Preliminary Study on the Effective Microbial Supplementation of Feed on the Infection of \textit{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 role of effective microbial supplementation to feed on the infection of \textit{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 $5 \times 10^{10}$ 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 \textit{Salmonella} using standard procedure. The occurrence of \textit{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 \textit{Salmonella} infection in the two group was significant ($\chi^2 = 13.01; p = 0.000$). The relative risk of \textit{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 \textit{Salmonella} in the lymph node of beef cattle, thereby potentially minimizing the economic and public health impacts of \textit{Salmonella} infection. Then, it was recommended to use EM as prevention and control option in \textit{Salmonella} carriage in cattle.
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 subdistrict. 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 below (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.
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 lowland 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 $\cdot$ $1^5$ 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 distributor 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 $5 \times 10^{10}$ 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.

\[ N = \frac{0.25}{SE^2}, \]

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 (\( \alpha \)) = 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 Vassiliadis (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) Simon’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 Chi². 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,

| Measures Effect | Variables | No of study animals | Proportion of *Salmonella* 95% CI* | \( \chi^2 \) | 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] | 0.37 (0.17 - 0.57) | 2.12 (1.41 - 3.20) | 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.

| 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 Salmonella 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 > | 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, χ² = 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 (χ² = 7.43; p = 0.006) and relative risk reduction (χ² = 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) | $\chi^2$ | 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.

| Treatment category | Prevalence of Salmonella (Control) | Prevalence of Salmonella (Treatment) | Risk Difference (Absolute Risk) | Relative Risk | Relative Risk Reduction |
|--------------------|------------------------------------|-------------------------------------|---------------------------------|---------------|-------------------------|
| 90 days            | 6/9 (0.67), [0.30 - 0.93]          | 11/33 (0.33), [0.18 - 0.52]        | 0.33 [-0.014 - 0.68]            | 2.00 [1.03 - 3.90] | 0.50 [0.029 - 0.74] |
| 100 days           | 6/9 (0.67), [0.30 - 0.93]          | 6/31 (0.19), [0.07 - 0.37]         | 0.47 [0.14 - 0.81]              | 2.47 [1.47 - 8.09] | 0.71 [0.32 - 0.88] |
| 115 Days           | 7/12 (0.58), [0.28 - 0.85]         | 0/36 (0.00), -                     | 0.58 [0.30 - 0.86]              |               | 1.00                   |
| $\chi^2$; 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, χ² = 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 (χ² = 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, χ² = 5.12; p = 0.023) and (RR: 0.93, 95% CI = 0.48, 0.99; χ² = 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* | χ² | 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.

| 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) |
| $\chi^2$; 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.
References

[1] FAO (Food and Agricultural Organization) (2006) Food Safety Risk Analysis: A Guide for National Food Safety Authorities. FAO Food and Nutrition Paper 87, Rome.

[2] Riemann, H.P. and Cliver, D.O. (2006) Food-Borne Infections and Intoxications. Third Edition, Food Science and Technology International Series, School of Veterinary Medicine University of California, Academic Press, Davis.

[3] Oosterom, J. (1991) Epidemiological Studies and Proposed Preventive Measures in the Fight against Salmonellosis. *The International Journal of Food Microbiology*, 12, 41-52. https://doi.org/10.1016/0168-1605(91)90046-R

[4] Edget, A., Dafnar, N. and Biruhtesfa, A. (2014) Review on Common Food-Borne Pathogens in Ethiopia. *African Journal of Microbiology Research*, 8, 4027-4040.

[5] FAO (Food and Agricultural Organization) (2010) FAO Expert Workshop on the Application of Bio Security Measures to Control Salmonella Contamination in Sustainable Aquaculture. Mangalore, India, 19-21 January 2010. Report No. 937.

[6] FAO (Food and Agricultural Organization) (2016) Intervention for the Control of Non-Thyphoidal Salmonella Spp in Beef and Pork. FAO and WHO Meeting Report and System Review. Microbiological Risk Assessment Series.

[7] Hiko, A., Irsigler, H., Ameni, G., Zessin, H. and Fries, R. (2016) Salmonella Serovars along Two Beef Chains in Ethiopia. *The Journal of Infection in Developing Countries*, 10, 1168-1176. https://doi.org/10.3855/jidc.6354

[8] Nyeleti, C., Molla, B., Hildebrand, G. and Kleer, J. (2000) The Prevalence and Distribution of Salmonella in Slaughter Cattle, Slaughter House Personnel and Minced Beef in Addis Ababa (Ethiopia). *Bulletin of Animal Health and Production in Africa*, 48, 19-24.

[9] Sibhat, B., Molla, B.Z., Zerihun, A., Mucke, A., Cole, L.A., Boerlin, P., Wilkie, E., Perets, A., Mistry, K. and Gebreyes, W.A. (2009) Salmonella Serovars and Antimicrobial Resistance Profiles in Beef Cattle, Slaughterhouse Personnel and Slaughterhouse Environment in Ethiopia. *Zoonoses Public Health*, 58, 102-109. https://doi.org/10.1111/j.1863-2378.2009.01305.x

[10] Teklu, A. and Negussie, H. (2011) Assessments of Risk Factor and Prevalence of Salmonella in Slaughtered Small Ruminant and Environments in an Export Abattoir, Modjo, Ethiopia. *American-Eurasian Journal of Agricultural & Environmental Sciences*, 10, 992-999.

[11] OWOA (Oda-Bultum Wereeda Office of Agriculture) (2016) Official Document of Annual Plan. Unpublished, Bedessa.

[12] Vipham, J.L. (2015) The Impact of Administration of a Lactobacillus Based Direct-Fed Microbial on the Presence, Concentration, and Ecology of Salmonella Enterica in Bovine Subiliac Lymph Nodes. A PhD Dissertation, Texas Tech University, Lubbock.

[13] WHLFDO (Western Harerghea Livestock and Fisheries Development Office) (2016) Official Document of Zonal Livelihoods and Production System Assessment. Unpublished, Chiro.

[14] Suiter, J. (1994) "Body Condition Score of Ruminants" Farm Note 69/1995. Department of Agriculture Western Australia.

[15] AARD (Alberta Agricultural and Rural Development) (2013) Guidelines for Age Verification in Cattle. Food Safety and Animal Health Division, Animal Health
Brunch, Edmonton.

[16] Sajjad, Z., Ahmed, M.S. and Abbasi, N.A. (2003) Effects of Phosphorous Level and Effective Microorganisms. *Sarhad Journal of Agriculture*, **19**, 193-197.

[17] OLFDB (Oromia Livestock and Fisheries Development Beoureo) (2012) The Improved Fattening Package Document. Official Training Manual for Experts, Finland.

[18] Arsham, H. (2002) Descriptive Sampling Data Analysis. Statistical Thinking for Managerial Decision-Making. [http://home.ubalt.edu/ntsbarsh/business-stat/stat-data/opre504.htm#rssss](http://home.ubalt.edu/ntsbarsh/business-stat/stat-data/opre504.htm#rssss)

[19] Gragg, S.E., Loneragan, G.H., Brashears, M.M., Arthur, T.M., Bosilevac, J.M., Kalchayanand, N., Wang, R., Schmidt, J.W., Brooks, J.C., Shackelford, S.D., Wheeler, T.L., Brown, T.R., Edrington, T.S. and Brichta-Harhay, D.M. (2013) Cross-Sectional Study Examining *Salmonella enterica* Carriage in Subiliac Lymph Nodes of Cull and Feedlot Cattle at Harvest. *Foodborne Pathogens and Disease*, **10**, 368-374. https://doi.org/10.1089/fpd.2012.1275

[20] Grimont, P.A.D. and Welll, F. (2007) Antigenic Formulae of the Salmonella Serovars. 9th Edition, World Health Organization Collaborating Centre for Reference and Research on Salmonella, 10. [http://www.pasteur.fr/sante/clre/cadrecnr/salmoms-index.html](http://www.pasteur.fr/sante/clre/cadrecnr/salmoms-index.html)

[21] Durand, A.M., Giesecke, W.H., Barnard, M.-L., Van Der Walt, M.L. and Steyn, H.C. (1990) Salmonella Isolated from Feeds and Feed Ingredients during the Period 1982-1988: Animal and Public Health Implications. *Onderstepoort Journal of Veterinary Research*, **57**, 175-181.

[22] Dodd, H.M. and Gasson, M.J. (1994) Bacteriocins of Lactic Acid Bacteria. In: Gasson, M.J. and de Vos, W.M., Eds., *Genetics and Biotechnology of Lactic Acid Bacteria*, Blackie Academic and Professional, Glasgow, 211-251. https://doi.org/10.1007/978-94-011-1340-3_5

[23] Langhendries, J.P., Detry, J., Van Hees, J., Lamboray, J.M., Darimont, J., Mozin, M.J., Secretin, M.C. and Senterre, J. (1995) Effect of a Fermented Infant Formula Containing Viable Bifidobacteria on the Faecal Flora Composition and pH of Healthy Full-Term Infants. *Journal of Pediatric Gastroenterology and Nutrition*, **21**, 177-181. https://doi.org/10.1097/00005176-199508000-00009

[24] Kailasapathy, K. and Chin, J. (2000) Survival and Therapeutic Potential of Probiotic Organisms with Reference to *Lactobacillus acidophilus* and Bifidobacterium spp. *Immunology & Cell Biology*, **78**, 80-88. https://doi.org/10.1046/j.1440-1711.2000.00886.x

[25] Rolfe, R.D. (2000) The Role of Probiotic Cultures in the Control of Gastrointestinal Health. *The Journal of Nutrition*, **130**, 396S-402S. https://doi.org/10.1093/jn/130.2.396S

[26] Demeria, D., Ewaschuk, J. and Madsen, K. (2009) Interactions of Lactobacillus with the Immune System. In: Ljungh, A. and Wadstrom, T., Eds., *Lactobacillus Molecular Biology: From Genomics to Probiotics*, Caister Academic Press, Norfolk, 139-148.

[27] Arthur, T.M., Brichta-Harhay, D.M., Bosilevac, J.M., Guerini, M.N., Kalchayanand, N., Wells, J.E., Shackelford, S.D., Wheeler, T.L. and Koohmaraie, M. (2008) Prevalence and Characterization of Salmonella in Bovine Lymph Nodes Potentially Destined for Use in Ground Beef. *Journal of Food Protection*, **71**, 1685-1688.

[28] CDC (Center for Disease Control) (2012) National Antimicrobial Monitoring Sys-
tem for Enteric Bacteria (NARMS): Human Isolates Final Report, 2010. US Department of Health and Human Services, Atlanta.

[29] Arthur, T.M., Brichta-Harhay, D.M., Bosilevac, J.M., Guerini, M.N., Kalchayanand, N., Wells, J.E., Shackelford, S.D., Wheeler, T.L. and Koohmaraie, M. (2008) Prevalence and Characterization of Salmonella in Bovine Lymph Nodes Potentially Destined for Use in Ground Beef. *Journal of Food Protection, 71*, 1685-1688. https://doi.org/10.4315/0362-028X-71.8.1685

[30] Tegegne, M. and Ashenafi, M. (1998) Microbial Load and Incidence of Salmonella Species in “Kitfo”, Traditional Ethiopian Spiced, Minced Meat Dish. *The Ethiopian Journal of Health Development, 12*, 135-140.

[31] Molla, B., Kleer, J. and Sinell, H.J. (2000) Occurrence, Distribution and Level of Salmonella in Selected Food Items in Addis Ababa, Ethiopia. *Fleischwirtschaft International, 4*, 37-39.

[32] Nel, S., Lues, J.F.R., Buys, E.M. and Venter, P. (2004) Microbial Population Associated with Meat from the Deboning Room of a High Throughput Red Meat Abattoir. *Meat Science, 66*, 667-674. https://doi.org/10.1016/S0309-1740(03)00187-6

[33] Stevens, A., Kaboré, Y., Perrier-Gros-Claude, J.-D., Millemann, Y., Brisabois, A., Catteau, M., Cavin, J.-F. and Dufour, B. (2006) Prevalence and Antibiotic-Resistance of Salmonella Isolated from Beef Sampled from the Slaughterhouse and from Retailers in Dakar (Senegal). *International Journal of Food Microbiology, 110*, 178-186. https://doi.org/10.1016/j.ijfoodmicro.2006.04.018