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Seroprevalence of Salmonellosis and Associated Risk Factors in Intensive Poultry Farms in Wolaita Sodo Town, Southern Ethiopia

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Abstract

Salmonellosis is a serious disease that affects poultry globally due to its high rate of morbidity, mortality and decreased productivity and results in significant financial losses. A cross-sectional study was carried out in Wolaita Sodo town, Southern Ethiopia, between October 2022 and April 2023, with the aim of determining the seroprevalence of salmonellosis and identifying the risk factors that contribute to its spread in the Bovace brown/exotic breeds from intensive poultry farms that were randomly selected. Three farms were randomly selected from study area and about a total of 384 blood samples were collected from the wing vein to prepare serum and serum slide agglutination test was performed following the test procedure. Based on this, out of 384 collected samples, 113 were tested positive for Salmonellosis with an overall prevalence 29.42%. A statistical investigation was conducted to assess the relationship between the infection rates of *Salmonella gallinarum* along with various factors. The results indicated a significant association ($P < 0.05$) between the age category of chickens, feed type and farm size. On the other hand, there is no discernible association ($p > 0.05$) between the housing system and the infection's prevalence. In conclusion, the aim of this study was to fill knowledge gaps regarding the seroprevalence and risk factors of salmonellosis in intensive poultry farms. By doing so, interventions to reduce the prevalence of salmonellosis can developed and effective control measures can be implemented, to improve poultry health and the safety of poultry products and improving our understanding of *Salmonella* transmission dynamics.

INTRODUCTION

Chickens (*Gallus domesticus*) are widely kept and make up the largest share in terms of numbers compared to other farm animals' genetic resources all over the world. They share the largest portion of the global economy in terms of poverty reduction and job creation (Fikadu^[1]). Ethiopia is home to an estimated 57 million chickens, of which 78.85 percent, 12.02 percent 9.11 percent are claimed to be native, hybrid and foreign breeds, respectively (CSA, 2021). According to Tsegaye^[2], of Ethiopia's enormous chicken population, laying hens account for the majority (34.26%), followed by chicks (32.86%), pullets (11.35%), cocks (11.12%), cockerels (5.74%) non-laying hens (4.59%).

Chicken farming is an important and fundamental part of most households in Ethiopia, as it is in other developing countries, in rural, urban and peri-urban settings., providing a significant positive impact on the economy, society, nutrition and culture (Dagnew^[3], Tadesse^[4]). The sector enables farmers to obtain high-quality protein in the form of meat and eggs while merely scavenging feed resources (Ahmad^[5] Abayneh^[6]).

Chicken production is hindered by a variety of problems, including the high frequency of infectious diseases, the low genetic potential of the native breeds and traditional rearing methods. One of the major global poultry industry challenges is salmonellosis, which is brought on by several *Salmonella* species (Ahmed^[7]). Due to increased morbidity, mortality and decreased productivity, the disease is known to cause the chicken sector to suffer large financial losses (Rostango^[8]). *Salmonella* is a motile rod-shaped gram-negative, facultatively anaerobic, non-spore-forming bacterium that is a member of the Enterobacteriaceae family. The majority of the bacteria are catalase-positive, oxidase-negative, non-lactose fermenters that use citrate as their only carbon source to create gas and acid from glucose (Haile^[9]). Within the genus, there are two species: *Salmonella enterica* and *Salmonella bongori*. Although it can infect people, *Salmonella bongori* is primarily found in animals that have cold blood. On the other hand, there are about 2600 serovars of the common pathogen *Salmonella enterica*, which are divided into typhoidal and non-typhoidal groups (Talukder *et al.*, 2023). Various chronic and acute conditions in chicken are caused by infections with *Salmonella* bacteria. According to Saif^[10], these illnesses still result in major financial losses for many countries and require substantial resources for testing and control programs in various nations. Poultry usually acquire salmonella by the fecal-oral route, which is primarily caused by consuming contaminated food or water. A wide variety of animal species, including reptiles, pigeons chickens,

may serve as this bacteria's reservoirs Isayas and Tefari^[11]. However, infected poultry and eggs are the primary source of *Salmonellae* Antunes^[12]. The management of chicken salmonellosis is more difficult due to the disease's capacity for both vertical and horizontal transmission (Gantoise^[13]). *Salmonella*'s environmental persistence contributes significantly to the epidemiology of the disease in chicken by facilitating opportunities for infection to spread across both inside and between flocks (Sarba *et al.*, 2020).

One of the most prevalent bacterial infections affecting poultry is fowl typhoid, which is caused by *Salmonella gallinarum* and may exist as septicemic, acute, or chronic. Adult birds are more susceptible to the disease than younger ones. It significantly endangers the chicken business and prevents the profitable production of chickens (Shivaprasad^[15] Salihi *at al* 2014., Shakir *et al.*, 2021). The clinical manifestations of fowl typhoid include poor quality and increased mortality in chicks produced from infected eggs, which are typical of a septicemic disease in poultry. Anemia, depression, dyspnea diarrhea, which cause excrement to adhere to the vent, are symptoms that elderly birds possess, Although serological testing are sufficient to identify and quantify the organism, the organism's isolation is necessary for a confirmed diagnosis (OIE, 2022). In Ethiopia Salmonellosis is a significant poultry disease that has been attributed to both domestic and exotic chickens (Sarba *et al*, 2020). The prevalence of *Salmonella* serotypes that have an impact on the poultry industry varies (Abayneh *et al.*, 2023). Nevertheless, information on the incidence, serotype distribution and phenotypic and genotypic relatedness of *Salmonella* isolated from poultry and human beings is poorly recorded and little effort is made to monitor the disease in chicken farms (Egualé, 2018). To develop an effective control strategy, local knowledge regarding the incidence of *Salmonella*, the distribution of serotypes and related risk factors is crucial Tekalign^[17].

According to earlier research conducted in various parts of Ethiopia, the prevalence of *Salmonella gallinarum* infection in chicken flocks can vary widely, ranging from 0.8% to 44.8% (Solomon^[18]). The isolation, characterization and prevalence of non-thypoid *Salmonella* spp. From poultry carcasses, poultry meat and broiler flocks in Ethiopia have all been the focus of numerous studies (Abunna^[19]). However, the prevalence and flock-level risk factors of salmonellosis caused by *Salmonella gallinarum* in Ethiopian poultry farms as a whole have not been thoroughly examined, as well as has the seroprevalence of these diseases based on serological investigations have not been examined in the current study area. Therefore in order to provide a better

indication of the prevalence of salmonellosis in poultry caused by *Salmonella gallinarum* in the defined study area, this study attempts to close this gap by collecting and evaluating data from carefully chosen poultry farms. Furthermore, a further study gap needs to be filled that is the investigation of particular risk factors related to *Salmonella* propagation in intensive chicken farming systems. A more comprehensive awareness of these risk variables will make it easier to identify efficient management strategies meant to lower the spread of *Salmonella* and in turn, lower the incidence of Salmonellosis.

Therefore, the Objectives of the Study were:

- To determine the seroprevalence of salmonellosis, caused by *Salmonella* species,
- To investigate the risk factors associated with the prevalence of the disease in the selected intensive poultry farms in Wolaita Sodo town, Southern Ethiopia.

MATERIALS AND METHODS

Description of the Study Area: The study was conducted in Wolaita Sodo town, in the Wolaita zone of Southern Ethiopia, between October 2022 and April 2023. The distances of Sodo town from Addis Ababa and Hawassa are, respectively, 390 km south and 167 km southwest. The town's coordinates are 6049" N latitude and 37045' E longitude. The city occupies an area of roughly 3,200 hectares overall. The town is separated into 99 villages, 11 kebeles three sub-cities. The city is located at the base of Mount Damotit slopes southward in elevation from these mountains. At its peak and lowest points, the town is located between 2,222-1,600 meters above sea level. The town receives 1,200 millimeters of rainfall and 20 degrees Celsius on average year. There are approximately 128,919 cattle, 29,191 sheep, 4,606 goats, 4,124 horses 55,278 chickens in the area (Zewde *et al.*, 2019).

Study Animals: The study's subjects were Bovans brown breeds, which are primarily found in intensive farm systems., consisting of different age groups selected from randomly selected intensive farms of Sodo ATVET college farm, Exodus farm GOLA farm. During sampling, the age, species and flock size, feed source and origin of the chickens were recorded.

Study Design: A cross-sectional study was conducted from October 2022-May 2023 in selected intensive poultry farms of Wolaita Sodo town to estimate the seroprevalence of salmonella species and determine the factors associated with the prevalence of the disease.

Sampling Method and Sample Size Determination:

Simple random sampling was applied to select chickens and selected chickens were separated into another class to minimize bias during sample collection. In the data collecting sheet, information was entered about the age, breed type, feeding types, water supply, house type, clinical state, production goal and total flock population. Based on the availability of chickens and the potential for poultry production, farms were purposefully chosen. Along with the collection of samples, information regarding the contribution of several risk variables to the occurrence of salmonellosis was also evaluated. The sample size was determined using the following formula given by Thrusfield, (2005). There was no previous study on seroprevalence of salmonellosis in the study area. Thus, a 50% prevalence with 5% desired level of precision and 95% of the confidence interval was considered to calculate the sample size.

$$N = \frac{1.96^2 [\text{pexpe}(1-\text{pexpe})]}{d^2}$$

Where N= sample size

P= expected prevalence

D= desired absolute precision

Hence, 384 chickens were sampled using random sampling method from the study area.

Study Methodology

Serum Sample collection, Transportation and Laboratory Examination: To prepare serum for seroprevalence, a total of 384 blood samples were aseptically taken from the wing veins of chickens. Each bird's wing vein was used to draw out about 3 milliliters of blood, which was then placed in a slanting vacutainer tube at room temperature to allow the blood to coagulate in the syringe. The sera underwent a 12-hour clotting period before being centrifuged for 10 minutes at 1000 rpm. Lastly, until testing, the cleared sera were kept in a deep freeze at -20 OC.

Latex Agglutination Test: The test procedure outlined by OIE (2022) was followed for performing the serum slide agglutination test in the Wolaita Sodo Regional Veterinary Laboratory. Before being used, all harvested sera and the reagent had been brought up to room temperature, which is 22±5°C. About 30 µl of *Salmonella gallinarum* antigens stained with crystal violet and an equal volume of serum were added to the sterile slide. Subsequently, the two contents were mixed and rotated for duration of two to three minutes. Next, the level of agglutination was measured and any level of agglutination was considered positive.

Data Management and Analysis: The process of managing and analyzing the data for this study was carefully designed and carried out to ensure dependable and precise outcomes. After the required information was gathered, the data was entered into an MS Excel spreadsheet application to construct a database. Descriptive statistics and multivariable logistic regression analysis were used by SPSS version 20 software to evaluate the coded data. Risk variables associated with Salmonella infection were identified and the prevalence of the bacteria on the farms was computed. Ultimately, $P \leq 0.05$ was shown to be a statistically significant indicator of possible risk.

RESULTS AND DISCUSSIONS

Over all prevalence of Salmonella Gallinarum: The current study revealed that out of the 384 chicken included in the current study, 29.42% (113/384), were tested positive for salmonella infection, which indicates Salmonellosis was prevalent in chicken production system in the study area. The study has also shown variation in prevalence of disease between tested farms, so that the GOLA farm had the highest prevalence, with 39.28% of chicken samples positive. AT VAT farm had the lowest prevalence at 23.24%, while EXODUS farm had an intermediate prevalence of 38.28% while the AT VAT farm had the lowest and the EXODUS farm had an intermediate prevalence.

Association of Risk Factors and Seroprevalence of Salmonella Gallinarum: The study showed a statistically significant association ($P < 0.05$) between the prevalence of Salmonellosis and the age category of poultry. Table 1 shows that around 38.29% of chickens at one-month-old and 41.4% of chickens at three months old tested positive for Salmonella, respectively. At fourteen months old, the infection was detected in approximately 8.59% of chickens. This suggests that the chickens' age may have an effect on the likelihood they are to contract salmonellosis.

According to the study, there is not a statistically significant association ($P > 0.05$) between the housing system and the prevalence of salmonellosis in intensive poultry farms. The prevalence of the disease was found to be relatively comparable in both cage and litter systems (floor). Out of the 28 samples examined in the cage system, 11 (or 39.28%) tested positive for salmonellosis, whereas the remaining 17 (60.72%) tested negative. Out of 356 samples, 102 (28.65%) tested positive for salmonellosis in the litter system (floor), while 254 (71.35%) tested negative. When both housing methods were taken into account, 113 (or 29.42%) of the 384 observed hens tested positive for salmonellosis, while 271 (70.58%) tested negative.

The association between the feeding system and the prevalence of salmonella infection showed that, of the 156 chickens that were seen to be fed commercial feed, 60 (38.46%) tested positive for salmonellosis and 96 (61.54%) tested negative. 175 (76.76%) of the 228 chickens that were watched and given locally produced feed tested negative for salmonellosis, while 53 (23.24%) tested positive. The feed type given to the chickens had no significant correlation ($P > 0.05$) with the frequency of salmonellosis. According to the findings, hens fed commercial feed have a larger percentage of positive cases than chickens fed locally produced feed (Table 3).

The prevalence of salmonellosis varies across different farm sizes indicating an insignificant association ($P < 0.05$) between farm size and the occurrence of salmonellosis in poultry farms (Table 4).

The association of farm origin with prevalence of disease has indicated a non-significant difference ($P > 0.05$) between salmonella infection and farms. As indicated in (table 5), the highest prevalence of salmonellosis was found in the GOLA farm, with 39.28% of chicken samples positive. AT VAT farm had the lowest prevalence at 23.24%, while EXODUS farm had an intermediate prevalence of 38.28% (Table 5).

In this study, from a total of 384 Samples examined in selected intensive poultry farms in Wolaita Sodo Town, 113 were identified as positive for salmonellosis. Hence the overall prevalence was 29.42%. The prevalence of the present finding was in agreement with the finding at Gondar which was found to be 28.6% (Molla *et al.*, 2019). However the current prevalence may be regarded higher as compared with prevalence rates of previous studies such as., Dereje (2002) who reported the prevalence of 1.5% at East Showa, Ethiopia, Melese (1991) who reported 10.05% seroprevalence in layers and pullets in Debre Zeit poultry farms., Kumar^[20] who reported 19.71% in Chicken Population of parts of Tigray and Addis Ababa.,

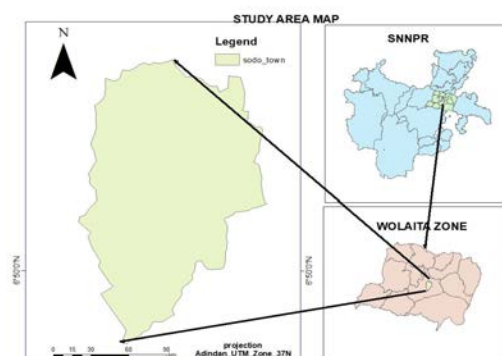


Fig. 1: Map of Wolaita Sodo town, SNNPR, Ethiopia. Source: Wolaita zone administrative and publication

Table 1: Prevalence of Salmonella gallinarum in the age category of poultry

Age in months	No. of Samples tested	No. of Chicken (+ve)	Percent (%)	X2	p-value
1	128	49	38.29	7.323	0.026
3	128	53	41.4		
14	128	11	8.59		
Total	384	113	29.42		

Table 2: Prevalence of Salmonella gallinarum in Poultry housing system

Housing system	No. of Samples tested	No. of Chickens (+ve)	Percent (%)	X2	p-value
Cage system	28	11	39.28	1.413	0.234
Litter (floor)	356	102	28.65		
Total	384	113	29.42		

Table 3: Prevalence of Salmonellosis in feed type and availability for Poultry

Feed type	No. of chickens observed	Positive		Negative		X2	p-value
		No. of Chickens (+ve)	Percent (%)	No. of Chickens(-ve)	Percent (%)		
Commercial	156	60	38.46	96	61.54	10.32	0.001
Locally formed	228	53	23.24	175	76.76		
Total	384	113	29.42	271	70.58		

Table 4: Prevalence of Salmonellosis in Poultry farm size

Farm size	No. of Samples tested	Positive		Negative		X2	p-value
		No. of Chickens (+ve)	Percent (%)	No. of Chickens (-ve)	Percent (%)		
Small (9<25)	28	11	39.28			10.337	0.006
Medium (250-2000)	228	53	23.42				
Large 9>2000)	128	49	38.28				
Total	384	113	29.42				

Table 5: prevalence of salmonellosis in three farm origins of Poultry

Origin	No. of chickens observed	Positive	
		No. of Chickens Samples (+ve)	Percent (%)
Exodus	128	49	38.28
Atvat	228	53	23.24
Gola	28	11	39.28
Total	384	113	29.42

whereas this prevalence was lower than studies from Edilu^[14] who reported 63.5% in selected districts of West Shoa, Ethiopia. The prevalence rates may differ depending on the area and methodology employed, which could be the cause of the variation in prevalence. These investigations were carried out in various parts of Ethiopia and with varying sampling techniques. The delayed emergence of agglutinating antibodies in the examined chickens, which occurs three to ten days or longer after infection, could also be the cause of the lack of antibody detection in the remaining samples (Salihu^[16]).

Concerning farms the present study revealed 39.28%, 38.28%) and 23.24% prevalence of, GOLA, EXODUS and ATVAT Farms respectively. However, the results of the current study revealed lower prevalence compared to research conducted by Bouzouba^[21] in Morocco, where they reported a 58% prevalence rate Adesiyun *et al.* (1984) in Nigeria, who reported a 43% prevalence rate among village chickens. This difference may result from variations in the contamination of the surrounding environment, the management practices employed, the lower sample size, the breed and parent stock differences of the chickens used currently the studies conducted by other investigators.

In this study, the presence of Salmonella antibodies in serum samples from different age category has indicated a significance association

$P < 0.05$ with the occurrence of salmonella infection among chickens. The current finding disagree with the finding of Genet *et al.* (2014) who reported non-significant association existed among age groups from apparently healthy chickens in eastern Ethiopia. The age ability of infection to survive increases with age and mortality is greatest in newly hatched chicks. Therefore, seroprevalence of *S. gallinarum* antibody found more in adult poultry (Teferi and Nejash^[22]).

In this study, the presence of Salmonella antibodies in serum samples from both commercial and locally formulated feed chickens indicated a significant association ($P < 0.05$) between feed chicken feed formulation and the occurrence of fowl typhoid in chicken farms. Studies have indicated that altering the components and nutrient makeup of feed can impact chickens' susceptibility to contracting Salmonella (Sarker^[23]).

In general, the study's findings indicate the sero-prevalence of fowl typhoid infection in chicken production systems, which suggest that fowl typhoid infection may pose a future threat to poultry production in the region. These findings are consistent with research conducted in various regions of the nation, where the prevalence of salmonellosis in poultry varies amongst studies due to factors like age, housing system, feed type farm size (Kassaye^[24] Endris^[25]). These findings emphasize the necessity of

efficient control strategies to lower the risk of salmonella transmission in systems of intensive chicken production. Enhancing biosecurity procedures, such as stringent hygienic protocols, routine observation appropriate feed management, can be extremely important in limiting the disease's spread. Furthermore, training chicken farmers about the risks of salmonellosis and how to avoid it is crucial for managing the disease.

CONCLUSION

Poultry farms are at risk of considerable threat from salmonellosis, which can result in economic losses from high rates of disease, mortality decreased productivity. In the current study, the seroprevalence of salmonellosis in the poultry investigated in Wolaita Sodo town, Southern Ethiopia, was 29.42%. According to this study, the chicken production system in the study area had the highest prevalence of salmonellosis, which is detrimental for the industry. Based on the findings, the following recommendations are suggested:

- Implementing thorough disease surveillance and monitoring program, encourage appropriate management practices and run awareness programs to reduce the risk of Salmonella infection and improve flock health.
- Invest resources on research and development to find out what triggers salmonellosis to be so common in chicken farms in the study area including studying the impact of feed types, farm sizes management systems on disease prevalence and identifying effective control measures.
- Further research is needed to investigate the prevalence of salmonellosis in other farming systems, such as backyard and free-range poultry in different districts of study site.

Data Availability: The data generated or analyzed during this investigation are all included in this paper; however, upon reasonable request, the authors may supply more material.

Author Contributions: Each author made a significant contribution to the work reported, whether it was through conception, study design, execution, data acquisition, analysis interpretation, or in all of these areas; they all agreed on the journal to which the article was submitted., they all agreed to take responsibility for the work in all its aspects and they all contributed to the article's draft, revision, or critical review.

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