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A survey on *Salmonella* infection among chicken flocks in Jimma town, Ethiopia

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A cross sectional study was conducted between November, 2011 and April, 2012 to determine the prevalence and risk factors associated with *Salmonella* infection among chicken flocks in Jimma town, Ethiopia. Management system, sex, season, age and breed of the chicken flocks examined in the study were considered as risk factors and evaluated whether they are associated with *Salmonella* infection or not. A bacteriological examination was carried out on 384 faecal samples originated from 232 exotic chicken flocks which were kept indoors and 152 local chicken flocks which were free ranging. The faecal samples were enriched with peptone water for 24 h and then seeded on the selective enrichment media, Rappaport Vassiliadis Soy Broth and incubated at 37°C for 24 h. Following selective enrichment, 0.1 ml of the pre enriched broth of the various dilutions were streaked aseptically into Xylose Lysine Desoxycholate (XLD) agar plates and incubated at 37°C for 24 h. Presumptive identification of *Salmonella* was done based on morphology and colour of the colonies on the culture media. Final identification and assignment of *Salmonella* was conducted by employing biochemical tests such indole production, citrate utilization and urease tests. The identification result proved the overall prevalence of *Salmonella* to be 41.9%. The prevalence of *Salmonella* infection were found to be higher in indoor chickens (42.7%) than chickens that were kept as free ranging (40.8%) but there was no statistically significant difference (p>0.05) on the prevalence of *Salmonella* infection in chicken flocks between management systems. The prevalence of *Salmonella* in female chicken flocks (43.0%) was higher than in male chicken flocks (39.0%) and there was no statistical significant difference (P>0.05) on the prevalence of *Salmonella* between sexes. The highest prevalence of *Salmonella* was recorded during spring (47.7%) followed by autumn (39.7%) and the lowest prevalence of *Salmonella* infection among chicken flocks was seen during winter (36.8%). Statistical analysis of the data showed that there was no statistical significant difference (P>0.05) on the prevalence of *Salmonella* in chicken flocks among seasons. Layers and cocks were proved to be highly infected with *Salmonella* (46.2%) followed by broilers (41.3%). The lowest prevalence of *Salmonella* infection was seen in chickens (40.8%). There was no statistical significant difference (P>0.05) on the prevalence of *Salmonella* in chicken flocks among ages. The prevalence of *Salmonella* was proved to be higher in exotic breed chicken flocks (42.7%) than chicken flocks which were kept as free ranging (40.8%). Analysis of the data showed there was no statistical significant difference (P>0.05) on the prevalence of *Salmonella* among chicken flocks between breeds. The prevalence of *Salmonella* among chicken flocks in Jimma town, Ethiopia, was proved to be very high. Hence, strengthening the knowledge, attitude and practice of chicken flock owners must be inaugurated in the area to mitigate the economic losses which could arise due to *Salmonella* infection in chicken flocks.

Key words: *Salmonella*, prevalence, chicken flocks, Jimma town, Ethiopia.

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INTRODUCTION

Chicken flocks are one of the most important sources of livelihood food and income in the world in general and in Ethiopia in particular. But the country, Ethiopia did not earn the expected production from its chicken flock population. The lion's share which contributes for the low productivity of chicken flocks in Ethiopia is attributed to salmonellosis (Mekonnen, 2007).

Salmonella orgamnisms live in the intestinal tracts of warm and cold blooded animals. Some species are ubiquitous. Other species are specifically adapted to a particular host (Oscar, 2004). Salmonellosis is a bacterial disease caused by Gram negative facultative rod shaped bacterium strains of *Salmonella* which is in the same proteobacterial family as *Escherichia coli,* the family Enterobacteriaceae, trivially known as "enteric" bacteria. It is a diarrheal disease, known more generically as gastroenteritis. The disease is quite common among chickens, ducks, and other poultry species (Holt and Chaubal, 2010).

In order to address the chicken flocks related challenges in production and marketing and to improve the livelihoods and food security of rural and urban households by enhancing the benefits from chicken flocks through appropriate production and marketing extension, it is essential to conduct a research that could generate appropriate technology, which is socially acceptable, environmentally sound and economically feasible (Wafaa et al., 2012).

In spite of the aforementioned prevailing situation and the presence of a number of economic losses due to *Salmonella* in Jimma town, Ethiopia, there is paucity of well documented information on the occurrence of the bacteria in chicken flocks. Therefore, this study was designed to determine the prevalence of *Salmonella* and risk factors associated with *Salmonella* infection among chicken flocks in Jimma town, Ethiopia.

MATERIALS AND METHODS

Study area

The study was conducted in Jimma town of Jimma Zone in Oromia Regional State, Ethiopia. Jimma town is found in Southwestern Ethiopia at the altitude and longitude of 7°40′N 36°50′E at 352 km from Addis Ababa, the capital of Ethiopia. Jimma Zone comprises of 13 Districts and has a population of 2.1 million. Jimma town, the capital of the Zone, has a population of over 100,000. The town has an average temperature of 20°C and a bimodal irregular raining system with an average annual rain fall of 200 mm. This irregular raining and temperature fluctuation has a great role for the outbreak of disease in animals and humans (CSA, 2007).

Study period and sampling technique

The study was carried out between November, 2011 and April 2012. The sampling technique which was employed in the current research was simple random sampling technique.

Study units and risk factors

The study units were those chicken flocks that were randomly selected from chicken flocks which were kept as indoor (n=232) and free ranging (n=152). Management system (indoor, free ranging), sex (male, female), season (autumn, winter, spring), age (chicken, broiler, layers and cocks) and breed (local, exotic) of the chicken flocks examined in the study were considered as risk factors and evaluated whether they are associated with *Salmonella* infection or not. Chicken flocks which were kept indoor were exotic breeds and those which were free ranging were local breeds.

Study design

A cross- sectional study was conducted from October 2011, to April, 2012 to determine the prevalence of *Salmonella* and risk factors associated with *Salmonella* infection among chicken flocks in Jimma town, Ethiopia.

Sample size determination

To calculate the total sample size, the following parameters were used: 95% level of confidence interval (CL), 5% desired level of precision and with the assumption of 50% expected prevalence of *Salmonella* among chicken flocks in Jimma town, the sample sizes were determined using the formula given in Thrusfield (2005) study.

$$n = \frac{1.962^{\circ}C\ Pexp\ (1\text{-}Pexp)}{d^2}$$

Where, n is the required sample size; P_{exp} is the expected prevalence; and d^2 is the desired absolute precision. Thus, the sample size was calculated to be 384.

Sample collection and transportation

Faecal samples were collected from chicken flocks that were kept indoor (n=232) and from chicken flocks that were free ranging (n=152) between November, 2011 and April, 2012. Sterile spatulas were used to collect fresh faecal samples from chicken flocks. Samples were aseptically collected and put into labeled sterile screw capped universal bottles and kept in an icebox containing ice packs. Aseptic collection samples were immediately transported to Microbiology laboratory, School of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia for bacteriological isolation and identification. Upon arrival, the samples were stored overnight in a refrigerator at 4°C until analyzed.

Isolation and identification of *Salmonella*

Isolation and identification of *Salmonella o*rganisms were carried out following the standard guidelines given by ISO-66579-3 (ISO, 66579-3: 2003) (Figure 1).

Non-selective enrichment

Faecal samples which were kept for overnight in a refrigerator at 4°C were thawed for 3-5 h at room temperature. Twenty five grams of each faecal sample was stirred separately into 225 ml of sterile

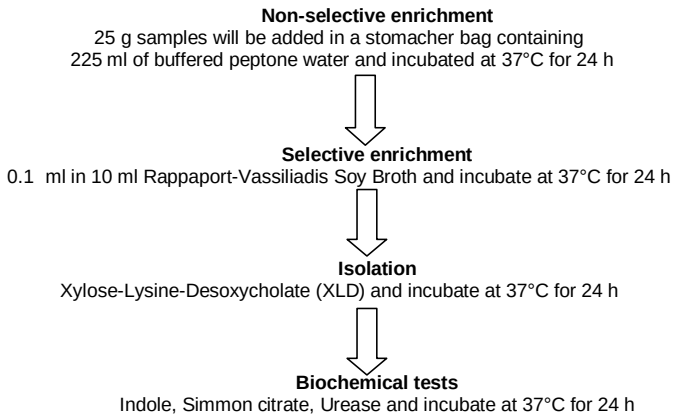

Figure 1. Flow chart of the ISO-6579 protocol for isolation and identification of *Salmonellla.*

buffered peptone water in a sterile stomacher bag. The pre enriched samples were homogenized in a stomacher for 2 min and incubated aerobically at 37°C for 24 h

Selective enrichment

Faecal samples were inoculated into Rappaport Vassiliadis Soy Broth and incubated at 37°C for 24 h, before plating out onto selective agar.

Spread on selective Xylose Lysine Desoxycholate agar plates

Following selective enrichment, 0.1 ml of the pre enriched broth of the various dilutions were streaked aseptically onto Xylose Lysine Desoxycholate (XLD) agar plates and the plates were incubated at 37^0C for 24 hours. Presumed *Salmonella* colonies were then selected and subjected for further tests. Data on bacterial growth and potential *Salmonella*e selected were recorded at the time of reading and verified by further bacteriological identification methods at regular intervals. Final identification of *Salmonella* organisms were done based on recommended biochemical tests.

Biochemical tests

Final identification of *Salmonella* organisms were done based on biochemical tests such as indole production, citrate utilization and urease tests**.**

Data management and analysis

Microsoft Excel was used for data management and computation of descriptive statistics was conducted using SPSS version 16.0. Descriptive statistics such as percentages, proportions and frequency distributions were applied to compute some of the data. The prevalence of *Salmonella* was calculated by dividing the number of chicken flocks positive for *Salmonella* infection by the number of chicken flocks examined. Pearson's Chi-square (χ^2) test at a significant level of 5 and 95% CI was used to measure association between prevalence of *Salmonella* with management system, sex, season, age and breed of the chicken flocks. The results were considered significant when P<0.05.

RESULTS

Overall prevalence of *Salmonella*

The overall prevalence of *Salmonella* among chicken flocks in Jimma town, Ethiopia was proved to be 41.9% (161/384). The prevalence of *Salmonella* was higher in indoor chicken flocks (42.7%) when compared to birds which were kept as free ranging (40.8%) but there was no statistical significant difference (*P*>0.05) on the prevalence of *Salmonella* among the chicken flocks examined between management system (Table 1).

The prevalence of *Salmonella* among female chicken flocks (43.0%) was higher than the prevalence of *Salmonella* infection among male chicken flocks (39.0%). There was no statistical significant difference (P>0.05) on the prevalence of *Salmonella* between sexes (Table 2).

The highest prevalence of *Salmonella* was recorded during spring (47.7%) followed by autumn (39.7%) and winter (36.8%). Statistical analysis of the data showed that there was no statistical significant difference (P>0.05) on the prevalence of *Salmonella* in chicken flocks among seasons (Table 3).

Layers and cocks were proved to be highly infected with *Salmonella* (46.2%) followed by broilers (41.3%). The lowest prevalence of *Salmonella* infection was seen in chickens (40.8%). Statistical analysis of the data showed that there was no statistical significant difference (P>0.05) on the prevalence of *Salmonella* in chicken flocks among ages (Table 4).

The prevalence of *Salmonella* was proved to be 40.8% among local breed chicken flocks and 42.7% among exotic breed chicken flocks. Analysis of the data showed there was no statistical significant difference (P>0.05) on the prevalence of *Salmonella* among chicken flocks between breeds (Table 5).

Prevalence of *Salmonella* among local chicken flocks

Of the total 152 local chicken flocks examined, 40.8% were proved to be infected with *Salmonella* organism of which 41.4% were females and 38.9% were males. Statistical analysis of the data showed that there was no statistical significant difference (P>0.05) on the prevalence of *Salmonella* infection among local chicken flocks between sexes (Table 6).

The highest prevalence of *Salmonella* among local chicken flocks was recorded during spring (45.8%) followed by autumn (40.0%) and winter (34.9%). Statistical analysis of the data showed that there was no statistical significant difference (P>0.05) on the prevalence of *Salmonella* in local breed chicken flocks among seasons (Table 7). The prevalence of *Salmonella* infection among local chick flocks was confirmed to be 41.4, 40.7 and 40.6% among chickens, broilers and layers and cocks, respectively. There was no statistical

Table 1. Overall prevalence of *Salmonella* among chicken flocks between management systems.

Managements system	Examined	Prevalence (%)	χ^2	P-value
Indoor	232	42.7	0.134	0.715
Free ranging	152	40.9		
Total	384	41.9		

Table 2. Overall prevalence of *Salmonella* among chicken flocks between sexes.

Sex	Examined	Prevalence (%)	χ2	P-value
Female	279	43.0	0.134	0.715
Male	105	39.0		
Total	384	41.9		

Table 3. Overall prevalence of *Salmonella* in chicken flocks among seasons.

Season	Examined	Prevalence (%)	χ^2	P-value
Spring	150	47.7		
Autumn	150	39.7	3.469	0.176
Winter	113	36.8		
Total	384	41.9		

Table 4. Overall prevalence of *Salmonella* in chicken flocks among different age groups.

Age	Examined	Prevalence (%)	χ2	p-value
Layers and Cocks	158	46.2		
Broiler	150	41.3	3.067	0.216
Chicken	76	40.8		
Total	384	41.9		

Table 5. Overall prevalence of *Salmonella* in chicken flocks between breeds.

Breed	Examined	Prevalence (%)	χ2	P-value
Local	152	40.8	0.134	0.715
Exotic	232	42.7		
Total	384	41.9		

Table 6. Prevalence of *Salmonella* among local chicken flocks between sexes.

Sex	Examined	Prevalence (%)	χ2	P-value
Female	116	41.4	0.071	0.791
Male	36	38.9		
Total	152	40.8		

Table 7. Prevalence of *Salmonella* in local chicken flocks among seasons.

Season	Examined	Prevalence (%)	χ2	P-value
Autumn	50	40.0		
Winter	43	34.9	1.238	0.538
Spring	59	45.8		
Total	152	40.8		

Table 8. Prevalence of *Salmonella* in local chicken flocks among age groups.

Age	Examined	Prevalence (%)	χ2	P-value
Chicken	29	41.4		
Broiler	59	40.7	0.005	0.997
Layers and cocks	64	40.6		
Total	152	59.2	40.8	

Table 9. Prevalence of *Salmonella* among exotic chicken flocks between sexes.

Sex	Examined	Prevalence (%)	χ2	p-value
Female	163	44.2.	0.504	0.478
Male	69	39.1		
Total	232	42.7		

significant variation (P>0.05) on the prevalence of *Salmonella* in local chicken flocks among age groups (Table 8).

Prevalence of *Salmonella* among exotic chicken flocks

The prevalence of *Salmonella* infection among exotic chicken flocks was confirmed to be 44.2%. The prevalence of *Salmonella* was proved to be higher in female exotic chicken flocks (44.2%) when compared to the prevalence of *Salmonella* among male exotic chicken flocks (39.1%). There was no statistical significant difference (p>0.05) on the prevalence of *Salmonella* among exotic chicken flocks between sexes (Table 9).

The seasonal prevalence of *Salmonella* in exotic chicken flocks was found to be 39.4, 36.6 and 50.0% during autumn, winter and spring, respectively. Statistical analysis of the data showed that there was no statistical significant difference (P>0.05) in the prevalence of *Salmonella* in exotic breed chicken flocks among seasons (Table 10).

The age specific prevalence of *Salmonella* among exotic chicken flocks was confirmed to be 40.4, 40.7 and 45.7% among chickens, broilers and in layers and cocks, respectively. There was no statistical significant variation (P>0.05) on the prevalence of *Salmonella* in exotic chicken flocks among age groups (Table 11).

DISCUSSION

The overall prevalence of *Salmonella* infection in the current research (41.9%) was higher than that reported by Kassaye et al. (2010) who reported 8% of *Salmonella* in Hawassa by direct swab plating technique from small scale chicken flock farms. The different prevalence of *Salmonella* from chicken flocks found in these reports could be explained by the different techniques used in these studies, differences in the origin of the samples or by geographical differences.

The prevalence of *Salmonella* was higher in indoor chicken flocks (42.7%) than chicken flocks which were kept as free ranging (40.8%). This fact could be best justified by the fact that all the chicken flocks which were kept indoor were exotic breeds and these breeds do have lower immunity against *Salmonella*. Besides the poor management experienced by the owners exposes birds to numerous potential sources of *Salmonella* contamination on chicken flocks which were kept indoor (Liljebjelke et al., 2005). Even if the potential exists for increased bacterial contamination on free ranging chicken flocks due to easier access to transmitting vectors, such as birds, rodents, insects, and/or wild animals local breeds are resistant to pathogenic organisms to develop infection (Forshell and Wierup, 2006).

The sex specific prevalence of *Salmonella* among chicken flocks in Jimma town, Ethiopia was known to be

Table 10. Prevalence of *Salmonella* in exotic chicken flocks among seasons.

Season	Examined	Prevalence (%)	χ2	P-value
Autumn	71	39.4	3.343	0.188
Winter	71	36.6		
Spring	90	50.0		
Total	232	42.7		

Table 11. Prevalence of *Salmonella* in exotic chicken flocks among age groups.

Age	Examined	Prevalence (%)	χ2	p-value
Chicken	47	40.4		
Broiler	91	40.7	0.610	0.737
Layers and cocks	94	45.7		
Total	232	42.7		

41.9% in both females males. The current finding on the prevalence of *Salmonella* infection was in agreement with the study of Sikder et al. (2005) who reported 40.5 and 41% prevalence of *Salmonella* among chicken flocks in Bangladish. Among local chicken flocks examined in this study, the sex specific prevalence of *Salmonella* was 38.9 in male and 41.4% in female local chicken flocks. The sex specific prevalence of *Salmonella* in exotic chicken flocks was 39.1% in males and 44.2% in females. Kang et al. (2002) explained that there could not be any sexual impact on the prevalence of *Salmonella* infection in male and female poultry.

The highest prevalence of *Salmonella* was recorded during spring (47.7%) followed by autumn (39.7%) and winter (36.8%). The highest prevalence of *Salmonella* in local chicken flocks was recorded during spring (45.8%) followed by autumn (40.0%) and winter (34.9%). The seasonal prevalence of *Salmonella* in exotic chicken flocks was found to be 39.4, 36.6 and 50.0% during autumn, winter and spring, respectively. Similarly higher prevalence of *Salmonella* during the rainy season (25.0%) and lower prevalence during winter (21.88%) has been reported in Bangladesh by Sikder et al. (2005). This fact could be justified by the fact that highest level of salmonellosis is seen after periods of high seasonal temperatures because temperature may be the major factor impacting the survival and proliferation of *Salmonella* (Korsak et al., 2006).

Layers and cocks were proved to be highly infected with *Salmonella* (46.2%) followed by broilers (41.3%). The lowest prevalence of *Salmonella* infection was seen in chickens (40.8%). The prevalence of *Salmonella* infection in local chick flocks was confirmed to be 41.4, 40.7 and 40.6% among chickens, broilers and layers and cocks, respectively. The prevalence of *Salmonella* infection in local chick flocks was confirmed to be 41.4, 40.7 and 40.6% among chickens, broilers and layers and cocks, respectively. The age specific prevalence of *Salmonella* in exotic chicken flocks was confirmed to be 40.4, 40.7 and 45.7% in chickens, broilers and in layers and cocks, respectively. The age specific prevalence of this study in broilers was lower than the 47% prevalence of *Salmonella* in broilers reported by Uyttendaele et al. (1998) in Belgium and the 74% prevalence of *Salmonella* in broilers reported by Mario (1990) in Mexico poultry farms. Lower prevalence of *Salmonella* among chicken flocks (11.1%) was recorded from Ethiopia by Endrias and Poppe (2009). The prevalence *Salmonella* infection was increased with the increase in age. *Salmonella* infection was found to be highest (30.76%) at 39 weeks of age and lowest (13.33%) at 32 weeks of age in the study of Sikder et al. (2005)

The higher prevalence of *Salmonella* infection in laying hens in this study might be attributed with the reality that layers are physiologically stressed during egg production and molting which significantly depress the immune response of layers and increase the susceptibility to *Salmonella* infection (Landers et al., 2005).

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