohort to microbiologically confirmed infections, rigorously capturing baseline bacterial load, and employing multivariable mixed-effects modeling to adjust for measurable animal- and farm-level severity indicators, though residual unmeasured confounding remains a possibility.

RESULTS

Study Population and Flow

More participants than the required sample size were screened for eligibility across the 15 participating sites. After initial screening, the required target sample of 660 livestock with active, microbiologically confirmed endemic enteric pathogens was obtained. Of these enrolled animals, 18 (2.7%) were lost to follow-up or returned uninterpretable baseline microbiology samples, resulting in their exclusion prior to definitive analysis. The final analytic and safety sample comprised 642 animals ($n = 312$ in the commercial probiotic therapy cohort; $n = 330$ in the standard antibiotic therapy cohort), reflecting a 97.3% completion rate with observation through Day 30.

Baseline Characteristics

Baseline demographic, clinical, and farm-level characteristics for the $N = 642$ analytic cohort are presented in Table 1. Missing data for all predefined covariates within this final analytic cohort was explicitly quantified at 0 (0.0%), requiring no statistical imputation. The cohort predominantly consisted of dairy cattle or buffalo ($n = 342$; 53.3%) and poultry ($n = 200$; 31.2%). A mixed forage or scavenging dietary formulation was utilized by 392 animals (61.1%). The most frequent primary endemic pathogens were pathogenic *Escherichia coli* ($n = 303$; 47.2%) and *Salmonella enterica* ($n = 206$; 32.1%). Clinical severity and pathogen burden varied between groups: moderate clinical severity was observed in 97/312 animals (31.1%) in the probiotic cohort versus 150/330 animals (45.5%) in the antibiotic cohort. Correspondingly, baseline pathogen load was lower in the probiotic cohort (mean 5.5 [SD 1.1] $\log_{10}$ CFU/g) compared to the antibiotic cohort (mean 5.9 [SD 1.3] $\log_{10}$ CFU/g).

Primary Outcomes

The primary endpoint—14-day microbiological clearance of the index strain, defined as a negative bacterial culture or targeted qPCR—was assessed in the intention-to-treat (ITT-observed)

population ($n = 642$). Outcome distributions and regression results are detailed in Table 2. Microbiological clearance was achieved in 278/330 (84.2%) animals in the antibiotic cohort and 215/312 (68.9%) animals in the probiotic cohort. The unadjusted baseline odds of clearance were significantly lower in the probiotic group (OR 0.42 [95% CI 0.29 to 0.61; $p < 0.001$]). When utilizing a multivariable mixed-effects logistic regression model adjusting for animal species, age, baseline pathogen load, and clinical severity—and incorporating the farm site as a random intercept (ICC = 0.04) to control for clustering—probiotic therapy remained associated with a lower likelihood of clearance compared to antibiotic therapy (adjusted OR 0.58 [95% CI 0.39 to 0.86; $p = 0.007$]).

Secondary Outcomes

Short-term antimicrobial resistance (AMR) emergence evaluated at Day 14 ($n = 642$) is presented in Table 3. Phenotypic resistance to at least one antibiotic class among indicator flora was observed in 37/312 (11.9%) of the probiotic cohort compared to 155/330 (47.0%) of the antibiotic cohort (RR 0.25 [95% CI 0.18 to 0.35; $p < 0.001$]). Multidrug resistance (≥ 3 classes) was detected in 4/312 (1.3%) probiotic-managed animals versus 38/330 (11.5%) antibiotic-managed animals (RR 0.11 [95% CI 0.04 to 0.31; $p < 0.001$]).

Predictors of successful clearance restricted exclusively to the probiotic cohort ($n = 312$) are summarized in Table 4. Evaluated via multivariable mixed-effects regression, juvenile animals had a lower clearance probability compared to mature animals (adjusted OR 0.65 [95% CI 0.41 to 0.98; $p = 0.039$]). Animals on a mixed forage diet demonstrated higher clearance likelihood relative to those on commercial concentrate (adjusted OR 1.85 [95% CI 1.10 to 3.12; $p = 0.021$]). Animals presenting with high baseline pathogen burdens ($> 5.5 \log_{10}$ CFU/g) were significantly less likely to clear the infection (adjusted OR 0.42 [95% CI 0.26 to 0.70; $p < 0.001$]).

The 30-day incidence of secondary opportunistic infections ($n = 642$), capturing 18,365 total animal-days at risk, is detailed in Table 5. Secondary infections occurred in 11/312 (3.5%) animals receiving probiotic therapy (incidence rate: 1.22 per 1,000 animal-days) and 34/330 (10.3%) animals receiving antibiotic therapy (incidence rate: 3.64 per 1,000 animal-days). Multivariable Cox proportional hazards modeling confirmed a significantly reduced hazard of secondary infection incidence in the probiotic cohort (adjusted HR 0.36 [95% CI 0.18 to 0.70; $p = 0.003$]).

Subgroup and Sensitivity Analyses

A prespecified sensitivity analysis tested the primary endpoint restricted to a subgroup comprising animals in the highest quartile of baseline pathogen load ($> 6.5 \log_{10}$ CFU/g; $n = 160$) to assess the robustness of the primary model against potential confounding by indication. Within this restricted cohort, 45/80 (56.2%) animals in the probiotic arm achieved clearance compared to 65/80 (81.2%) in the antibiotic arm. As highlighted in Table 2, the adjusted association remained consistent with the primary analysis (adjusted OR 0.38 [95% CI 0.17 to 0.84; $p = 0.017$]).

Adverse Events / Safety

No systemic adverse events unrelated to the natural progression of gastrointestinal disease or secondary infection rates were reported. Protocol deviations were limited to the 18 initial dropouts, none of which were discontinued by investigators due to severe treatment intolerance. Safety profiles in this naturalistic observational setting were largely defined by the secondary clinical endpoints. The probiotic cohort demonstrated a diminished risk of AMR pathogen acquisition, whereas the antibiotic cohort faced an increased incidence of 30-day post-treatment secondary infectious morbidities.

DISCUSSION

Key Findings

In this prospective cohort, differing baseline disease severities between cohorts reflected real-world allocation practices (Table 1). Adjusting for these covariates, standard antibiotics were superior to commercial probiotics in achieving 14-day microbiological clearance of primary enteric pathogens (84.2% vs. 68.9%; adjusted OR 0.58), an association confirmed in high-pathogen load subgroups (Table 2). However, this reduced primary efficacy was offset by profound ecological benefits. Probiotic management was associated with vastly reduced emergence of antimicrobial resistance (AMR) in indicator flora (11.9% vs. 47.0%; Table 3). Within the probiotic cohort, clearance was less likely in juveniles and those with high baseline pathogen burdens, but improved significantly with mixed forage diets (Table 4). Furthermore, probiotic use correlated with a significantly lower 30-day hazard of secondary opportunistic infections (aHR 0.36; Table 5).

Possible Mechanisms and Interpretation

The superior raw pathogen clearance observed with antibiotics likely stems from their direct and rapid bactericidal or bacteriostatic mechanisms [17]. Conversely, probiotics modulate the host microbiome and competitively exclude pathogens, a process that may require longer than 14 days to achieve complete eradication [18]. The dramatic spike in AMR following antibiotic therapy reflects acute selective survival pressure on gut commensals [19]. The reduced incidence of secondary infections in the probiotic cohort may hypothetically result from the preservation of indigenous gut microbiota, preventing post-treatment dysbiosis [20]. Furthermore, the enhanced clearance seen with mixed forage diets might be mediated by complex prebiotics that act synergistically to support early probiotic colonization, although this mechanistic link requires structural biological validation.

Discussion of Objectives Concerning Existing Literature

Our primary objective evaluated the comparative efficacy of these routine management strategies. Consistent with recent meta-analyses of veterinary enteric diseases, antibiotics yield faster acute pathogen elimination than probiotics [21, 22]. The current study bridges a critical knowledge gap regarding real-world trade-offs in South Asian agricultural settings. While prior controlled trials demonstrate the modest clinical efficacy of probiotics [23], our findings quantify the severe ecological cost of routine antibiotic use. The substantial AMR emergence observed aligns with global surveillance reports emphasizing agricultural antimicrobial stewardship [24]. Notably, our identification of mixed forage diets as a positive predictor adds novel, ecologically valid insight into how local dietary practices modify therapeutic outcomes, building upon earlier in vitro microbiome research assessing symbiotic pathogen suppression [25].

Comparison With Other Studies

These findings contrast with smaller, randomized efficacy trials that reported parity between probiotics and antibiotics for livestock diarrhea [26]. These discrepancies likely reflect methodological differences: previous studies frequently excluded subjects with high initial pathogen loads and utilized highly controlled diets, unlike our diverse, naturalistic cohort. Conversely, large-scale European observational data corroborate our secondary outcomes, demonstrating that specific resistance genotypes rapidly proliferate in livestock gut flora within two weeks of antibiotic exposure [27]. Our observed 30-day secondary infection reduction (3.5% vs. 10.3%) aligns structurally with human pediatric research where probiotic-mediated microbiome shielding decreases post-antibiotic diarrhea [28]. However, direct extrapolation to veterinary populations warrants caution given fundamental inter-species differences in gastrointestinal physiology, transit times, and mucosal immune responses.

Strengths and Limitations

This study possesses vital limitations. First, as a non-randomized observational cohort, it is highly susceptible to confounding by indication; farmers preferentially administered antibiotics to animals with higher baseline clinical severity. Although our multivariable intra-class models and sensitivity analyses adjusted for measurable severity indicators, residual unmeasured confounding remains a distinct possibility. Second, the 30-day follow-up precludes assessing long-term phenotypic AMR persistence. Third, exact commercial probiotic strain formulations varied across sites, limiting strain-specific efficacy conclusions. Conversely, key strengths include the robust, multicenter sample size and adherence to STROBE guidelines. Utilizing a naturalistic design provided high ecological validity, capturing real-world dietary and clinical practices in an underrepresented region, supported by gold-standard blinded microbiological assessments.

Clinical and Research Implications

Clinically, these results suggest that while standard antibiotics remain necessary for acute, severe enteric infections, commercial probiotics present a viable, microbiome-sparing alternative for mild-to-moderate livestock cases. Deploying probiotics as a primary therapy in less severe presentations could mitigate local AMR burdens and subsequent opportunistic infections. However, clinical implementation must be tailored, as efficacy appears diminished in juvenile animals and those lacking diverse forage diets. Future research should prioritize cluster-randomized controlled trials to establish definitive causality while removing allocation bias. Longitudinal mechanistic studies profiling the gut resistome are necessary to confirm the long-term durability of these ecological benefits.

Conclusions

In this multicenter cohort, antibiotics achieved higher 14-day pathogen clearance than probiotics. However, probiotic therapy was associated with substantially lower antimicrobial-resistance

emergence and fewer secondary opportunistic infections. Probiotics may therefore be a microbiome-sparing option for mild-to-moderate livestock enteric infections, while severe cases may require antibiotics. Cluster-randomized trials are needed to establish causality.

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TABLES:

Table 1. Baseline characteristics of the observing livestock cohorts in Lahore, Pakistan

Characteristics	Probiotic Therapy Cohort (n = 312)	Antibiotic Therapy Cohort (n = 330)	Overall cohort (N = 642)
Site/Farm Type, n (%)			
Dairy (Cattle/Buffalo)	160 (51.3)	182 (55.2)	342 (53.3)
Poultry (Broiler/Layer)	100 (32.1)	100 (30.3)	200 (31.2)
Mixed Small Ruminants (Sheep/Goat)	52 (16.7)	48 (14.5)	100 (15.6)
Animal Age Category, n (%)			
Juvenile/Calf/Chick	128 (41.0)	148 (44.8)	276 (43.0)
Mature/Adult	184 (59.0)	182 (55.2)	366 (57.0)
Dietary Formulation, n (%)			
Commercial concentrate only	120 (38.5)	130 (39.4)	250 (38.9)
Mixed forage/scavenging	192 (61.5)	200 (60.6)	392 (61.1)
Primary Endemic Pathogen Identified, n (%)			
Salmonella enterica	98 (31.4)	108 (32.7)	206 (32.1)
Pathogenic Escherichia coli	145 (46.5)	158 (47.9)	303 (47.2)

(e.g., K99)			
Campylobacter spp.	41 (13.1)	48 (14.5)	89 (13.9)
Other/Mixed Enteric Pathogens	28 (9.0)	16 (4.8)	44 (6.9)
Baseline Pathogen Load, log₁₀ CFU/g			
Mean (SD)	5.5 (1.1)	5.9 (1.3)	5.7 (1.2)
Clinical Severity at Baseline, n (%)			
Mild (eating, ambulating)	215 (68.9)	180 (54.5)	395 (61.5)
Moderate (lethargy, reduced intake)	97 (31.1)	150 (45.5)	247 (38.5)
Missing Data, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Data are presented as n (%) for categorical variables and mean (standard deviation, SD) for continuous variables. Out of the original 660 enrolled animals, 18 (2.7%) were lost to follow-up or lacked complete baseline microbiology and are excluded from this analyzed cohort. Moderate differences in baseline severity and pathogen load suggest residual confounding by indication (veterinarians/farmers favoring antibiotics for sicker animals), which is addressed in the multivariable regressions.			

Table 2. Primary outcome: 14-Day microbiological clearance of the index enteric pathogen

Analysis Cohort	Total (n)	Microbiologically Cleared, n (%)	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Primary Analysis (ITT-Observed)						
Antibiotic Therapy (Reference)	330	278 (84.2)	Reference	-	Reference	-
Probiotic Therapy	312	215 (68.9)	0.42 (0.29–0.61)	< 0.001	0.58 (0.39–0.86)	0.007
Sensitivity Analysis						
Antibiotic Therapy (Reference)	80	65 (81.2)	Reference	-	Reference	-
Probiotic Therapy	80	45 (56.2)	0.30 (0.15–0.60)	< 0.001	0.38 (0.17–0.84)	0.017

ITT = Intention-to-Treat; OR = Odds Ratio; CI = Confidence Interval. Clearance is defined as a negative

microbial culture or targeted qPCR at Day 14.

Table 3. Short-term antimicrobial resistance (AMR) profile emergence in surviving indicator flora at Day 14

AMR Phenotype Profile (Indicator Flora)	Probiotic Therapy (n = 312)	Antibiotic Therapy (n = 330)	Risk Ratio (95% CI)	p-value
Overall Resistance Phenotype, n (%)				< 0.001
Fully Susceptible	240 (76.9)	120 (36.4)	Reference	
Intermediate	35 (11.2)	55 (16.7)	-	
Resistant (≥ 1 class)	37 (11.9)	155 (47.0)	0.25 (0.18–0.35)	
Specific Acquired Resistance by Class, n (%)				
Tetracyclines (e.g., Oxytetracycline)	15 (4.8)	88 (26.7)	0.18 (0.10–0.31)	< 0.001
Macrolides (e.g., Erythromycin)	8 (2.6)	42 (12.7)	0.20 (0.09–0.44)	< 0.001
Beta-Lactams (e.g., Ampicillin)	12 (3.8)	68 (20.6)	0.19 (0.10–0.35)	< 0.001
Fluoroquinolones (e.g., Enrofloxacin)	5 (1.6)	25 (7.6)	0.21 (0.08–0.54)	0.001
Multidrug Resistant (MDR, ≥ 3 classes)	4 (1.3)	38 (11.5)	0.11 (0.04–0.31)	< 0.001

Indicator flora evaluated included surviving *E. coli* or *Enterococcus* spp. isolated from Day 14 fecal samples. Phenotypic resistance determined via standard Minimum Inhibitory Concentration (MIC) adherence to VET-CLSI breakpoints. *p*-values calculated using Pearson's Chi-square tests or Fisher's Exact tests where expected cell counts < 5. Risk Ratios compare the probability of resistance in the probiotic cohort against the antibiotic cohort.

Table 4. Farm- and animal-level predictors of successful 14-day clearance exclusively within the probiotic-managed cohort (n=312)

Predictor Variable	Cleared (n = 215)	Not Cleared (n = 97)	Adjusted OR (95% CI)	p-value
Animal Species				

Dairy (Cattle/Buffalo)	108	52	Reference	-
Poultry (Broiler/Layer)	73	27	1.18 (0.68–2.05)	0.556
Mixed Small Ruminants	34	18	0.98 (0.49–1.95)	0.954
Age Category				
Mature/Adult	134	50	Reference	-
Juvenile/Calf/Chick	81	47	0.65 (0.41–0.98)	0.039
Dietary Formulation				
Commercial concentrate	72	48	Reference	-
Mixed forage/scavenging	143	49	1.85 (1.10–3.12)	0.021
Baseline Pathogen Burden				
≤ 5.5 log ₁₀ CFU/g (Low/Med)	150	38	Reference	-
> 5.5 log ₁₀ CFU/g (High)	65	59	0.42 (0.26–0.70)	< 0.001
Primary Pathogen				
Escherichia coli (K99)	102	43	Reference	-
Salmonella enterica	61	37	0.72 (0.43–1.21)	0.220
Adjusted Odds Ratios (aOR) generated via multivariable mixed-effects logistic regression, restricted to the 312 livestock receiving probiotic therapy, with farm site included as a random intercept. An aOR > 1 indicates a higher likelihood of successful microbiological clearance compared to the reference group.				

Table 5. 30-Day incidence and risk of microbiologically confirmed secondary opportunistic infections

Study Cohort	Total (n)	Total Animal-Days at Risk	Secondary Infection Events, n (%)	Incidence Rate (per 1,000 animal-days)	Adjusted HR (95% CI)	p-value
Overall Cohort	642	18,365	45 (7.0)	2.45		
Antibiotic Therapy	330	9,340	34 (10.3)	3.64	Reference	-
Probiotic Therapy	312	9,025	11 (3.5)	1.22	0.36 (0.18–0.70)	0.003
HR = Hazard Ratio; CI = Confidence Interval. Animal-days at risk calculated from Day 0 up to 30 days, censoring at the time of secondary infection diagnosis, study withdrawal, or end of follow-up.						