

Isolation of *Salmonella* in Commercial Chicken Feeds in Ilala District

Siha Mdemu^{a*}, Julius Maina Mathara^b, Zachariah Ephraim Makondo^c

^{a,c}Department of Bacteriology, Central Veterinary Laboratory, Tanzania Veterinary Laboratory Agency, P.O. Box 9254, Dar es Salaam, Tanzania.

^bDepartment of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000, Nairobi, Kenya.

^aEmail: sihamdemu@yahoo.com

^bEmail: juliusmaina_2000@yahoo.com

^cEmail: zmakondo@yahoo.com

Abstract

Salmonella is one of important hazardous pathogens causing salmonellosis in both humans and animals. In Tanzania, commercial chicken farming is a rapidly growing industry and salmonellosis is a serious problem. A study on *Salmonella* was conducted in commercially produced chicken feeds from feed mills in Ilala, Dar es Salaam, Tanzania between October 2015 and January 2016. The objective of the study was to estimate the prevalence of *Salmonella* contamination in commercial chicken feeds. Feed samples were collected from a total of 197 randomly selected feed bags of different types from 3 feed mills to estimate the contamination prevalence. Cultural and biochemical tests were performed for the presence of *Salmonella* in the samples. The overall prevalence of *Salmonella* in the study was 29.4%. The prevalence of *Salmonella* in broiler starter mash, broiler grower mash, broiler finisher mash and layers mash were confirmed to be 30.8%, 38.1%, 33.3% and 21.1% respectively and prevalence of *Salmonella* in batches 1 and 2 were 27.8% and 30.5% respectively. Prevalence of *Salmonella* contamination in feed mills A, B and C, was 22.2%, 48.1% and 14.7% respectively. Significantly higher ($p = 0.001$) prevalence of *Salmonella* contamination was seen in feed mill B when compared to the other two. The presence of *Salmonella* in commercial chicken feeds in Ilala presents a contamination hazard for both humans and *Salmonella*-free flocks, and therefore, calls for improvement of hygienic processing and handling of feeds for effective control measures.

* Corresponding author.

Keywords: Salmonellosis; *Salmonella*; Poultry mash; Feed mills; Ilala.

1. Introduction

Salmonella infection is one of the most important diseases worldwide [1]. Although *Salmonella* bacteria normally inhabit the intestines of most animal species (including humans and birds) they can potentially be found widely in the environment [2]. These bacteria can survive for prolonged periods of time without multiplication on materials with low moisture contents [3] therefore providing for the possibilities of the bacteria to be mechanically transmitted from one site to another through fomites, including contaminated feeds [4]. Feeds are formulated from different ingredients with different possible levels of *Salmonella* contamination. A study in cereal ingredients in the United Kingdom [5] showed that cereal ingredients for animal feeds were contaminated with *Salmonella* at the farm level, whereas 92% of the meat and bone meal samples tested in the United States were contaminated with *Salmonella* [6] and in the Netherlands 31% *Salmonella* contamination was recorded in fishmeal samples [7]. Feed manufacturing facilities are therefore considered as critical contamination points where *Salmonella* enters the food chain [8].

Chicken rearing is a very common practice in Ilala and the District has the highest number of chickens (51%) in the Region [9]. The increase in chicken production has resulted in high demand of feeds, and consequently, proliferation of feed mills, some of which operates under substandard conditions. This may result into packaging of feed contaminated with pathogens and thereby spreading diseases to both humans and farms. Studies show that feeds can be possible source of *Salmonella* infection to humans [10] as well as contamination in chicken farms [11]. The prevalence of *Salmonella* in poultry feeds varies widely between studies mainly due to the difficulty in detection as well as differences in sampling and testing methods [12]. The study on contamination of commercial chicken feeds has been used as an ideal step in the control of different diseases including salmonellosis. Salmonellosis is an important disease worldwide [13], affecting both humans and animals and, is associated with high costs of control [14]. A study conducted in Tanzania [15] indicated that salmonellosis is a disease with high risk of death among children with increasing trend of typhoid fever [16]. Fowl Typhoid has been reported as the most important disease in commercial chicken industry in Tanzania [17] with a prevalence of up to more than 50% of the flocks [18].

Effective control measures of any infectious disease requires identification of the possible sources of the disease causing agent [19]. However, no study have been done to identify feed as potential source of *Salmonella* contamination in Tanzania. This jeopardizes the overall efficiency on the control of salmonellosis and thus the disease continues to escalate in the country. Investigation of the possibility of *Salmonella* contamination in commercial chicken feeds in Ilala was taken as an ideal step on generating knowledge on the feed bio-security. The investigation involved overall prevalence and contamination differences between different types and brands of feed.

2. Materials and methods

A cross-sectional study was conducted in Ilala District, Dar es Salaam, Tanzania. The district has a land area of

273 km² located at 6°48' S, 39°17' E with a tropical hot and humid weather [9]. A total of 197 feed samples were collected from three feed mills during the period of October 2015 to January 2016. Selection of feed bags was done using systematic sampling method and the interval of selection was determined according to the expected number of bags from the finished product bins ready for bagging. Approximately 50 grams of feed were collected from each of the selected bag into a zip-lock bag.

2.1. Salmonella analysis

Detection of *Salmonella* in feed samples was done in Tanzania Veterinary Laboratory Agency (TVLA) according to the standard culture methods [20,21]. A 25 g portion of feed sample was pre-enriched in 225 ml of buffered peptone water (Himedia, Mumbai, India) and incubated at 37°C for 24 hr.

Then 0.1 ml of the pre-enrichment culture was added to 10ml of Rappaport-Vassiliadis broth (Himedia, Mumbai, India) and incubated at 41.5°C for 24 hr. Loopful inoculums were subsequently streaked into Xylose Lysine Deoxychocolate Agar (XLD-Agar, Scharlau Chemie S.A., Barcelona Spain) and McConkey's agar (Himedia, Mumbai, India) and incubated at 37°C for 24 - 48 hr to obtain only single type of colonies. The isolates were identified as *Salmonella* species based on the colony appearance, Gram stain, triple-sugar-iron (TSI) reaction, indole reaction, methyl-red (MR) reaction, Voges-Proskauer (VP), and citrate utilisation according to [22].

2.2. Data Analysis

Data were analyzed using IBM SPSS Statistics version 20 computer program. The Pearson's chi-square (χ^2) test at a significance level of 5% was used to determine the prevalence of *Salmonella* contamination among different feeds, between batches and among feed mills. The difference was considered statistically significant if the p-value was less than or equal to 0.05.

3. Results and discussion

The overall prevalence of *Salmonella* contamination was 29.4% (58/197) as shown in the tables 1 - 3. This is less than the prevalence of 71.43% reported in the study on poultry feeds from farms and markets in Bangladesh [23] but agrees with the 22.2% prevalence recorded in the study from feed outlets in Nigeria [24]. However, a lesser prevalence of 10.9% *Salmonella* contamination was recorded in animal feed from abattoirs in Namibia [10] and a much lower prevalence of 4.4%, 3.6% and 3.3% were reported from animal feeds in Brazil [25], swine feeds in the USA [26] and broiler feeds in Iran [27] respectively. Although detection of *Salmonella* in feeds is common, studies in some countries have reported feeds free of *Salmonella* [28]. The variation of *Salmonella* contamination levels between studies, however, might be due to the difficulty in detection as well as differences in sampling and testing methods [12]. In this study, difficulties in obtaining *Salmonella* pure isolates in some of the samples were overcome by repeated culturing and duplicating the test samples.

Table1: Prevalence of Salmonella contamination among batches

Batch	Prevalence	χ^2 -value	p-value
1	27.8	0.161 ^a	0.688
2	30.5		
Total	29.4		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 23.26.

Table2: Prevalence of Salmonella contamination among feed types

Feed type	Prevalence	χ^2 -value	p-value
Broiler Starter Mash	30.8	3.137 ^a	0.371
Broiler Grower Mash	38.1		
Broiler Finisher Mash	33.3		
Layers Mash	21.1		
Total	29.4		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.18.

Table3: Prevalence of Salmonella contamination among feed mills (brands)

Feed type	Prevalence	χ^2 -value	p-value
A	22.2	21.848 ^a	0.000
B	48.1		
C	14.7		
Total	29.4		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 13.25

The prevalence of *Salmonella* contamination was 27.8% and 30.5% in batches 1 and 2 respectively. Although batch 2 showed a slightly higher prevalence than batch 1, statistical analysis of the data indicated that there was no significant difference ($\chi^2 = 0.161$, P = 0.688) on the prevalence of *Salmonella* contamination between batches 1 and 2 (Table 1). This may indicate consistency in the operation of the feed mills in the area.

Salmonella isolated from different feed types were 30.8%, 38.1%, 33.3% and 21.1 in broiler starter mash, broiler grower mash, broiler finisher mash and layers mash, respectively. This is in partial agreement with the authors [24] who reported a prevalence of 40%, 0%, 25%, and 20% in broiler starter mash, broiler grower mash, broiler finisher mash and layers mash, respectively. Statistical analysis of the data showed that there was no

significant difference ($\chi^2 = 3.137$, $P = 0.371$) on the prevalence of *Salmonella* contamination among broiler starter mash, broiler grower mash, broiler finisher mash and layers mash (Table 2).

The prevalence of *Salmonella* contamination was 22.2%, 48.1% and 14.7% in the feed mills A, B and C, respectively. Statistical analysis of the data indicated that there was significant difference ($\chi^2 = 21.848$, $P = 0.001$) on the prevalence of *Salmonella* contamination among feed mill A, feed mill B and feed mill C (Table 3). Feed mill B was more likely to formulate *Salmonella* contaminated feeds (48.1%) as compared to mills A (22.2%) and C (14.7%).

The rates of *Salmonella* contamination are considered to be related to management practices of a particular feed mill [8]. The authors in [29] reported different levels of *Salmonella* contamination in the environment of ten feed mills in Great Britain suggesting that feeds can be contaminated at the feed mill. Although all the feed mills involved in this study were small scale producing less than twenty tones a day, feed mill B had more strict hygienic procedures including strict entry regulations, clean premises, clearly partitioned loading/unloading area, ingredient storage area and formulated feed storage area. According to the authors in [30] feed mill premises are critical in the control of *Salmonella* in feeds.

Different feed mills also use different ingredients in formulating their feeds. The commonly used feed ingredients in the formulation of feeds in developing countries include maize and maize products, animal fats, vegetable oils, soybean meal, fishmeal, meat and bone meal, limestone, shell grit, dicalcium phosphate, defluorinated rock phosphate, and other additives such as antibiotics [31]. In Tanzania, blood meal, cottonseed meal and sunflower meal are also used. Although all feed mills in the present study were using similar ingredients, they were supplied to the feed mills independently suggesting that they can reach the feed mills at different levels of contamination [5]. The data in this study therefore suggest that feed mills play a major role in the contamination of feed.

4. Conclusion

The study confirmed that there was significant difference in the prevalence of *Salmonella* in feeds among the tested feed mills in Ilala and it is shown that *Salmonella* contamination is more prevalent in feed mill B, suggesting that feed mills play a major role in the contamination of feed. It is therefore recommended that *Salmonella* monitoring in feed mills should be instituted to reduce the incidence of *Salmonella* contaminated feeds in the district. Besides, feed mills management should improve the hygienic practices in the feed mill premises and establish systems for regular testing of the ingredients and formulated feeds for the presence of *Salmonella*.

Acknowledgement

The researchers are grateful to The Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) and The Central Veterinary Laboratory, Tanzania Veterinary Laboratory Agency (TVLA) for supporting this work. We wish to extend our profound gratitude to Ms. G. Banda of the Department of Bacteriology, Central Veterinary Laboratory, TVLA for her technical support.

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