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Preliminary Study on the Effective Microbial Supplementation of Feed on the Infection of *Salmonella* in Two Lymph Nodes of Beef Cattle in Eastern Ethiopia

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Abstract

A double-blinded randomized controlled field trial based on parallel group design was conducted from January, 2018 to July, 2018 in Chercher Oda-Bultum Farmers Union beef Farm. The present study was conducted to evaluate the roll of effective microbial supplementation to feed on the infection of *Salmonella* in the mesenteric and sub-iliac lymph nodes of beef cattle. In order to undertake the study, 130 beef cattle kept by the farm were used to establish a cohort. The study animals were randomly assigned to the treatment group (n = 100) and control group (n = 30). The feed of treatment group was mixed with EM at dose of 5×10^{10} cfu/day/head for 90, 100 and 115 days while that of the control group was mixed with molasses, which acts as placebo. Both the treatment and control were slaughtered and two lymph nodes were collected from each animal under strict sterile condition and processed for the isolation and identification of *Salmonella* using standard procedure. The occurrence of *Salmonella* was 70% (CI = 51% - 85%) in control group while it was 33% (CI = 24% - 43%) in treatment group. The difference in the proportion of *Salmonella* infection in the two group was significant (x^2 = 13.01; p = 0.000). The relative risk of *Salmonella* isolation in the control was 2.12 (1.41 - 3.20) compared to treatment group. The absolute and relative risk reduction in the treatment were 37% (CI = 17% - 57%) and 53% (CI = 29% - 69%), respectively. This preliminary study indicated that effective microbial supplementation of beef cattle feed reduced the occurrences of *Salmonella* in the lymph node of beef cattle, thereby potentially minimizing the economic and public health impacts of *Salmonella* infection. Then, it was recommended to use EM as prevention and control option in *Salmonella* carriage in cattle.

Keywords

Salmonella, Lymph Node, Effective Microbial, Risk Reduction

1. Introduction

Gradual increase in world population and change in lifestyles have resulted in demands for quality oriented foods of animal origin. Meanwhile, the number of incidences of food poisoning cases is increasing throughout the world. On the other hand, ensuring food safety to protect public health and promote economic development remains a significant challenge in both developing and developed countries. Considerable progress to strengthen food safety systems has been achieved in many countries, highlighting the opportunities to reduce and prevent food-borne disease. However, unacceptable rates of food borne illness still remain and new hazards continue to enter the food supply [1]. In this regard, many emerging and re-emerging pathogens those associated with fresh or raw meat can be mentioned including *Salmonella* [2].

In Ethiopia, like other developing countries, it is difficult to evaluate the burden of food-borne pathogens. This is because of the limited scope of studies and lack of coordinated epidemiological surveillance systems. In addition, under-reporting of cases and the presence of other diseases considered to be of high priority may have overshadowed the problem of food-borne pathogens [3] [4]. The widespread habit of raw beef consumption is a possible potential cause for the spread of food-borne illnesses in Ethiopia [4].

The current principles of HACCP could not answer *Salmonella* related food safety issues [5]. Current estimates indicate that exposure to non-typhoidal *Salmonella* results in 93.76 million GIT illnesses and 155,000 deaths worldwide each year [6]. These and related issues rise the necessity of establishing important food safety measures.

In Ethiopia, the prevalence of *Salmonella* 26.6%, 23.5%, and 8.8% has been reported in abattoir line, animals' feces and lymph nodes respectively [7]. Positive results from the lymph nodes indicate the infection status of the animals. Positive environmental samples ranged between 30.7% in knives and 60% in refrigerators. The same study reported 8.3%, 45.5% and 32.4% *Salmonella* prevalence from cleaning water, meat transporting track and raw beef from butcheries respectively [7]. Approximately the same rates were reported in the same or related chain of beef in Ethiopia [8] [9] [10]. The primary source of *Salmonella* for cattle occurs at the farm level. On-farm control of *Salmonella* may thus contribute to the whole food chain continuum of measures for reducing the food safety risk of *Salmonella* in beef [6]. Therefore, the purpose of this study was to provide prevention and control options for *Salmonella* carriage in cattle. The Effective Microbial (EM) supplement to the diet of beef cattle was used as a means of

intervention, to reduce the pathogen in cattle thereby ground beef.

2. Materials and Methodology

2.1. Description of the Study Area

The study was carried out in Oda Bultum district of Western Harerghea Zone, Oromia Regional State; Eastern Ethiopia. The specific site was Charcher Oda Bultum Farmers Cooperative Union farm, which is found in Gode-Hora sub-district. Oda Bultum district is located at approximately 375 km far from Addis Ababa and 50 km from zonal capital, Chiro. Geographically this area has an altitude of 1400 - 3100 m.a.s.l and the specific location of the site is provided bellow (Figure 1). The area has a mean temperature ranging from 22˚C - 28˚C. It receives an average annual rainfall of 900 mm - 1200 mm with bimodal distribution of the seasonal pattern peaking in mid-April and mid-August of the year; however there is a variation from year to year [11]. Currently, the Farm is being operated with 200 local breed in fattening, 50 Cross breed and 140 Borena breed in dairy Farms. The Farm is equipped with production facilities like feed chopper, feed mixer, milking machine, basic veterinary equipment for clinical diagnosis and modern housing for both fattening and dairy which is suitable lay out for the purpose of the study.

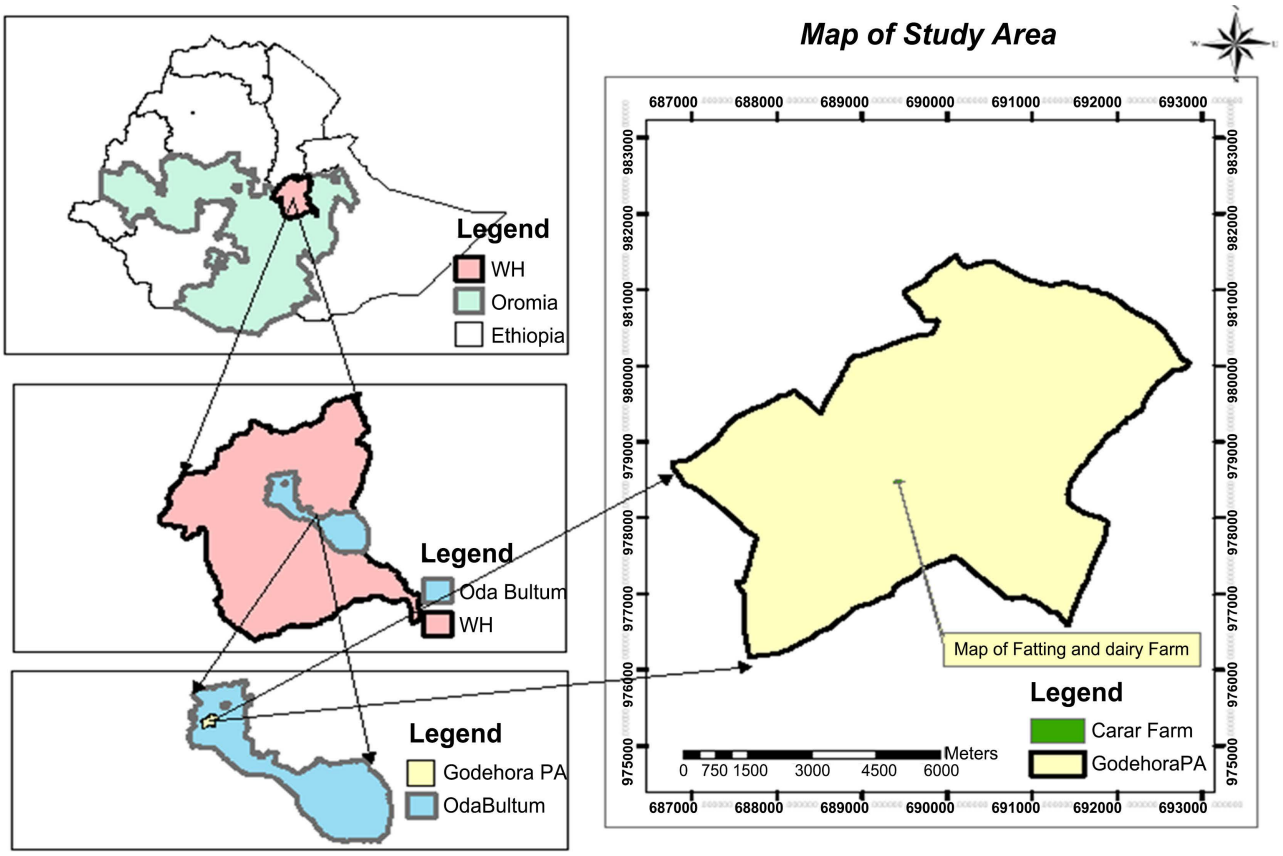

Figure 1. The study site location in Ethiopia.

2.2. Study Animals

In order to meet the specific objective, analogous to [12], the study was conducted in one commercial feedlot setting with two pens of 100 and 30 animals each. The Farm is found in the study area stated above. All the bulls of study subjects were zebu breed of those mainly produced by the local small holders. They were bought from the local markets Baddessa town surrounded by highlands whereas Boke, Gabiba and Milkae were from Wabi-Shebelle basin lowland areas of Harerghea where these three lowland areas are 30 km, 60 km and 120 km distance from the study Farm. The production system in low land is pastoral and in highland managed under zero grazing by the small holders [13]. The Chercher Oda-Bultum Farmers Union collect these animals for the purpose of finishing and supplying beef to abattoir or butchers of central Ethiopian markets like Addis Ababa, Mojo, Adama and large institutes including Haramaya and Oda-Bultum Universities.

For connivance of the following exposed and non-exposed animal at abattoir, all exposed animals and the randomized control groups, which were slaughtered at Haramaya University Abattoir, were examined for samples.

2.3. The Study Animal's Management

Body condition scoring [14], and age determination of the study animals were done according to the standards developed by Canadian Food Inspection Agency [15]. Both control and treatment cohorts of feedlot cattle were received a starter diet and a finishing diet during the feeding period. The treatment diets were differed from the control cattle diet by the addition of EM · 1© inoculants (EM Research Organization Japan, Inc. #3600-01-007771) and the control group was used molasses as placebo as the color of two liquids are similar as well as used as owner blinding.

The product was supplied by *EM-Woljejii Agricultural Industry PLC*, which is accredited distributer in domestic market. They were supplied in the form of feed mixed with EM, molasses and warm water (chlorine free) in the ratio of 1:1:18 liter, according to manufacturer's recommendation, with the target dose being 5×10^{10} cfu/day/head of *Lactobacillus* bacteria [16]. For 90,100 and 115 days based on batch of animals to be slaughtered. Treatment and control diets were administered for the duration of the feeding period and separate feeding trucks were used to administer the two different diets. Other than the treatment feed for treatment groups, the rest were the same in terms of natural challenge and local feed including hay, teff straw and "frushka", coffee husk. Close supervision and monitoring were in place by using tools like checklists.

2.4. The Study Design and Sampling Method

Double blinded parallel-group-designed and randomized controlled field trial (RCFT) were conducted in which the treatment EM were supplemented to treatment group (n = 100) and control (n = 30) animals in each pen. Within

pens the animals were clustered based on their biological differences considering animal age determination [15], body condition [14], body weight, sources and exposure time. Hence, all animals were tagged and registered for onset of the study. For these purpose, animals' attendants and employed workers were trained on how to prepare and feed EM-microbial inoculants.

Following the study animals at study abattoir in both cohorts of the study, one sample of SLN and one sample MLN per carcass were collected from both treatment and control groups of animals at study abattoir immediately after slaughter. Thus, 130 samples of LNs each from both groups of animal (N = 260) were collected for laboratory examination. The cattle were slaughtered in three groups and therefore housed at the feedlot for 90, 100, and 115 days of exposure, respectively based on national fattening package [17]. Thus, pair of samples (SLN and MLN) from each the sampled animal were collected aseptically and separately.

The Farm was sampled by convenience sampling as it was accessible, manageable and convenient for group harvesting and sample collection within the project time frame.

2.5. Sample Size Determination

The prospective randomized control field trial in parallel-groups-designed study based on feed supplemented by EM and non-EM supplemented group. Sample size was calculated by using the formula given by [18], which is appropriate in comparison of effects.

$$N = 0.25/\mathrm{SE}^2,$$

where: N = sample size, SE (standard error) = 5%; Hence, the required sample sizes were (n = 100) for treatment and (n = 30) animals for control cohort. Assuming that, confidence interval (CI) = 95%; desired absolute precision (d) = 0.05; Power (P) = 96%.

2.6. Sample Collection

Following specific identification given during the feeding, the samples of SLN and MLN were aseptically collected and registered with same identification code used while animals were alive at the farm. On 90th day (n = 42), 100th day (n = 40) and 115th day (n = 48) animals were slaughtered from which (n = 84), (n = 80) and (n = 96) samples were collected separately on 90th, 100th and 115th days respectively. The samples were transported to Veterinary Microbiology Laboratory, College of Veterinary Medicine of Haramaya University for immediate process on the date of sampling. Sample collection and processing were done aseptically (flaming the sampled LNs before processing) but blinded using the coding system that has been given at the beginnings of study. Thus, codes were lifted in to Excel sheet after data collection in order to conduct statistical analysis.

2.7. *Salmonella* Isolation and Characterization

Sampled LNs were processed as previously described [12] [19]. Surrounding fat and fascia were trimmed from LN samples, which were weighed, surface sterilized by surface flaming, placed into individual filtered sample bags, and pulverized using a stomacher (model: 400 stomacher, Seward, Worthington, UK) at 230 rpm for 2 minutes. The isolation and identification of *Salmonella* were undertaken following conventional cultural methods. Briefly, each processed sample was pre-enriched in BPW (BM020, Sisco Research Laboratories; India), (1: 9) and incubated for 16 - 20 h at 37˚C. From the pre-enrichment broth, 100 micro liters were transferred into 9.9 ml of Rappaport Vassilliadis (RV) (Oxoid) broth and incubated at 42˚C for 24 h. A loop full of the inoculums from RV was streaked side by side onto Xylose Lysine Deoxycholate agar (XLD) (M031-500G, HiMedia Laboratories Pvt. Ltd.), and brilliant green phenol red lactose sucrose (BPLS) (Merck) agar plates and incubated at 37˚C for 24 h. The Presumptive *Salmonella* colonies were purified on fresh nutrient agar (HiMedia, India) and further characterized using conventional biochemical tests. Isolated *Salmonella* colonies were inoculated onto triple sugar iron agar (TSI) (M021-500G, HiMedia Lab. Pvt. Ltd., India) lysine iron agar (LIA) (CM081, Oxoid Ltd., England) Simmon's citrate (M099, HiMedia Lab. Pvt. Ltd., India) and Moreover, two or more colonies from pure isolates were inoculated on urea broth (SRL, India) and incubated at 37˚C for 24 h for confirmation according to [20].

2.8. Data Analysis

Raw data were interred to Microsoft Excel 2007© and analyzed using STATA 12.1. For qualitative data, r/n (events/trials) binomial response variables was created for each control and treatment groups, where r is the number of positives and n is the number of lymph nodes to be assayed. Cohort study risk ratio was used grossly at pen level relative risk estimation. Multi-level-mixed effects Model, extension to Mixed-effects linear regression was constructed in which risk factors were considered a random variable and used to screen the potential confounding factors. Model estimation was achieved using maximum likelihood method and Wald Chi^2. To account for potential within and among pen dependency residuals (*i.e.*, clustered outcomes) a random effect regression model was used. The mean prevalence for the treatment by time interaction was analyzed and data were used for estimation of percent efficacy. Relative risks (RR), relative risk reductions (RRR) and absolute risk (AR) at 95% confidence intervals (C.I. 95%) were calculated where ($p < 0.05$) was considered as significant *Salmonella* prevalence.

3. Result and Discussions

3.1. *Salmonella* in the Study Animals

The total number of lymph nodes ($N = 260$), where, $n = 130$ and 130 of them

were MLN and SLN respectively; 200 were collected from 100 cattle administered EM2 (Treatment) and 60 were collected from 30 cattle in the control group. A total of 33/100 (33%) and 21/30 (70%) LNs collected from cattle in the treatment and control groups were found *Salmonella* positive respectively. A greater percent of positives (70%) were observed LNs collected from cattle in the control group (Table 1).

A significant reduction in *Salmonella* prevalence in LNs (MLN and SLN) was observed from cattle administered EM ($p = 0.000$). The risk of *Salmonella* harborage in LNs of EM supplemented group was 53% less than the counterpart and 37% of the risk could be reduced by EM supplementation (Table 1). On a percentage basis the amount of positive animals in the treatment group was 33%. A higher percentage of positives 70% were observed in cattle from control group. These results agree with the slight difference in treatment group from similar study by [13], which reported prevalence of 57% and 76.3% in treatment and control respectively.

In this study relative reduction in the prevalence of *Salmonella* within MLN and SLNs was observed in the study cattle presented for harvest after treated by EM. The data reported herein indicate that administering EM to cattle during the feeding period has an effect in reduction of *Salmonella* detected in MLN and SLNs of beef cattle. These preliminary data are impactful for the beef industry as well as public health, since lymph nodes, including SLNs within beef are commonly incorporated into beef trim destined for ground beef production.

As shown in (Table 2), variable proportions of *Salmonella* were observed in MLN and SLN of both control and EM-feed animals except for those EM-feed for 115 days those with good body condition. As age of animals increases the prevalence of *Salmonella* become increasing in both MLN and SLN of control groups but reduction in SLN of EM-feed animals were observed. Significant reductions in *Salmonella* prevalence with increase in treatment time for EM-feed group were observed.

3.1.1. *Salmonella* Risk Reduction in Mesenteric Lymph Node (MLN)

Variations in positive MLNs by other risk factors like age group, body condition, body weight, source of animals and duration of time in treatment were observed,

Table 1. The effect of feeding of EM in clearing of salmonella in specific lymph node of studied animals.

Measures Effect Variables	No of study animals	Proportion of *Salmonella* 95% CI*	χ^2	P-Value
Non-Treated Group (Control)	30	21/30 (0.70), [0.51 - 0.85]	13.01	0.000
EM Feed Group (Treatment)	100	33/100 (0.33), [0.24 - 0.43]		
Risk Difference (Absolute Risk)	*	0.37 (0.17 - 0.57)		
Relative Risk	*	2.12 (1.41 - 3.20)		
Relative Risk Reduction	*	0.53 (0.29 - 0.69)		

CI* = Confidence Interval.

Table 2. Prevalence of salmonella in specific lymph node across studied risk factors in studied animals.

	Proportions of *Salmonella* positive in (MLN)*		Proportions of *Salmonella* positive in (SLN)**	
Risk factors	**Control**	**Treatment**	**Control**	**Treatment**
Age (Years)				
2 - 3.5	6/11 (0.55)	6/37 (0.16)	3/11 (0.27)	8/37 (0.22)
3.5 - 4.5	6/12 (0.50)	9/55 (0.16)	8/12 (0.67)	12/55 (0.23)
>4.5	7/7 (1.00)	2/8 (0.25)	5/17(0.71)	1/8 (0.13)
Body Condition				
Poor	11/14 (0.78)	6/35 (0.17)	9/14 (0.64)	11/35 (0.31)
Medium	6/10 (0.60)	10/55 (0.18)	7/10 (0.70)	8/55 (0.15)
Good	2/6 (0.33)	1/10 (0.10)	0/6 (0.00)	2/10 (0.20)
Body weight				
177 - 200	7/13 (0.54)	8/40 (0.20)	6/13 (0.46)	10/40 (0.25)
200 - 214	9/11 (0.82)	2/15 (0.13)	8/11 (0.72)	4/15 (0.27)
214 - 225	3/6 (0.50)	7/45 (0.16)	2/6 (0.33)	7/45 (0.16)
Animals Source				
Low Land	10/17 (0.59)	12/61 (0.19)	10/17 (0.59)	15/61 (0.25)
Highland	9/13 (0.69)	5/39 (0.13)	6/13 (0.46)	6/39 (0.15)
Treatment time				
90 days	6/9 (0.67)	11/33 (0.33)	5/9 (0.56)	12/33 (0.36)
100 days	6/9 (0.67)	6/31 (0.19)	6/9 (0.67)	8/31 (0.26)
115 days	7/12 (0.58)	0/36 (0.00)	5/12 (0.42)	1/36 (0.03)
Total proportion	19/30 (0.63)	17/100 (0.17)	16/30 (0.53)	21/100 (0.21)
95% CI* **	[0.43 - 0.80]	[0.10 - 0.26]	[0.34 - 0.72]	[0.13 - 0.30]

*MLN = Mesenteric Lymph Node; SLN** = Sub-iliac Lymph Node; CI*** = Confidence Interval.

with 7/7 (100%) in age group above 4.5 and 2/8 (25%) in the treatment group of the same age. Regards to body condition in poor 11/14 (78%) and 6/35 (17%) MLN were positive in control and treatment respectively (Table 2). However, statistically significant reductions were associated with EM supplement, duration of treatment and age groups (Table 3).

Regardless of few numbers current sample the 63% observed in the control group revealed the natural S*almonella* history of the farm and higher compared to 23.5% from animal feces by [7] and 19% found in rumen contents reported by [9]. The difference among these reports might be attributable to the sample type in the current study was lymph nodes where bacteria concentrated due to the action of immune system and the others were at carcass level. In addition, *Salmonella*

Table 3. The salmonella risk difference across risk factors in mesenteric lymph node.

Variable	Risk Difference	z-value	P > |Z|	95% CI	Wald test	P-Value
Pen	0.43	−5.13	0.000	0.26 - 0.59	**56.71**	**0.000**
Age	0.029	2.88	0.004	0.009 - 0.049		
Body Condition	0.009	−0.91	0.364	0.011 - 0.031		
Source	0.003	0.05	0.963	0.141 - 0.134		
Weight	0.004	−0.57	0.571	0.01 - 0.02		
Time	0.023	−3.44	0.001	0.009 - 0.035		

are versatile enteric pathogens noted for their ability to invade and survive within host lymphoid tissues [21]. In the current study, we also observed that *Salmonella* could be recovered from MLN of positive cases. Those from the control group indicated the infection status of the animals.

A significant reduction in *Salmonella* prevalence in MLN was observed from cattle administered EM with a relative risk reduction of (RR: 0.73; 95% CI = 0.55, 0.84, χ^2 = 24.74 and p = 0.000), (**Table 4**). In addition, significant differences were also observed across time of treatment (Days of harvesting) but not on (90 days) of treatment (p = 0.070). The relative risk reduction on post (100 days) of treatment (χ^2 = 7.43; p = 0.006) and relative risk reduction (χ^2 = 24.59 and p = 0.000) on (115 days), (**Table 5**). There were also significant absolute risk reduction in age groups (p = 0.005; 0.008; 0.000) in cattle age of (2 - 3.5), (3.5 - 4.5) and (>4.5) yrs respectively (**Table 6**). We haven't come across with the report specific to the effect of EM on *Salmonella* in MLN this might be related to its low food safety importance.

Therefore, this directs us to the hypothesis on the potential modes of actions by which *Lactobacilli* exert their protective or therapeutic effect. The *lactobacilli* achieve this effect through production of antimicrobial compounds [22], reduction of gut pH by stimulating the lactic acid producing microflora [23], competition for binding of receptor sites that pathogens occupy [24], stimulation of immunomodulatory cells [25]. [26] supports this observation by indicating that many strains of *Lactobacillus* are capable of eliciting different immune responses; from enhanced epithelial resistance to increased antibody production and competition with pathogens for available nutrients [25]. The EM in current study might have done one or more of actions listed above. Generally, it is important to note the complexity of the bovine lymphatic system in order to fully understand the limitations of our data and the inferences that can be made from it. Further investigations into the ecology of *Salmonella* within the bovine lymphatic system should be a goal for future research and will provide a more in-depth understanding of this issue.

3.1.2. *Salmonella* Risk Reduction in Sub-Iliac Lymph Node (SLN)

Salmonella prevalence in SLN also varies across the blocks of risk factors with

Table 4. The effect of feeding EM on salmonella in mesenteric lymph node (MLN).

Measures Effect Variables	No of study Animals	Proportion of *Salmonella* Positive (95% CI)	χ^2	P-Value
Non-Treated Group (Control)	30	19/30 (0.63), [0.44 - 0.80]	24.74	**0.000**
EM Feed Group (Treatment)	100	17/100 (0.17), [0.10 - 0.26]		
Risk Difference (Absolute Risk)	*	0.46 (0.28 - 0.65)		
Relative Risk	*	3.72 (2.21 - 6.27)		
Relative Risk Reduction	*	0.73 (0.55 - 0.84)		

Table 5. Effect of feeding EM on salmonella reduction across time in mesenteric lymph node.

Proportion of *Salmonella* Positive Animals on Days of Harvesting			
Treatment category	**90 days**	**100 days**	**115 Days**
Prevalence of *Salmonella* (Control)	6/9 (0.67), [0.30 - 0.93]	6/9 (0.67), [0.30 - 0.93]	7/12 (0.58), [0.28 - 0.85]
Prevalence of *Salmonella* (Treatment)	11/33 (0.33), [0.18 - 0.52]	6/31 (0.19), [0.07 - 0.37]	0/36 (0.00), -
Risk Difference (Absolute Risk)	0.33 [−0.014 - 0.68]	0.47 [0.14 - 0.81]	0.58 [0.30 - 0.86]
Relative Risk	2.00 [1.03 - 3.90]	2.47 [1.47 - 8.09]	*
Relative Risk Reduction	0.50 [0.029 - 0.74]	0.71 [0.32 - 0.88]	1.00
χ^2; P-Value	3.26, 0.070	7.43; 0.006	24.59; 0.000

Table 6. The salmonella risk difference across age in mesenteric lymph node.

Age	Risk Difference	z-value	P > |Z|	95% CI	Wald test
2 - 3.5	0.38	2.78	0.005	0.11 - 0.65	7.71
3.5 - 4.5	0.34	2.66	0.008	0.08 - 0.58	7.09
>4.5	0.75	4.58	0.000	0.43 - 1.1	21.00

5/17 (0.71) and 1/8 (0.13) for cattle above 4 years of control and treatment groups respectively in the same age. Treatment group had reduction to as few as 1 positive (0.03%) 115th days of treatment but as many as 12 positive (0.36%) in the same category of 90th day. The variation was observed amongst risk factors in the control group with 0 positive (0.00%) as many as 7 positive (70%) in the same group. The total prevalence of *Salmonella* in SLN was 16/30 (0.53) in control and 21/100 (0.21) in treated group (Table 2).

The previous researches have shown that cattle peripheral LNs including SLN can serve as a vehicle for *Salmonella* contamination; if fat trim containing these nodes are incorporated into ground beef directly have food safety implication [27] [28]. Contaminated LNs may explain the difference in *Salmonella* prevalence between post-intervention carcasses or trim, and ground beef [29]. The current studies 53% prevalence of *Salmonella* in SLN of control group might confirm the idea above theorized by [29]. In that it was higher than 32.4% from row beef at butchery reported by [7] as well as 40% and 42% prevalence reported from minced meat (locally known as "kitfo") while the samples were collected

from different hotels, bars and restaurants in Addis Ababa [30] [31]. This finding was comparable with the 60% rate found among samples from a South African slaughterhouse [32], and lower than the 87.4% rate reported by [33] from retail beef in Senegal.

However, the statistical significances of risk differences were associated with treatment (EM) and time of treatment (Table 7). A significant reduction in *Salmonella* prevalence in SLNs were observed from cattle administered EM with a relative risk reduction (RR: 0.61; 95% CI = 0.33, 0.77, χ^2 = 11.85 and p = 0.000). The EM supplemented group had 61% less likely to harbor *Salmonella* in their SLNs compared to non-supplemented ones as well as 32% risk reduction was attributable to EM (Table 8). On the other hand, the effect varied across slaughter days (Time of treatment) with no significant reduction (χ^2 = 1.08, p = 0.298) on first day. However, significant differences were observed on the second and third days with (RR: 0.61, 95% CI = 0.18, 0.82, χ^2 = 5.12; p = 0.023) and (RR: 0.93, 95% CI = 0.48, 0.99; χ^2 = 12.44, p = 0.000) respectively (Table 9). To our knowledge there is no data available in Ethiopia making it difficult to create meaningful comparison of interaction observed in this study in domestic. The limited number of the animals blocked to different risk factors based on biological difference in the beef farm is a limiting factor in our ability to make inferences to Ethiopia even if we consider grouping of cattle in the study farm according to the source of population, and it is important when interpreting these data to consider this limitation. Considering the absolute risk reduction between the treatment and control study animals allows for a better frame of reference for interpretation.

Table 7. The salmonella risk difference across risk factors in sub-iliac lymph node.

Variable	Risk Difference	z-value	P > |Z|	95% CI	Wald test	P-Value
Pen	0.308	−3.45	0.001	0.133 - 0.48	33.99	0.0000
Age	0.013	1.20	0.232	0.034 - 0.008		
Body Condition	0.012	−1.09	0.274	0.009 - 0.035		
Source	0.049	−0.66	0.511	0.098 - 0.197		
Weight	0.007	−0.93	0.351	0.008 - 0.022		
Time	0.024	−3.4	0.001	0.01 - 0.038		

Table 8. The effect of feeding EM on salmonella in sub-iliac lymph node.

Measure Effect Variables	No of study Animals	Prevalence of *Salmonella* (%), 95% CI*	χ^2	P-Value
Non-Treated Group(Control)	30	16/30 (0.53), [0.34 - 0.72]	**11.85**	**0.000**
EM Feed Group (Treatment)	100	21/100 (0.21), [0.13 - 0.30]		
Risk Difference (Absolute Risk)	*	0.32 (0.14 - 0.51)		
Relative Risk	*	2.54 (1.49 - 4.33)		
Relative Risk Reduction	*	0.61 (0.33 - 0.77)		

*Confidence Interval.

Table 9. The effect of feeding EM on salmonella reduction across time in sub-iliac lymph node.

Proportion of *Salmonella* Positive animals and Days of Harvesting			
Treatment category	**90 days (95% CI*)**	**100 days (95% CI*)**	**115 Days (95% CI*)**
Prevalence of *Salmonella* (Control)	5/9 (0.56), [0.21 - 0.86]	6/9 (0.67), [0.30 - 0.93]	5/12 (0.42), [0.15 - 0.72]
Prevalence of *Salmonella* (Treatment)	12/33 (0.36), [0.20 - 0.55]	8/31 (0.26), [0.12- 0.45]	1/36 (0.03), [0.001 - 0.15]
Risk Difference (Absolute Risk)	0.19 (-0.17 - 0.56)	0.41(0.06 - 0.75)	0.39 (0.10 - 0.67)
Relative Risk	1.53 (0.73 - 3.19)	2.58 (1.21 - 5.49)	15 (1.94 - 115.9)
Relative Risk Reduction	0.35 (−0.37 - 0.68)	0.61 (0.18 - 0.82)	0.93 (0.48 - 0.99)
χ^2; P-Value	1.08, 0.298	5.12; 0.023	12.44; 0.000

However, the current study reduction agrees with the 50%, 31% and 10% across three slaughter days by [12], from USA. The slight difference lay on the difference in duration of the treatment which is 90 days based on the fattening package in Ethiopia and above 129 days in USA that might be due to agro ecological and beef breed difference.

However, in current study the EM supplemented group was 39% less likely to be infected by *Salmonella* (RR: 0.61, 95% CI = 0.33, 0.77) (**Table 9**), the finding agrees in principle with 82% reported by [12] in USA and lower in magnitude of effect. The difference shown might be attributed to the ground and setting of study animals in which the current study subjects were in natural challenge and the later in research farm as well as high difference in sample size among the two studies.

4. Conclusion and Recommendation

The study demonstrated that effective microbial supplement in the diet of beef cattle reduces the risk of infection in sub-iliac and mesenteric lymph nodes by *Salmonella.* This showed the potential of effective microbial supplement in minimizing the contamination of beef with *Salmonella* organism. However, additional data should be generated to substantiate the result of this study before effective microbial supplement is recommended for use widely. Therefore, a well-designed and blinded randomized control trial is recommended for the evaluation of the effect of effective microbial supplement in beef diet in *Salmonella* in beef.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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