JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Salmonellosis prevalence and risk factors in chicken breeding farms in and around Arba Minch town, Gamo Zone, Ethiopia

Edget Abayneh[1], Henok Goba[2], Mesfin Shurbe[3]

[1] College of Agricultural Science, Animal Health Science Department, Arba Minch University, Arba Minch, Ethiopia
[2] Arba Minch Zuria District, Livestock and Fishery Department, Arba Minch, Ethiopia
[3] Arba Minch University Livestock and Fishery Resource Research Center, Arba Minch, Ethiopia

Abstract

Introduction: Salmonellosis is one of the diseases affecting chicken breeding farms in research locations. This study aimed to estimate the prevalence of *Salmonella*, its risk factors, and the distribution of antibiotic resistance in chicken breeding farms in and around Arba Minch town, Southern Ethiopia.

Methodology: A total of 390 samples were obtained from the chicks selected using stratified random selection from the breeding farms. Each chick's rectum was sampled for cloacal swabs and fecal samples, which were later analyzed for *Salmonella* using microbial culture and serological methods. Drug sensitivity testing was done using disk diffusion techniques.

Result: *Salmonella* isolates were found in 7/285 (2.45%) of fecal dropping and 14/105 (13.33%) of cloacal swabs. *S. Anatum* 6/21 (28.57%), *S. Saintpaul* 5/21 (23.8%), *S. Typhimurium* 4/21 (19.04%), *S. Kentucky* 4/21(19.04%), and *S. Haifa* 2/21 (9.52%) were the identified serotypes with a prevalence of 21/390 (5.38%) (95% CI = 2.2-8). According to a multivariate logistic regression analysis of the risk factors, the source of feed, contact with other farms, chick breed, and management were statistically significant influences on the presence of *Salmonella* in chicks ($p < 0.05$). The 8 antimicrobials tested were found to be ineffective against 90.47% of the isolates. These antimicrobials are used in both human and animal medicine.

Conclusions: Our findings confirmed that risk factors such as feed source, breed, contact with other farms, and management had a significant effect on the occurrence of salmonellosis in chicks, and disease control in the study area requires special attention.

Key words: antimicrobial; resistance; chicks; prevalence; risk; *Salmonella*.

J Infect Dev Ctries 2023; 17(2):226-235. doi:10.3855/jidc.17553

(Received 17 October 2022 – Accepted 15 November 2022)

Introduction

Poultry production is one of Ethiopia's most important livestock subsectors. It is crucial in terms of creating job opportunities, improving family nutrition, and empowering women [1]. It is an appropriate business for low-income households due to the small amount of land required and the low investment required to start and run the operation [2]. Ethiopia's current poultry population is estimated to be around 60 million, with the majority (37.9% or 22.7 million) being chicks and only 33.6 percent (20.2 million) being laying hens. Approximately 56% (9.6 million) of Ethiopian households have poultry holdings of varying flock sizes. Approximately 80% of poultry-owning households have 1 to 9 chickens [1].

Ethiopia's poultry sector can be classified into village or backyard, small-scale and commercial poultry production systems. Poultry production is important for the economies of the majority of the country's regions [3]. They are important sources of cash income for women, and some of the proceeds from the sale of family chickens are used to pay for children's school fees [4]. Chicken is also important to the society as a whole. During the holidays and the festive season, they are consumed in every household. As a result, the Ministry of Livestock and Fisheries' livestock master plan prioritized upgrading village chicken production to improve family poultry demand [5].

The growth of Ethiopia's poultry industry has been hampered by a number of factors, including the prevalence of salmonellosis in poultry. Disease-related poultry mortalities are estimated to range between 20 and 50%, but can reach as high as 80% during epidemics [6]. *Salmonella* causes significant economic loss in the poultry industry through mortality and reduced production [7], as well as the various direct expenses producers incur as a result of infections in their flocks. On the other hand, there are additional

costs associated with the treatment of sick birds, such as the cost of medicines and increased labor costs for the management of affected stock [8].

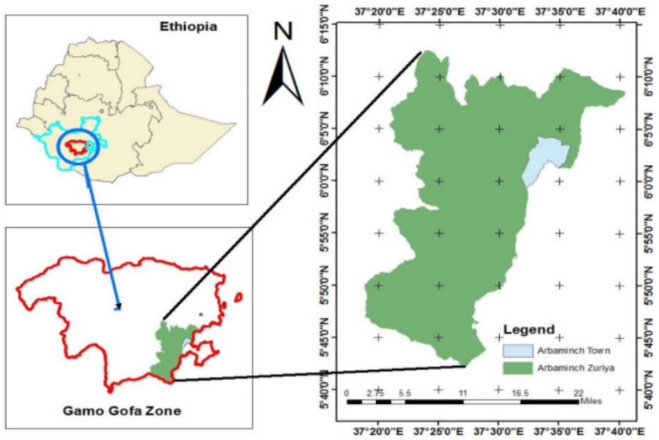
Ethiopia's chicken flock population is not as productive as expected. Salmonellosis is a primary reason for the low productivity of Ethiopian chicken flocks [9]. Day old chicken growing in breeding farms in and around Arba Minch town has been one of the main practices of poultry production in urban and peri-urban areas in recent years. Growing day-old chickens to pullets and cockerels is primarily done for immediate income and job opportunities. There hasn't been much research on salmonellosis in the day-old chicken rearing (breeding farms) system, which is widely used in the current study area after the development of a livestock master plan with greater emphasis than before, despite the relatively substantial information on the epidemiology and prevalence of *Salmonella* in poultry provided by a few research studies on various production systems in different regions of the country [8,10,11,12,13]. The main goals of this study were to estimate the prevalence of salmonellosis in breeding farms, identify any relevant risk factors for the illness, and determine the distribution of antibiotic resistance in and around Arba Minch town.

Methodology

Description of study area

The town of Arba Minch, in southern Ethiopia, was the focus of the study. Arba Minch settlement serves as the administrative hub of the Gamo zone, which is bound on the north by the Wolayta zone, on the east by Lake Abaya and Chamo, and on the south by Segene and a piece of South Omo. Addis Ababa and Arba Minch Town are separated by 446 kilometers. It is situated between latitudes 5°57″ and 6°71″ N and longitudes 36°37″ and 37°98″ E, not far from the region's geographic center. It is located at an elevation of 1285 m above sea level, receives 600–1600 mm of precipitation each year, and has temperatures between 10-34 °C [14].

Figure 1. Location of study area on map (Source: Own preparation using Arc GIS, 2021).



The Arba Minch settlement is surrounded by the Arba Minch Zuria district. Bimodal rainfall occurs in the Arba Minch Zuria district and adjacent areas of the Gamo zone, with the short rainy season lasting from January to April and the long rainy season lasting from June to September. The average annual temperature is 26.33 °C, while the annual rainfall ranges from 800 to 1200 mm in the Arba Minch Zuria district. The district is located between 1001 and 2500 m above mean sea level. There are 95,373 and 320,206 people living in Arba Minch town and Zuria district, respectively [46]. According to the Arba Minch Zuria district and Gamo zone livestock and fisheries resource office report from 2020 [14;15], the district has a total estimated cattle population of 101,628, sheep population of 27,339, goat population of 42,662, horse population of 3,204, and poultry population of 140,050. There are 40 chicken breeding farms in Arba Minch town, and there are around 27 day-old chicken breeding farms in the Arba Minch Zuria district [14] (Figure 1).

Study design and population

A cross-sectional study was conducted from December 2020 to May 2021 to estimate the prevalence, associated risk factors, and drug resistance pattern of *Salmonella* in chicken breeding farms in and around Arba Minch town, Gamo Zone, Ethiopia. The chickens used in the study were raised in small-scale poultry breeding farms that raised commercial exotic chickens. The bacteriological study included kebeles and breeding farms with exotic breeds and chicks ranging in age from 1 day old to 45 days old. Kebeles that do not have breeding farms were excluded from the study.

Determining sample size and sampling technique

The study region was chosen from the Gamo Zone based on past poultry production history relating to breeding and accessibility [21]. Breeding farms were located in 21 of the kebeles that surround Arba Minch town. Of them, 10 were located in the Arba Minch Zuria area and 11 in the town of Arba Minch. Stratified sampling was used to choose an acceptable sample by using breeding farms as strata from which a proportionate number of chicks were taken. Only 10 kebeles were chosen at random to participate in the study due to the project's objectives and resource

constraints. The study included 19 breeding farms from these 10 kebeles.

According to the Arba Minch yearly report on Ethiopian chicken farms [21], the maximum and minimum number of chicks per farm were 3000 and 500, respectively. Sample sizes for proportionately allocated stratified samples were computed using the techniques suited to simple random samples, according to Levy and Lemeshow [45] as described by Thrusfield [16]. In order to ensure the homogeneity of sample collection to the chosen farms, 384 chicks were chosen and 390 samples were taken, with a targeted absolute precision of 5% at a 95% confidence interval and an expected prevalence of 50%. This number was proportionally distributed to each breeding farm based on the size of their flock (Table 1).

Questionnaire poll

The probability proportionate sampling technique was used to determine the sample size for estimating households for the questionnaire survey [17]:

$n = z2xp(q)/d2$

When the population household is 10,000, n is the desired sample size according to Cochran [17], z is the standard normal deviation (1.96 for 95% confidence interval), p is 0.1 (proportion of the population to be included in the sample, i.e., 10%), q is 1 - p, which is 0.9 (90%), and d is the degree of accuracy desired (0.05) or 5% error term. A total of 390 households were included in the study and polled. The questionnaire survey included the owners and practitioners of the breeding farms in the study area.

A semi-structured questionnaire was used to gather demographic, management-related, and other fundamental data regarding the production system and management practice in the study area. The epidemiology questionnaire looked into possible risk factors such the husbandry system (housing, management practices), access to other farms, the source of feed and water, the use of antibiotics by poultry producers, the environment, and farm staff/owner awareness. The housing, litter type, litter change frequency, use of disinfectants as foot-baths at gates, and cleanliness of the feeding and watering equipment used in farms were used in this study as criteria to assess farm management.

Bacteriological testing

Swab samples

After soaking in 10 mL buffered peptone water, a sterile cotton tipped swab with a 3 cm shaft was rubbed inside the chickens' cloaca. The cotton swab was removed from the wooden shaft and soaked in buffered peptone water before being placed in the universal bottle [18].

Fecal droppings

Fecal samples were aseptically taken from the chicken houses, labeled, and placed in sterile, screw-capped, universal bottles. These bottles were then maintained in an icebox with ice packs and sent to Arba Minch University for bacterial isolation and identification. Minor modifications were made to the technique recommended by the International Organization for Standardization [19] for the isolation and identification of *Salmonella*. The Kirby-Bauer disc diffusion method was used for the antimicrobial susceptibility test, and the bacteriological media were prepared in accordance with the manufacturer's instructions.

Pre-enrichment and selective enrichment

Swab samples were pre-enriched in the proper quantity of buffered peptone water in a ratio of (1:9), and they were then incubated at 37 °C for 24 hours. The samples were selectively enriched using Rappaport-Vasiliadis medium (RV) (BD Difco, Heidelberg, Germany). A small portion of the pre-enriched sample, around 0.1 mL, was put into a tube with 10 mL of RV broth and incubated for 24 hours at 42 °C.

Plating out and identification

For plating out and identification, xylose lysine desoxycholate (XLD) agar was utilized. Inoculums from RV broth cultures were looped onto XLD and incubated there for 24 hours at 37 °C. After incubation, the plates were checked to see if any questionable or typical colonies were present. In contrast to hydrogen sulfide (HS) negative variations developed on XLD agar, which are pink with a darker pink center, typical colonies of *Salmonella* grown on XLD-agar have a black center and a weakly translucent zone of reddish color. *Salmonella* that had tested positive for lactose on

Table 1. The distribution of sample size among breeding farms.

Flock size of the farm	No of farms	Sample size to be taken	Sample type	
			Cloacal swab	Fresh feces
500-1000	6	10-15	10	4
1000-2000	7	15-20	15	5
> 2000	6	20-30	20	5-8

XLD agar was yellow with or without blackening. Suspected isolates were sub-cultured on XLD agar to produce pure isolates [19].

Biochemical tests

All possible non-lactose fermenting *Salmonella* colonies were selected from the XLD agar and inoculated into the following biochemical tubes for identification: triple sugar iron (TSI) agar, Simmon's citrate agar, Lysine iron agar, SIM agar, and MR-VP broth (Sifin, Berlin, Germany). These biochemical tubes were incubated for 24 or 48 hours at 37 °C. *Salmonella* was thought to be present in colonies that produced an alkaline slant with acid (yellow color) butt on the TSI with hydrogen sulfide production, positive for lysine (purple color), negative for tryptophan utilization (indole test) (yellow-brown ring), negative for Voges-Proskauer, and positive for citrate utilization [20].

Serological identification

Sero-grouping of identified bacterial isolates was performed in the National Veterinary Institute (NVI) Debre Zeit according to the Kauffmann–White method [44].

Antimicrobial susceptibility testing

As advised by the National Committee for Clinical Laboratory Standards [22], antibiotic susceptibility of the isolates was evaluated using the disc diffusion technique. Isolated colonies were added to tubes filled with sterile saline water and stirred to make a smooth suspension in order to meet the 0.5 McFarland turbidity requirements. A sterile cotton swab was dipped into the solution, rotated a few times, and then swabbed uniformly across the Muller Hinton agar plate's surface. To remove extra inoculums, firm pressure was applied to the tube's interior wall above the fluid level. The plates were exposed to room temperature drying for 30 minutes. The following antibiotic discs were tested for the susceptibility of the isolates: Ten grams each of the following drugs: ampicillin (AMP), ciprofloxacin (CIP), chloramphenicol (C), tetracycline (TE), and streptomycin (S), 30g of erythromycin (E), amoxicillin (AML), and cefoxitin (FOX) were positioned at least 15 mm apart and away from the border of the plates to prevent overlapping inhibitory zones. The plates were incubated for 24 hours at 37 °C. The diameter of the inhibitory zones was categorized as being resistant, moderate, or susceptible in accordance with the Clinical Laboratory Standards Institute's interpretation [22].

Data management and analysis

The entire data set was cleaned, entered into a Microsoft Office Excel spreadsheet, and then subjected to STATA 14 analysis. *Salmonella* status was the dependent variable, and categorical independent variables were employed to analyze the connection between them using multivariable logistic regression. The degree to which risk variables and the prevalence of *Salmonella* are correlated was investigated using an odds ratio. The goodness-of-fit of the model was then assessed using the Hosmer-Lemeshow method. A p value < 0.05 was used as the statistical significance cutoff point and the examined data were presented using tables, percentages, and odds ratios.

Results

Salmonella serotype, prevalence and distribution

Salmonella was found in 21/390 (5.38%) samples, of which *S. Anatum* 6/21 (28.57%), *S. Saintpaul* 5/21 (23.8%), *S. Typhimurium* 4/21 (19.04%), *S. Kentucky* 4/21 (19.04%) and *S. Haifa* 2/21(9.55%) were the serotypes identified (Table 2). The levels of *Salmonella* serotype varied among the sampling sites and types. The level of *Salmonella* serotype in the Arba Minch town was not significantly higher than the level of *Salmonella* serotype in the Arba Minch Zuria (p > 0.05). The level of *Salmonella* serotype in cloacal swabs from the Arba Minch town was slightly higher than the level of *Salmonella* serotype in fecal drooping from Arba Minch Zuria. Table 2 depicts the distribution of *Salmonella* serotype prevalence by sample type and collection site.

Risk factors linked to the prevalence of Salmonella

Thirteen potential risk factors were evaluated using multivariable logistic regression analysis: flock size, age in days, litter type, frequency of litter change, water source, feed source, contact with other farms, treatment history, breed, type of sample, disease history, management, and site of sample collection.

Four of the risk factors including the source of feed, contact with other farms, breed of the chicks and management were found to be statistically significant (p < 0.05) factors for *Salmonella* prevalence in multivariable logistic regression analysis and predictors of *Salmonella* in breeding farms (p < 0.05) (Table 3). The data fit of the model was also evaluated. The model fit the data according to the Hosmer-Lemeshow goodness-of-fit test (2 = 7.79; p = 0.45) (Supplementary Table 1).

Table 2. *Salmonella* prevalence, serotypes isolated from poultry farms in study site, sample type and farm flock size.

Characteristics	No. examined	Positive %	Serotype				
			S. Typhimurium	*S. Saintpaul*	*S. Kentucky*	*S. Haifa*	*S. Anatum*
Study site	390	**21 (5.38%)**	4/21 (19.04%)	5/21 (23.8%)	4/21 (19.04%)	2/21 (9.55%)	6/21 (28.57%)
Arba Minch Town	191	11 (2.82%)	3/11 (27.27%)	3/11 (27.27%)	2/11 (18.19%)	0	3/11 (27.27%)
A/Minch Zuria	199	10 (2.56%)	1/10 (10%)	2 (20%)	2 (20%)	2 (20%)	3 (30%)
Sample type	390	**21 (5.38%)**	4/21 (19.04%)	5/21 (23.8%)	4/21 (19.04%)	2/21 (9.55%)	6/21 (28.57%)
Cloacal swab	297	14 (3.59%)	2/14 (14.29%)	4/14 (28.57%)	4/14 (28.57%)	0	4/14 (28.57%)
Fecal droop	93	7 (1.79%)	2/7 (28.57%)	1/7 (14.29%)	0	2/7 (28.57%)	2/7 (28.57%)
Farm flock size	390	21 (5.38%)	4/21 (19.04%)	5/21 (23.8%)	4/21 (19.04%)	2/21 (9.55%)	6/21 (28.57%)
< 1000	91	3 (0.77%)	0	0	0	0	3/3 (100%)
1001-2000	124	8 (2.05%)	3/8 (37.5%)	1/8 (12.5%)	1/8 (12.5 %)	1/8 (12.5%)	2/8 (25%)
> 2000	175	10 (2.56%)	1/10 (10%)	4/10 (40%)	3/10 (30%)	1/10 (10%)	1/10 (10%)

Table 3. Multivariable logistic regression analysis found that potential risk factors were significantly associated with *Salmonella* prevalence.

Risk Factors		Examined	No +ve	P (%)	OR	CI (95%)	*p* value
Flock Size	< 1000	91	3	3.29	Ref		
	1001-2000	124	8	6.45	2.02	0.52-7.85	0.308
	> 2000	175	10	5.71	1.77	0.47-6.62	0.391
Age	1-14days	141	3	2.12	Ref		
	15-28days	135	11	8.14	4.08	1.12-14.96	0.134
	28-45days	114	7	6.14	3	0.76-11.91	0.117
Litter type	wood shaving	320	19	5.93	Ref		
	wood shaving and straw	70	7	10	0.46	0.10-2.04	0.312
Frequency of litter change	once per month	120	8	6.67	Ref		
	twice per month	148	8	5.4	0.8	0.29-2.19	0.665
	three and more/month	122	5	4.09	0.59	0.19-1.88	0.38
Water source	tap	257	14	5.45	Ref		
	tap and additional sources	133	7	5.3	0.96	0.38-2.45	0.939
Feed source	chick provider	185	4	2.16	Ref		
	chick provider plus home by products	205	17	8.29	3.03	2.60-12.0	0.003
Contact with other birds	no	199	5	2.51	Ref		
	yes	191	16	8.37	2.23	0.60-9.90	0.005
Treatment history	no	271	18	28.7	Ref		
	treated with antibacterial	91	2	2.19	0.31	0.07-1.38	0.127
	treated with anticcocidial	28	1	3.57	0.52	0.06-4.05	0.533
Breed	Saso	259	9	3.47	Ref		
	Bovans	131	12	9.16	2.8	1.15-6.85	0.024
Type of sample	cloacal swab	297	14	4.71	Ref		
	fresh dropping	93	7	7.52	1.64	0.64-8.86	0.299
Disease history	no/absent	189	5	2.64	Ref		
	yes/present	201	16	7.96	3.18	1.14-8.86	0.027
Management	poor	159	15	9.43	Ref		
	satisfactory/good	231	6	2.59	-3.12	-11.90-2.7	0.002
Site of collection	Arba Minch town	199	11	5.52	Ref		
	Arba Minch Zuria Kebeles	191	10	5.23	0.86	0.35-2.08	0.748

Table 4. *Salmonella* isolates' antimicrobial susceptibility to common medications.

Type of Antibiotics	N	No. of *Salmonella* isolates		
		Susceptible (%)	Intermediate (%)	Resistant (%)
Ciprofloxacin (CIP)	21	19 (90.5)	2 (9.5)	0 (0)
Ampicillin (AMP)	21	8 (38.09)	7 (33.34)	6 (28.57)
Cefoxitine (FOX)	21	13 (61.9)	5 (23.8)	3 (14.28)
Streptomycin (S)	21	16 (76.19)	3 (14.28)	2 (9.52)
Chloranphenicol (C)	21	10 (47.61)	3 (14.28)	8 (38.09)
Erytromycin (E)	21	11 (52.38)	7 (33.34)	2 (9.52)
Tetracycline (TE)	21	2 (9.52)	4 (19.05)	15 (71.42)
Amoxacilin (AML)	21	8 (38.09)	3 (14.28)	10 (47.61)

Salmonella isolates antimicrobial susceptibility profile

All of the isolates from the study (n = 21) were tested with eight different antimicrobials that were commonly used and available on the market.

15 isolates were resistant to tetracycline (15/21) followed by amoxacilin (10/21), chloranphenicol (8/21), ampicillin (6/21), cefoxitine (3/21), and erytromycin + streptomycin (2/21). However, 90.5% (19/21) of the isolates were found to be sensitive to ciprofloxacin (Table 4). Nineteen of the twenty-one isolates (90.5%) were resistant to one or more of the tested antimicrobials. Table 4 summarizes the isolates' responses to the eight different antimicrobials.

In the case of multidrug resistance tests, resistance to five antibiotics (AMP, S, E, TE, and AML) was the most common multidrug resistance (MDR) found. MDR to three antibiotics was found in 46.67% (7/15) isolates (Table 5).

Table 5. *Salmonella* isolates have a trend of multi-drug resistance.

No of antimicrobials	Antimicrobial resistance pattern (no of isolates)	Number of isolates (%)
Three	AMP, FOX, TE (1) C, TE, AML (3) AMP, TE, AML (1) E, TE, AML (1) AMP, C, TE (1)	7 (43.75)
Four	S, E, TE, FOX (1)	1 (6.67)
Five	AMP, S, E, TE, AML (1)	1 (6.67)

AMP: ampicillin; CIP: ciprofloxacin; C: chloramphenicol; TE: tetracycline; S: etreptomycine; E: erythromycin; AML: amoxacillin; FOX:cefoxitin.

Discussion

The current incidence of *Salmonella* was lower than in other studies from Ethiopia: 15.12% in poultry farms from Modjo [23] and 42.7% in indoor chicken flocks from Jimma town [24], 11.9% in southern and central Ethiopia [8]. Previous research on the frequency of *Salmonella* did not particularly address farms that raise chickens, but they did take into account age as a risk factor.

A higher prevalence of *Salmonella* was also reported from various countries, which differed significantly from the current results in poultry farms. Among these, Khudor *et al.,* [25] reported 25.7% prevalence of *Salmonella* in breeding farms in Iraq, 94% in broiler farms, and 17.9% in layer farms in the Netherlands [26]; and 36.1% in broiler farms in Japan [27]. The higher prevalence reported by different studies from different countries could be due to differences in *Salmonella* contamination, sample type, location, sampling techniques, and detection methods used. One study's results, based on *Salmonella* isolation from chickens in Khartoum was slightly higher than 2.9%. The prevalence found in this study was 5.38%, which was a little higher than the prevalence found in [12]. The current study's findings surpassed a 0.8% prevalence report from Hawassa's small-scale chicken flock farms, which was discovered utilizing the direct swab plating method [29].

The overall level of *Salmonella* serotype was higher in fecal dropping than cloacal swab of the previous study by Fiseha [8], who found that fecal dropping had a greater prevalence (16.4%) than cloacal swabs (10.4%) which is consistent with the present study. The prevalence of *Salmonella* in cloacal swab samples in this investigation was greater than the reports by Kassaye *et al.* (0.8%) who utilized the same sample [30] and Mdegela *et al.* (2.6%) from commercial farms in Tanzania [31]. It was lower than the report by Islam *et al.,* [33] from Bangladesh which was 16.7%. This might be caused by variations in the production process, isolation technique and difference in biosecurity measures.

This study identified that the prevalence of *Salmonella* was higher in chicks aged 14 to 28 days. *Salmonella* was found to be 4.83 times more likely in chicks of this age category than in chicks aged 1 to 14 days. This finding contrasted with that of Khudor *et al*. in Basrah, Iraq, who reported an isolation rate of 25.7% in the first week of the chicks [25]. However, the prevalence of *Salmonella* in chicks older than 28 days was lower than in chicks aged 15 to 28 days. *Salmonella* is also less likely in this group than in the previous one. However, there was no statistically significant relationship ($p > 0.05$). This could be because of the increased immunity that comes with age and exposure.

According to the current findings, the incidence of *Salmonella* was practically identical in Arba Minch and the nearby kebeles, which contrasts with findings from prior research that indicated the prevalence varied depending on the location of sample collection [13;33;34]. The location of sample collection and the prevalence of *Salmonella* were not statistically significantly correlated ($p > 0.05$). The management practices of the owners and various biosecurity measures may, however, expose the birds to a variety of potential sources of *Salmonella* contamination in indoor chickens that have spent more time on the farms than breeding farms. This may account for the differences between the study areas in earlier studies.

The current study's findings revealed that the source of feed for the chicks was statistically associated with *Salmonella* prevalence in breeding farms, with slightly higher prevalence in farms that provide an

additional feed from home byproducts to the chicks in breeding, 8.29%, than in farms that only provide the feed provided with the chicks by the hatchery, 2.16%. The likelihood of *Salmonella* in chicks fed additional feed from home byproducts was 3.4 times greater than the likelihood of *Salmonella* in chicks fed hatchery feed. Thus, *Salmonella* infection in breeding farms may result from household waste products that may have come into contact with other *Salmonella* sources as a result of contact with a different production system.

Salmonella contamination of poultry in pre-harvest environments can usually be traced back to production issues such as contaminated poultry feed or pathogen introduction into the facilities via a variety of carriers such as house pets, wild animals, and insects [35]. There are several reasons for the increased focus on poultry feeds as a source of *Salmonella*. To begin with, one *Salmonella* organism per gram of feed can colonize in young chicks; low or undetectable numbers of *Salmonella* represent a high risk for infection in these birds, which is exacerbated by increased feed mixing and incorporation of feed ingredients from a variety of sources. This is especially concerning if the breeder flock hatchlings are exposed, as they are the starting point for all commercial flocks and household poultry keepers [36].

Salmonella was shown to be more common where breeding farms were in contact with other farms and free-ranging chickens in surrounding homes. On farms with contact, the prevalence was 8.37%, compared to 2.51% on farms without contact. Compared to farms with no contact, farms with contact had a 4.13 times higher chance of having *Salmonella*. This result is consistent with the idea that contact with carriers or sick birds can spread *Salmonella* horizontally among chickens [37]. No-contact farms included those with a separate feed storage facility, farms without one, and residences with backyard chicken coops close to the breeding site.

In this study, breeding farms with good biosecurity practices were classified as well-managed, while breeding farms without biosecurity measures were classified as poorly managed. *Salmonella* prevalence varied depending on how breeding farms were managed. *Salmonella* was found to be more common in poorly managed breeding farms (9.43%) than in well-managed farms (2.59%). In properly managed breeding farms, the likelihood of *Salmonella* was 0.231 times lower than in improperly managed breeding farms.

In the current investigation, it was discovered that *Salmonella* isolates had resistance to the following antibiotics: tetracycline (71.42%), amoxicillin (47.61%), chloramphenicol (38.09%), ampicillin (28.57%), cefoxitin (14.28%), streptomycin, and erythromycin (9.52%). Tetracycline demonstrated the greatest resistance in this study, which was also consistent with the findings of Destaw *et al.* [13], who found that 82% of antimicrobials were resistant to *Salmonella* isolated from the caecal contents of exotic chicken in Debre Zeit and Modjo, Ethiopia. The most recent discovery was more important than earlier studies on *Salmonella* drug resistance isolated from food animals, animal products, and personnel. In one study, resistance to tetracycline was 41.2%, 30% to chloramphenicol [38], and 46.9% to tetracycline [39]; however, another study reported 45.5% resistance to chloramphenicol and 100% to tetracycline [39].

According to Okeke *et al*. [40], the high level of *Salmonella* antibiotic resistance may be due to the fact that antimicrobials can be purchased without a prescription in many Sub-Saharan African countries, and indiscriminate use of antimicrobial agents by unskilled practitioners in both the veterinary and public health sectors is common. The lack of resistance to ciprofloxacin, streptomycin (9.52%), and erythromycin (9.52%) in this study suggests that these medications are relatively the most effective against *Salmonella* in poultry production. The fact that ciprofloxacin and similar medications are not frequently used in poultry in nations like Ethiopia and other African nations may explain why they are effective [20]. The finding of 100% susceptibility to ciprofloxacin concur with a previous report of 100% susceptibility in *Salmonella* isolated from poultry farms in Modjo [23], but differ from a study that found 11.9% resistance to ciprofloxacin in samples related to poultry at particular locations in central and southern Ethiopia [34].

The level of multidrug resistance found in the current study was lower than 97.7% [40], 83.3% [20], and 52.5% [37], who reported that *Salmonella* isolates from chicken carcass and giblet displayed multiple-drug resistance, which was higher than the finding of this study in breeding farms. In contrast, the current finding was higher than 44.8% [41] who isolated from food of animal origin, humans, and animals, as well as 32.65% [39] from food items and personnel. This finding differed from that of [23], who discovered the highest MDR registered to seven antimicrobials in *Salmonella* isolated from poultry farms in Modjo. The pattern of resistance to five antibiotics out of eight in this study suggests that chicken may be a source of single and multiple antimicrobial-resistant *Salmonella* infection, which is a serious public health concern.

The irrational use of antibiotics against poultry diseases by breeding farmers and other chicken production farms was a major contributor to MDR. The questionnaire survey also revealed that oxytetracycline and sulfonamides were the most commonly used antibiotics in the study area. The area's cattle and fowl may also use the drugs improperly, leaving antibiotic residues in the environment. The resistance gene is then acquired by *Salmonella* isolates from other bacterial species or the environment. When drug misuse becomes excessive, it exerts pressure on the emergence of drug resistance. *Salmonella* can develop resistance by transformation, conjugation, or transduction, all of which involve the exchange of genetic material between living things [42]. Transduction involves the use of a bacteriophage to accomplish this task[Supplementary Table 2].

Conclusions

One of the biggest problems for the breeding farms in the research area is salmonellosis. *Salmonella* was found to be prevalent in 5.38% of cases and *S. Anatum* 6 (1.53%), *S. Saintpaul* 5 (1.28%), *S. Typhimurium* 4 (1.02%), *S. Kentucky* 4 (1.02%) and *S. Haifa* 2 (0.50%) were the identified serotypes in the study area. The type of feed used, interaction with other farms, the breed of the chicks, and management were all factors in its dissemination. This study found that *Salmonella* is not only frequent in breeding farms but also that the isolates have antibiotic resistance. Nineteen of the 21 isolates, or 90.47 percent, were resistant to at least one of the tested antibiotics. 84.21% (16 of 19) of the tested samples were also shown to have the resistant isolates exhibiting multidrug resistance. This study also found that breeding farms and other chicken-producing facilities utilize antimicrobials against poultry diseases irrationally and do not vaccinate their animals against salmonellosis. Finally, breeding farmers should receive adequate training on fundamental poultry-keeping skills and management-related issues, awareness creation on the inappropriate use of antibiotics, and establishment of standardized monitoring systems in poultry farms should be necessary. Regular epidemiological surveillance should be carried out to monitor the occurrence and distribution of *Salmonella* in breeding farms.

Acknowledgements

The authors are appreciative of Arba Minch University's College of Agricultural Science and College of Natural Science for allowing them to use their lab spaces.

References

1. FAO (2019) Poultry Sector Ethiopia. FAO Animal production and Health Livestock Country Review Available: https://www.fao.org/documents/card/ar/c/CA3716EN/. Accessed: 1 September 2021.
2. FAO (2019) Poultry development review. Available: https://www.fao.org/3/i3531e/i3531e.pdf. Accessed: 1 September 2021.
3. Fisseha M, Azage T, Tadelle D (2010) Indigenes chicken production and marketing system in Ethiopia: characteristic and opportunities for market-oriented development. IPMS (improving productivity and market success) of Ethiopian Farmers Project Working Paper 24. Nairobi, Kenya, ILRI press. 66 p.
4. FAO (2019) Poultry Sector Ethiopia. FAO Animal production and Health Livestock Country Reviews. Available: https://scholar.google.com/scholar?q=FAO+(2019)+Poultry+Sector+Ethiopia.+FAO+Animal+production+and+Health+Livestock+Country+Reviews+11,+Rome+17&hl=en&as_sdt=0&as_vis=1&oi=scholart. Accessed: 4 September 2021.
5. Shapiro BI, Gebru G, Desta S, Negassa A, Nigussie K, Aboset G, Mechal H (2015). Ethiopia livestock master plan. ILRI Project Report. Nairobi, Kenya: International Livestock Research Institute (ILRI). Available: https://www.researchgate.net/publication/283781705_Ethiopia_livestock_master_plan_Roadmaps_for_growth_and_transformation. Accessed: 2 September 2021.
6. Tadelle D, Ogle B (2001) Village poultry production system in the Central Highlands of Ethiopia. Trop Anim Health Prod 33: 521- 537.
7. Mares M (2017) Current topics in *Salmonella* and Salmonellosis. London, United Kingdom, IntechOpen. Available: https://www.intechopen.com/books/5464. Accessed: 10 September 2021.
8. Fiseha M (2015) *Salmonella* isolates and drug resistance epidemiology in poultry related samples in selected sites of central and south Ethiopia. MSc Thesis. Addis Ababa University. 1-100.
9. Gebre-Egziabher MM (2007) Characterization of smallholder poultry production and marketing system of Dale, Wonsho and Loka Abaya Weredas of Southern Ethiopia. MSc thesis (Animal Production) Awassa (Ethiopia) Hawassa University Available: https://hdl.handle.net/10568/701. Accessed: 5 September 2021.
10. Bekele B, Ashenafi M (2010) Distribution of drug resistance among enterococci and *Salmonella* from poultry and cattle in Ethiopia. Trop Anim Health Prod 42: 857–864.
11. Alebachew K, Mekonnen A (2013) A survey on *Salmonella* infection among chicken flocks in Jimma town, Ethiopia. Afr. J. Microbiol Res 7: 1239-1245.
12. Eguale T (2018) Non-typhoidal *Salmonella* serovars in poultry farms in central Ethiopia: prevalence and antimicrobial resistance. BMC Vet Res 14: 217.
13. Destaw AA, Belege T, Aragaw E (2020) Prevalence and antibiotic resistance pattern of *Salmonella* isolated from caecal contents of exotic chicken in Debre Zeit and Modjo, Ethiopia. Int J Microbiol: 6: 1910630.

14. Arbaminch Zuria District Livestock and Fishery Resource Office (2018) Report from Arbaminch Zuria district livestock and fishery resource office, Gamo Zone, SNNPR (Unpublished report) Arba Minch Zuria. 32p.
15. Gamo Zone Livestock and Fishery Resource Department (2020) Gamo Zone Livestock and Fishery Resource Department, Gamo Zone, SNNPR (Unpublished report). Arba Minch. 15p.
16. Thrusfield M, Christley R, Brown H, Diggle P, French F, Howe K, Kelly L, Connor K, Sargeant N, Wood H (2018) Veterinary Epidemiology, 4th edition. Royal School of Veterinary Studies University of Edinburgh: Willey Blackwell 276 p.
17. Cochran WG (1977) Sampling Techniques, 3rd edition. New York: John Wiley and Sons. 442 p.
18. ISO-17604 (2003) Microbiology of food and animal feeding stuffs: carcass sampling for microbiological analysis. Available: https://www.iso.org/standard/33146.html. Accessed: 25 September 2021.
19. ISO-6579 (2002) Microbiology of food and animal feeding stuffs: horizontal method for the detection of *Salmonella* spp. ISO. Available: https://www.iso.org/obp/ui/#iso:std:iso:6579:en. Accessed: 25 September 2021.
20. Zelalem A, Nigatu K, Zufan W, Haile G, Alehegne Y, Tesfu K (2011) Prevalence and antimicrobial resistance of *Salmonella* isolated from lactating cows and in contact humans in dairy farms of Addis Ababa. BMC Infect Dis 11: 222.
21. Ethio-chicken Farms Arba Minch (2020) Annual report of sales and breeding farms. Unpublished.
22. Clinical and Laboratory Standards Institute (2012) Performance standards for antimicrobial susceptibility testing. 17th Informational Supplement CLSI Document M100-S17 27. 32nd. 362 p.
23. Abunna F, Bedasa M, Beyene T, Ayana D, Mamo B, Duguma R (2017) *Salmonella*: isolation and antimicrobial susceptibility tests on isolates collected from poultry farms in and around Modjo, Central Oromia, and Ethiopia. JAPSC 5: 21-35.
24. Kindu A, Addis M (2013) A survey on *Salmonella* infection among chicken flocks in Jimma town, Ethiopia. Afri J of Microbiol Res 7: 1240-1245.
25. Khudor MH, Ali AA, Nael MO (2014) Isolation and identification of *Salmonella* spp. from poultry farms by using different techniques and evaluation of their antimicrobial susceptibilities. Bas J Vet Res 1: 246-259.
26. Adeline HS, Marianne C, Sophie Le B, Franc L, Isabelle P, Sandra R, Virginie M, Philippe F, Nicolas R (2009) Risk factors for *Salmonella enterica* subsp. *enterica* contamination in 519 French laying hen flocks at the end of the laying period. Preve Vet Med 89: 51-58.
27. Ishihara K, Takahashi T, Morioka A, Kojima A, Kijima M, Asai T, Tamura Y (2009) National surveillance of *Salmonella enterica* in food-producing animals in Japan. Acta Vet Scandinavica 51: 35.
28. Hiba HMA (2007) Isolation and Identification of *Salmonella* species from Chickens in Khartoum State. University of Khartoum, A thesis submitted to University of Khartoum in Partial Fulfillment of the Requirements for the Degree of Master of Science in Microbiology by Courses and Supplementary Research.
29. Aragaw k, Lencho T, Abera M (2010) Prevalence of *Salmonella* infection in intensive poultry farms in Hawassa and isolation of *Salmonella* species from sick and dead chickens. Ethiop Vet J 14:115-124.
30. Mdegela RH, Yongolo MGS, Minga UM, Olsen JE (2000) Molecular epidemiology of *Salmonella gallinarum* in chickens in Tanzania. Avian Pathol 29: 457-463.
31. Islam MM, Haider MG, Chowdhury EH, Kamruzzaman M, Hossain MM, (2006) Seroprevalence and pathological study of *Salmonella* infections in layer chickens and isolation and identification of causal agents. Bangl J Vet Med 4: 79-85.
32. Mengistie F (2015) *Salmonella* isolates and drug resistance epidemiology. In Poultry related samples in selected sites of Central And South Ethiopia. MSc Thesis. Addis Ababa University College of Agriculture and Veterinary Medicine.
33. Abdi RD, Fisseha M, Ashenafi FB, Takele B, Hika W, Bedasso M, Dinka A, Fufa A (2017) Determination of the sources and antimicrobial resistance patterns of *Salmonella* isolated from the poultry industry in Southern Ethiopia. BMC Infect Dis 17: 352.
34. Park SY, Woodward CL, Kubena LF, Nisbet DJ, Birkhold SG, Ricke SC (2008) Environmental dissemination of foodborne *Salmonella* in preharvest poultry production: reservoirs, critical factors and research strategies. Crit Rev Environ Sci Technol 38: 73-111.
35. Jones FT (2011) A review of practical *Salmonella* control measures in animal feed. J Appl Poult Res 20: 102–113.
36. Tabo DA, Diguuimbaye CD, Granier SA, Moury F, Brisabois A, Elgroud R, Millemann Y (2013) Prevalence and antimicrobial resistance of non-typhoidal *Salmonella* farms in N'Djamena. Chad Vet Microbiol 166: 293-298.
37. Molla B, Mesfin A, Alemayehu D (2003) Multiple antimicrobial-resistant *Salmonella* serotypes isolated from chicken carcass and giblets in Debre Zeit and Addis Ababa, Ethiopia. Ethiopian Journal of Health Development 17: 131-149.
38. Zewudu E, Cornelius P (2009) Antimicrobial resistance pattern of *Salmonella* serotypes isolated from food items and personnel in Addis Ababa. Ethiopian Tropical Animal Health and Production 41: 241-249.
39. Ferede B (2014) Isolation, identification, antimicrobial susceptibility test and public awareness of *Salmonella* on raw goat meat at Dire Dawa municipal abattoir, eastern Ethiopia. Addis Ababa University College of Veterinary Medicine and Agriculture. Msc Thesis. 91 p.
40. Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, Pablos-Mendez A (2005) Antimicrobial resistance in developing countries. Part I: Recent trends and current status. Lancet Infec Dis 5: 481-493.
41. Molla W (2004). Cross sectional study on *Salmonella* in apparently healthy slaughtered sheep and goat at Addis Ababa and Modjo abattoirs. MSc thesis. Addis Ababa University Faculty of Veterinary Medicine, Debrezeit, Ethiopia.
42. Courvalin P (1996) Molecular and epidemiologic aspects of the resistance to antibiotics: example of glycopeptides on Enterococci. C R Seances Soc Biol Fil 190: 467-469.
43. Mezene W, Getahun EA, Balako G, Dinka A, Gezahegn M (2020) Antibiotic use in poultry production in selected districts of East Showa Zone, Central Ethiopia: from antibiotic stewardship perspective. Am-Euras J Sci Res 15: 101-111.
44. Aribam SD, Elsheimer-Matulova M, Matsui H, Hirota J, Shiraiwa K, Ogawa Y, Hikono H, Shimoji Y, Eguchi M (2015) Variation in antigen-antibody affinity among serotypes of *Salmonella* O4 serogroup, determined using specific antisera. FEMS Microbiol Let 362: 362–368.
45. Levy PS, Lemeshow S (2008) Sampling of populations: methods and applications, 4th Edition Willy. 616p.

46. European Commission's Joint Research Centre (2021) Work on the GHS built-up grid. Available: https://data.jrc.ec.europa.eu/collection/ghsl. Accessed: 10 September 2021

Corresponding author
Edget Abayneh, DVM, MVSc
Instructor in Arba Minch University, Arba Minch, SNNPR, Ethiopia P.O. Box 21. Arba Minch University
Tel: +251913347763
Email: edgetabayneh@gmail.com; edget.abayneh@amu.edu.et

Conflict of interests: No conflict of interests is declared.

Annex – Supplementary Items

Supplementary Table 1. Univariable logistic regression analysis result.

Risk Factors	No. Examined	No positive	Prevalence (%)	OR	CI (95%)	p-Value
Flock Size						
<1000	91	3	3.29	Ref		
1001-2000	124	8	6.45	2.02	0.52-7.85	0.308
>2000	175	10	5.71	1.77	0.47-6.62	0.391
Age						
1-14days	141	3	2.12	Ref		
15-28days	135	11	8.14	4.08	1.12-14.96	0.034
28-45days	114	7	6.14	3	0.76-11.91	0.117
Litter Type						
wood shaving	320	19	5.93	Ref		
wood shaving and straw	70	7	10	0.46	0.10-2.04	0.312
Frequency of litter change						
once per month	120	8	6.67	Ref		
twice per month	148	8	5.4	0.8	0.29-2.19	0.665
three and more/month	122	5	4.09	0.59	0.19-1.88	0.38
Water source						
tap	257	14	5.45	Ref		
tap and additional sources	133	7	5.3	0.96	0.38-2.45	0.939
Feed source						
chick provider	185	4	2.16	Ref		
chick provider plus home by products	205	17	8.29	4.09	1.35-12.39	0.013
Contact with other birds						
No	199	5	2.51	Ref		
Yes	191	16	8.37	3.54	1.27-12.39	0.015
Treatment history						
No	271	18	28.7	Ref		
Treated with antibacterial	91	2	2.19	0.31	0.07-1.38	0.127
Treated with anticcocidial	28	1	3.57	0.52	0.06-4.05	0.533
Breed						
Saso	259	9	3.47	Ref		
Bovans	131	12	9.16	2.8	1.15-6.85	0.024
Type of sample						
Cloacal swab	297	14	4.71	Ref		
fresh dropping	93	7	7.52	1.64	0.64-8.86	0.299
Disease history						
No/absent	189	5	2.64	Ref		
Yes/present	201	16	7.96	3.18	1.14-8.86	0.027
Management						
Poor	159	15	9.43	Ref		
satisfactory/good	231	6	2.59	0.256	0.09-0.67	0.006
Site of collection						
Arba minch town	199	11	5.52	Ref		
Arba minchzuria kebeles	191	10	5.23	0.86	0.35-2.08	0.748

Supplementary Table 2. Questionnaire survey result.

Variables	No. of respondents	Distributions
Poultry keeping experience		
<1year	10	7.24
1-5years	42	30.43
>5years	86	62.31
Source of day-old chicks		
Private hatchery	68	49.27
Gov't hatchery	0	
Ngo's	70	50.73
Recommended house construction		
No	36	26.08
Yes	102	73.92
Breed of chicks		
Saso	79	57.24
Bovans red island	46	33.34
Local	13	9.42
Type of litter used		
Wood shaving	67	48.55
Teff straw	71	51.45
Frequency of litter change		
Once/month	34	24.63
Twice/month	20	14.5
Three and more	12	8.69
Major problems farms face		
Disease	103	74.63
Enviromental stress	12	8.69
Management related	53	38.4
Major disease signs		
Diarrhea	24	17.39
Depression	56	40.58
Loss of opetite	89	64.49
Loss of condition	61	44.2
Others	23	16.67
Training		
No	29	21.01
Yes	109	78.98
Awareness		
No	98	71.02
Yes	40	28.98
Vaccination		
No	25	18.12
Yes	113	81.88
Type of vaccines		
Newcastle	113	81.88
Fowl pox	113	81.88
Treatment		
No	14	10.14
Yes	124	89.85
Type of treatment used		
Oxytetracycline	87	63.04
Sulfonamides	76	55.07
Amprolium	23	16.67
Vitamins	34	24.63
Poultry farm in vicinity		
No	26	18.84
Yes	112	81.16
Contact		
No	18	13.04
Yes	120	86.95
Point of contact		
Water source	78	56.52
Feed source	64	46.37
Market place	78	56.52
Others	72	52.17
Predisposing point		
Feed source	54	39.13
Market place	67	48.55
Water source	21	15.21
Working equipment	52	37.68