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# Evaluating the relative resistance of different poultry breeds to *Salmonella* Typhimurium

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**Salmonella infections originating from poultry are one of the major causes of food-borne diseases. To control Salmonella in poultry multifactorial approach is likely to be effective, and genetic resistance of poultry breeds to Salmonella infections may have valuable contribution. Experimental research was conducted to evaluate relative resistance of poultry breeds: Local, Fayoumi and Koekoek to *Salmonella* Typhimurium from November 2012 to May 2013. Total of 48 chicks were taken and half from each was randomly allocated to treatment and control groups. Treatment groups were orally infected with $10^{7-9}$ *S.* Typhimurium at two week's age. Samples were taken from week one post infection and continued every week till week four. Liver, spleen and lung were considered to determine relative bacterial loads and feces were added to identify *S.* Typhimurium. Highest bacterial load was found in Koekoek (1.95 log CFU/ gram) breeds than Local (0.71 log CFU/ gram) and Fayoumi (1.00 log CFU/ gram) breeds (P<0.05). However, no statistically significant difference of bacterial load between Local and Fayoumi was found. There was significant bacterial load difference among liver (2.01 log CFU/ gram), lung (0.95 log CFU/ gram) and spleen (0.69 log CFU/ gram). According to bacterial growth on XLD, Koekoek was found to be 4.26 times more susceptible to Local breed. There was no statistically significant difference between Local and Fayoumi breeds. In conclusion, local breeds were relatively found to be the most resistant among the three poultry breeds to *S.* Typhimurium.**

**Key words:** Bacterial load, breed, Ethiopia, isolation, organs, Salmo*nella* Typhimurium.

## INTRODUCTION

The world poultry population has been estimated to be about 16.2 billion of which 71.6% are found in developing countries producing 67, 718,544 and 57,861,747 metric tons of chicken meat and eggs, respectively (Gueye, 2005). In Africa, village poultry contributes over 70% of poultry products and 20% of animal protein intake. In East Africa over 80% of human population live in rural areas and over 75% of these households keep indigenous chickens which Ethiopia could not be different to this situation (Kitalyi, 1998).

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Rural poultry production in Ethiopia represents a significant part of the national economy in general and the rural economy in particular. Currently, there is a rapid expansion in the number of private commercial poultry enterprises of various sizes and using exotic stocks. Before about twenty years, 99% of the poultry population consists of Local breed types under individual farm household management (Alamargot, 1987) contributing 98.5 and 99.2% of the national egg and poultry meat production, respectively, with an annual output of 72,300 metric tons of meat and 78,000 metric tons of eggs (AACMC, 1984).

On the other time, Tadelle et al. (2003) indicated that from the total population of chicken in Ethiopia, 99% are raised under the traditional back yard system of management, while 1% is under intensive management system. However, as the demand of the higher production potential increases, the proportion of Local poultry population has decreased through time. According to the CSA (2009), the country has 42 million chicken populations with an estimated value of around 40.6 million native chickens representing 96.6% Local, 0.55% hybrid and 2.84% exotic breeds mainly kept in urban and peri-urban areas.

Poultry production plays an important role especially to the disfavored sector of the society (women and children) as it requires little labor and initial investment costs as compared with other farm activities (Dessie and Ogle, 1996). A number of authors have outlined that rural poultry play a significant role through their contribution to the cultural and social life of rural people (Veluw, 1987; Sonaiya, 1990; Dessie and Ogle, 1996). Although there is a great demand for poultry and poultry products in the country, infectious diseases are imposing the poultry industry both from the production and public health points. These diseases, either they do not get treatment or they are not appropriately treated which leads to the development of drug resistance (Mola et al., 2003; Bekele and Ashenafi, 2010).

Salmonellosis is among the food-borne diseases most widespread global public health problems, and their implication for health and economy is increasingly recognized (Van der Venter, 1999). According to the World Health Organization reports of 1995, 88% of all food-borne diseases were caused by *Salmonella* (Gabert et al., 1999).

It is a common intestinal illness caused by numerous *Salmonella* serovars and manifested clinically in animals (Radostits et al., 2000) and humans as an acute enteritis and chronic enteritis, an acute septicemic disease or as subclinical infections (Acha and Szyfres, 2001). *Salmonella* contamination of poultry meat and eggs continues to be a global threat to public health (Barrow, 1997).

*Salmonella* has both host specific like the *S.* Pullorum and *S.* Gallinarum in chickens and non-host specific pathogens such as *S.* Typhimurium and *S.* Enteritidis. From the public health aspect, *S.* Typhimurium and *S.* Enteritidis have a significant role especially from the food products and by-products of chicken. According to Bouvet et al. (2002), *S.* Enteritidis alone, which infects the eggs of contaminated hens, is responsible for one third of the human food poisoning cases in France.

In Ethiopia, considerable researchers are able to find *Salmonella* both from chicken, egg and human beings which indicate the presence of higher risk of the disease. Molla et al. (1999) reported a prevalence of 28.6, 22.6, and 15.4% in chicken gizzard, liver, and heart, respectively. Similarly, Molla and Mesfin (2003) and Endrias (2004) have determined the prevalence of *Salmonella* in chicken as 21.1 and 13.9%, respectively. Assefa et al. (2011) have found a *Salmonella* prevalence of 11.5% from eggs. There are also some reports of *Salmonella* infection in human beings. Enderia (2004) claimed a prevalence of 7.4% from supermarket workers. Likewise, Nyeleti et al. (2000) indicated a prevalence of 6% in Addis Ababa abattoir workers.

Some poultry diseases which have public health significance are symptomatic at poultry farm level so that it is easier for control and prevention as food-borne zoonoses. However others are asymptomatic which make the control and prevention at poultry farm level difficult. *S.* Typhimurium is one of the asymptomatic poultry diseases which have great impact on both the production and public health aspects (Velge et al., 2005).

On the other hand chronic subtherapeutic use of antimicrobial agents in domestic livestock may control bacterial diseases as well as promote growth. Nevertheless, this approach may be a factor in transmitting antibiotic-resistant *Salmonella* to humans through the food chain (White et al., 2001). If the frequency of drug resistance increases, the choice of antimicrobials for treatment of systemic salmonellosis in humans becomes more limited. Globally, the three main causes of antimicrobial resistance have been identified as use of antimicrobial agents in agriculture, over-prescribing by physicians, and misuse by patients (IFT, 2003). *S.* Typhimurium DT 104 has a broad host reservoir and is usually resistant to five antibiotics (ampicillin, chloramphinicol, streptomycin, sulphonamides, and tetracycline) and can be resistant to others (e.g., fluoroquinolones) (IFT, 2003). So the increasing expansion of exotic chicken breeds, the public health importance, asymptomatic property and creating the drug resistance make *S.* Typhimurium more difficult to control both in chicken and human beings. To solve this problem there should be an option that is developing a poultry breed which has relatively better resistance to the disease.

Previous studies of genetic resistance in domestic animals have focused on two broad aims. The first is to reduce the levels of disease and economic losses through high mortality rates of systemic Salmonellosis caused by *S. serovars* such as *S.* Choleraesuis in pigs

(Van Diemen et al., 2002), or *S.* Gallinarum in poultry (Wigley et al., 2002). The second is to control colonization in domestic animals and subsequent transmission of food-poisoning serovars such as *S.* Typhimurium or *Salmonella* Enteritidis through consumption of meat or eggs (Lamont et al., 2002).

Initial studies on genetic resistance centered largely on resistance of various chicken breeds to fowl typhoid (*S.* Gallinarum) and Pullorum (S. Pullorum) disease (Hutt and Crawford, 1960). Another studies in inbred White Leghorn chickens revealed massive differences in the $LD_{50}$, pathogenesis and numbers of *Salmonella* in organs following oral or intravenous infection with *S.* Gallinarum or *S.* Typhimurium (Bumstead and Barrow, 1993).

In Ethiopia, using the breeding strategy to control specific livestock diseases is not common so far. But, the International Livestock Research Institute (ILRI) has conducted a research for several years to develop some trypanotolerant cattle breeds in Ethiopia and it has got a promising result (Lemecha et al., 2006) indicating that the breeding aspect can contribute great importance especially for diseases which are creating a drug resistance problem. Therefore the objectives of this paper were summarized as follows:

(i) To evaluate the relative resistance of three poultry breeds to *S.* Typhimurium.
(ii) To determine the bacterial load (*S. typhimurium*) in the poultry breeds
(iii) To evaluate the relative carriage of *S. typhimurium* in the organs (offals) of the poultry breeds

## MATERIALS AND METHODS

### Study area

The research was conducted in Debre-Zeit Agricultural Research Center and Sebeta National Animal Health Diagnostic and Surveillance Center, Ethiopia. Debre Zeit is located about 45 km South-east of Addis Ababa, just on the escarpment of the Great Rift Valley and the geography of the area is marked by creator lakes. It is found at 9°N latitude and 40°E longitude and at an altitude of 1850 m above sea level in the central high lands of Ethiopia. It has a human population of about 95,000.

It experiences a bimodal pattern of rainfall with the main rainy season extending from June to September (of which 84% of rain is expected) and a short rainy season from March to May with an average annual rainfall of 800 mm. The mean annual minimum and maximum temperatures are 12.3 and 27.7°C, respectively, with an overall average of 18.7°C. The mean relative humidity is 61.3% (CSA, 2006).

Sebeta National Animal Health Diagnostic and Surveillance Center is found in Sebeta town which is located 25 Km west of Addis Ababa. It is a town and separate woreda in central Ethiopia located in the Oromia Special Zone Surrounding Finfinne of the Oromia Region. This town has a latitude and longitude of 8°55′N 38°37′E and an elevation of 2,356 m above sea level. The 2007 national census reported a total population for Sebeta of 49,331, of whom 24,356 were men and 24,975 were women (CSA, 2007).

### Study population

The experimental animals were Local, Fayoumi and Koekoek breeds of chickens. Because of low performance of indigenous chicken breeds of Ethiopia, different exotic chicken breeds (White Leghorn, Brown Leghorn, New Hampshire, Rhode Island Red, Fayoumi and Koekoek, etc) have been introduced to Ethiopia (Alemu and Tadelle, 1998; Tadelle et al., 2003; Demeke, 2004; Wilson, 2010).

Fayoumi chicken breed has been imported with the expectation of better productivity, adaptation and disease resistance. They are originated in Egypt, reported to be a hardy breed and particularly well suited to hot climates (Heinrichs, 2007). They are also very good foragers, and if left to their own devices on a free range basis they can fend for themselves in a nearly feral manner. Fayoumi hens are good layers of small white eggs. They are not given to broodiness as pullets, but can be when they reach two or three years of age. The breed is fast to mature, with hens that lay by four and half months (Ekarius, 2007).

Similarly, Koekoek breed is originated from South Africa and introduced to Ethiopia in the intention of improving the production and productivity of the Local chickens. According to Fourie and Grobbelaar (2003), Koekoek was bred at the Potchefstroom Agricultural College during the 1950s by a researcher named Marais. Although it is developed in South Africa, it is found from the cross breed of Black Australorp and the White Leghorn of the Australia and Italy, respectively. This breed is very popular among rural farmers in South Africa and neighboring countries for egg and meat production as well as their ability to hatch their own offspring (Grobbelaar, 2008).

For the Local chickens, fertile Local eggs were brought from Chefe Donsa wereda which is found in the East Shewa and taken to the Debre-Zeit Agricultural Research Center for incubation. According to Tadelle et al. (2003), this was one of the places selected in the research of Village chicken production systems in Ethiopia, flock characteristics and performance, intending that no prior distribution of exotic breeds were undertaken. Eggs of the Fayoumi and Koekoek were found from the Debre-Zeit Agricultural Research Center. After egg and fecal swabs were taken and prescreened for *Salmonella*, eggs of the three breeds were incubated at the same time in the research center.

### Study type

A single factorial experimental design that is randomized multi-group with a post-test design was used to determine the relative resistance of the breeds to *S.* Typhimurium. Three groups of chickens, the Local, the Fayoumi and the Koekoek, were formed. Within each group, two sub groups, the treatment and the control groups, were also formed. From each breed, 16 chickens were selected and half of them were randomly allocated to the treatment and the rest to the control group using a lottery method. Control groups were used to observe whether there was contamination before and during the experiment time other than the experimentally given *S.* Typhimurium.

### Sample size

To determine the sample size for the experimental design type, as it is a single group experiment, the following formula is used (Dell et al., 2000).

$$n = \log ß / \log p$$

Where 1- ß is the chosen power and *p* represents the proportion of

the animals in the colony that are not infected. The power of an experiment is the probability that the effect will be detected. It is usually and arbitrarily set to 0.8 or 0.9 (that is, the investigator seeks an 80 or 90% chance of finding statistical significance if the specified effect exists). Note that 1-power, symbolized as ß, is the chance of obtaining a false-negative result (that is, the experiment failed to reject an untrue null hypothesis or to detect the specified treatment effect). The proportion not infected is used in the formula. Accordingly, 50% was taken as the probability of infection and a 95% chance of detecting that infection, and then the number of animals that need to be sampled (N) is

$N = \log ß / \log p$
$N = \log 0.05 / \log 0.5$
$N = 4.32$

So with an approximate number, 5 chickens were to be taken from each group. However, to increase the accuracy of the experiment, 8 chickens were taken from each breed for the treatment and 8 chickens for the control with a total of 48 individuals (24 for treatment and 24 for control).

## Study methodology

### *Chicken house preparation and challenging*

First two houses (one for the experimental and the other for control) 4x3 m with 3 m high were prepared in Sebeta National Animal health diagnostic and investigation Center. It was cleaned and fumigated using potassium permanganate (20 g) and formalin (30 ml) for one cubic meter and was closed for three days. The houses were designed for poultry research purpose. They were naturally ventilated with meshes at the top of their walls. All the materials which were intended to be used were fumigated together. Day old chickens of three breeds (Local, Fayoumi and Koekoek) were taken from Debre-Zeeit Agricultural Research Center (DzARC) to Sebeta National Animal health diagnostic and investigation Center. All chickens were tagged on their two wings and the number in the tag was registered. They were fed with chicken starters feed formulated in DzARC. Both water and feed were given as an ad libitum. After two weeks of age, half of all the three breeds (8-Local, 8-Fayoumi and 8-Koekoek) were randomly selected using a lottery method. One group of the chickens (the treatment group) was allocated at one house and the other group (the control group) at another house.

After chickens were separated as treatment and control groups, all the treatment groups were challenged with $10^7$-$10^9$ *S.* Typhimurium bacteria orally. The *S.* Typhimurium was Microtrol discs obtained from Becton Dickinson, France in which one disc has $10^7$-$10^9$ *S.* Typhimurium. The discs were inserted in to the already prepared 1 ml nutrient broth and incubated in 37°C for about 30 min to initiate the growth of the bacteria. To observe its viability, turbidity of the bacteria was checked in all tubes. Using the appropriate biosafety way, chickens of the three breeds were given the *S.* Typhimurium orally with syringe. From the time of bacterial challenge, all materials intended to be used including water, feeds were used independently. No any material was taken in either direction and always first, the poultry attendant should serve the control groups and then the treatment ones to prevent from cross contamination.

### *Sample collection*

A week after challenging, two chickens from each breed as well as control and treatment groups were taken randomly to be slaughtered every one week until all the chickens were finished. Sterilized slaughtering materials were used for every individual chicken and its organ to prevent cross contamination. From every chicken, liver, lung, spleen organs and feces were taken aseptically. Samples taken from organs were used for both bacterial count and (culture method) whereas samples taken from feces were used for only bacterial growth.

### *Bacteriological examination*

The technique recommended by the International Organization for Standardization ISO 6579 (1998) was employed to isolate and identify *Salmonella* organisms. The bacteriological media used for the study was prepared following the instructions of the manufacturers. For chicken organ samples from 3 different parts (liver, spleen, and lung) were cut, mixed thoroughly and the appropriate analytical units (2 g) were withdrawn. These were aseptically cut into smaller fine pieces with sterile scalpel blades. 1 g from each sample was taken to sterile capped tubes and 9 ml physiological saline water (0.85% NaCl) was added and agitated using vortex for 2 min to determine the bacterial load. The remaining 1 g from the organs and fecal swabs were processed similarly to determine the bacterial isolates.

**Bacterial load determination (direct count):** A 9 ml of physiological saline water (0.85% NaCl) was added to one gram of the thoroughly grinded samples from the organs and was mixed by vortex. Then three sterilized test tubes and Petri dishes were prepared for each sample labeled from 1 to 3. The test tubes were filled with 9 ml physiological saline water (0.85% NaCl) solution. On the other hand plate count agar was prepared according to the instruction of the manufacturer (Mumbai, India). From the initial solution, 1 ml was transferred to test tube labeled number 1. After mixed thoroughly, 1 ml of the solution was transferred to the second test tube and similarly from the second test tube to the third one. At last, 1 ml solution was discarded from the last test tube. From all the three solutions, 0.1 ml was taken and poured to their respective Petri dishes. As soon as the temperature of the plate count agar reached 50°C from an autoclave, 20 ml of it was poured to each petridishes and was shacked gently to be mixed. Finally, the Petri dishes were incubated at 37°C for 24 h (Figure 1).

**Bacterial count was calculated as follows:** At the initial sample 1 g was diluted to 9 ml physiological saline water to give 10 ml solution and number of bacteria in one ml became 1/10 of the solution. When the 1 ml transfers to the 9 ml test tube 1, it also gave 1/10 of the solution and continued to test tube 3. When 0.1 ml solution from test tubes transfer to respected Petri dishes, it gave a dilution factor of 100 (1/100). So Petri dishes 1, 2 and 3 had a total dilution factors of 10, 000 (1/10 * 1/10 * 1/100), 100,000 (1/10*1/10*1/10*1/100) and 1,000,000 (1/10*1/10*1/10*1/10*1/100), respectively. As a result, a Petri dish with number of bacterial colony between 30-300 was considered and was multiplied with its respective dilution factor and reported as colony forming unites (CFU) per 1 g (FSSAI, 2012).

**Bacterial growth (culture method):** A one gram sample from liver, lung and spleen, and fecal swab was taken and pre-enriched with buffered peptone water (BPW). It was incubated for 16 to 20 h at 37°C. Following this, 0.1 ml aliquot of the pre-enriched broth was transferred aseptically into 10 ml of Rappaport-Vassilliadis (RV) broth, mixed and then incubated for 18 to 24 h at 42°C. Then a loop full of each culture was streaked onto plate of Xylose Lysine Desoxycholate (XLD, Bhiwadi, Rajasthan, India) medium and incubated at 37°C for 24 to 48 h. The plate (XLD) was examined for the presence of *Salmonella* colonies. On XLD medium the majorities of *Salmonella* serotypes produces hydrogen sulphide

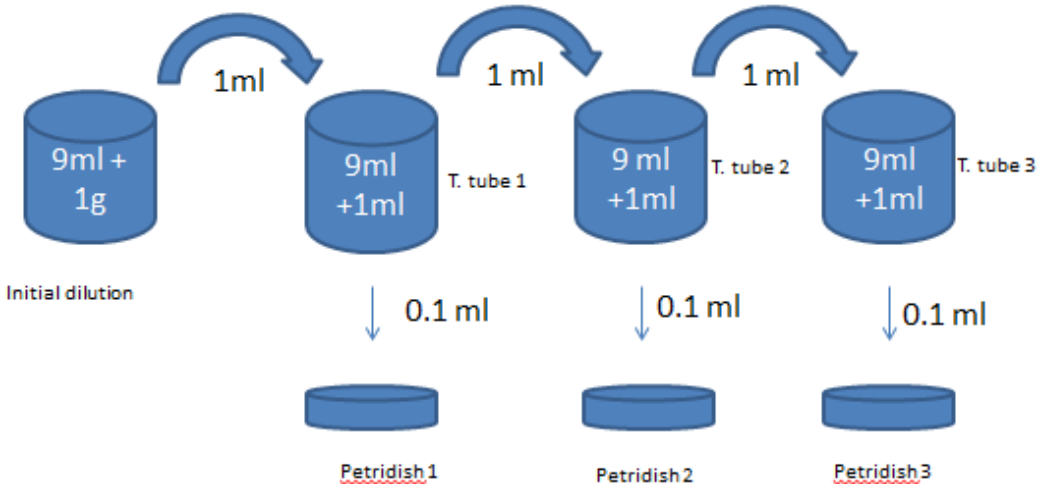


**Figure 1.** The dilution process from the initial sample to test tubes and subsequent petridishes.

($H_2S$) and have red colonies with a black center (Quinn et al., 1994). Some of the positive colonies showing red color with a black center on XLD were further examined using molecular technique (PCR) for confirmation.

**Polymerase chain reaction (PCR):** DNA extraction was conducted according to QIAamp DNA Mini and Blood Mini Handbook. Bacterial isolates were grown in nutrient broth at 37°C overnight (QIAGEN, 2010b). Exactly 1 ml of the culture was centrifuged for 5 min at 300 x g in a 1.5 ml microcentrifuge tube and the supernatant was discarded. Then the pellet was resuspended in Phosphate-buffered saline (PBS) to a final volume of 200 and 20 µl QIAGEN Protease (or proteinase K) was added. A 200 µl AL Buffer was added to the sample and mixed by pulse-vortexing for 15 s. After incubating at 56°C for 10 min, it was centrifuged to remove drops from the inside of the lid then 200 µl 100% ethanol was added and mixed by pulse-vortexing for 15 s. After mixing, it was centrifuged to remove drops from the inside of the lid.

The mixture was added to the QIAamp Mini spin column. After centrifuging at 6000 x g for 1 min, it was placed in a clean 2 ml collection tube. The QIAamp Mini spin column was opened and 500 µl AW1 Buffer was added. It was centrifuged at 6000 x g for 1 min and placed in a clean 2 ml collection tube. The QIAamp Mini spin column was opened and added 500 µl AW2 Buffer and was centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min. The QIAamp Mini spin column was placed in a new 2 ml collection tube and centrifuged at full speed for 1 min. The QIAamp Mini spin column was placed in a clean 1.5 ml microcentrifuge tube. The QIAamp Mini spin column opened and 100 µl AE Buffer was added (QIAGEN, 2010a). Finally, it was incubated at room temperature (15 to 25°C) for 1 min and was centrifuged at 6000 x g for 1 min. Nuclease free water was used in PCR tests as negative controls.

The extracted DNA was amplified using the oligonucleotide primer set specific for Fli15 gene of *S.* Typhimurium. The sequences of the two primers were S. Typhimurium Fli15 forward: 5´-CGGGTGTGCCCAGGTTGGTAAT-3´ and *S.* Typhimurium Tym reverse: 5´ ACTCTTGCTGGCGGTGCGACTT-3´ (Soumet et al., 1999). The negative control was a PCR mixture without DNA template. Thermal cycling was done in a thermo cycler (Applied Biosystems 2720, USA) using 4 ul of DNA template, 15 µl of PCR IQ super mix (BIO-RAD, California, USA) and 0.5 µl of each primer (0.1 mM) in a volume of 20 µl of reaction mix (Sánchez-Jiménez and Cardona-Castro, 2004). The initial denaturation step was at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min followed by a final extension cycle at 72°C for 10 min. The PCR products were electrophoresed in 1% agarose gel, stained with ethidium bromide and DNA fragments were visualized under UV light. Samples were considered positive when 559-bp band was detected.

***Data management and analysis***

Microsoft Excel was employed for data entry which was found from the culture. Bacterial load was considered to be counted when the range of the colony was between 30-300 colony forming units (CFU). For the purpose of bacterial load determination, the bacterial count was converted to log colony forming units for statistical analysis using the Microsoft Excel ("=log10(X)" where X is the bacterial count before analysis. Bacterial growth was considered when at least one CFU was grown at XLD. Descriptive statistics such as percentage, proportion and mean were applied to compute some of the data. Differences in bacterial count attributable to breeds and organs/feces were tested using one way ANOVA with GLM models (SPSS 15).

Logistic regression using STATA 11 (2009) was used to compare the effect of breed on the susceptibility to *S.* Typhimurium. It was also used to compare the susceptibility of organs (liver, lung and spleen) towards the disease. The Pearson's chi-square test was used to determine the significance of difference or variation. A difference was statistically significant if the P-value was less than 0.05.

## RESULTS

### Bacterial load comparison

### *Bacterial load comparison among the breeds*

Following the oral inoculation of the chickens with the *S.* Typhimurium, bacteria were taken differently by the organs of the different chicken breeds. Koekoek breed was found to be the most susceptible to *S.* Typhimurium than both the Local and Fayoumi breeds (Table 1). On average Local chickens had bacterial count of 0.71 log

**Table 1.** Breed susceptibility to *S.* Typhimurium (mean log cfu).

| Breed | Total organs examined | Bacterial load Mean ± SE | 95% confidence interval for mean | | F | P-value |
|---|---|---|---|---|---|---|
| Local | 24 | 0.71± 0.30 [a] | 0.10 | 1.33 | 4.03 | 0.022 |
| Fayoumi | 24 | 1.00±0.32 [a] | 0.31 | 1.66 | | |
| Koekoek | 24 | 1.95± 0.35[b] | 1.23 | 2.68 | | |
| Total | 72 | 1.22 ± 0.20 | 0.83 | 1.61 | | |

* [a b]Bacterial load values (means) with the same letters are not significantly different each other at 0.05 level.

CFU per gram of *S.* Typhimurium whereas Fayoumi and Koekoek had caught about 1.00 log CFU and 1.95 log CFU per gram, respectively. As indicated in Table 1 there was no statistically significant difference bacterial load between Local and Fayoumi breeds. However, Koekoek breeds had statistically significant difference (with p-value less than 0.05) bacterial loads among the others.

### *Bacterial load comparison among the chicken organs*

According the study, all organs of interest (liver, spleen and lung) had harbored *S.* Typhimurium. However, they did have different susceptibility to the causative agent. On average, liver had 2.01 log CFU per gram which was the highest of spleen (0.69 log CFU per gram) and lung (0.95 log CFU per gram). The statistical analysis is indicated in Table 2.

## Bacterial growth comparison

### *Bacterial growth comparison among breeds*

To compare the bacterial growth among the breeds, samples were taken from liver, lung spleen and feces from each breed. According to the bacterial growth at XLD, Koekoek breed was the most susceptible to *S.* Typhimurium than the rest with statistically significant difference with both breeds. It was found to be 4.26 (CI, 1.49-12.19) times more susceptible to the Local breed. However, there was no statistically significant difference between the Local and Fayoumi breeds (Table 3).

### *Bacterial growth comparison among organs and feces*

The bacterial growth among organs (lung, spleen and liver) and feces on XLD was also evaluated to compare their relative susceptibility to *S.* Typhimurium. In greater than half of (15/24 (62.5%) and 13/24 (54.17%) to feces and liver, respectively) the breeds' feces and liver were found to be positive for the causative agent. Lung was the least to be affected (5/24 (20.83)) followed by spleen (8/24 (33.33)). *S.* Typhimurium was found more likely in feces by 6.33 (CI, 1.75-22.91) times that was found in lung. Comparing to organs (offals), liver was highly affected more than lung which exceeded about 4.49 (with CI, 1.26-16.00). However, there was no statistically significant difference comparing spleen to lung, liver and feces (Table 4). To see the comparison of the organs and feces, it is illustrated in Figure 2.

### *Bacterial growth comparison among individual organs of breeds*

*S.* Typhimurium was grown in all organs of the three breeds except in lung of the Local chickens. Organs and feces of Koekoek had statistically higher bacterial growth than both Local and Fayoumi chicken breeds (liver, lung, spleen and feces with $X^2$=6.0, P=0.014; $X^2$=5.81, P=0.016; $X^2$=4.31, P=0.038; $X^2$=4.09, P=0.043, respectively which had different letters in their proportion) (Table 5). However, bacterial growth of the organs and feces of Local and Fayoumi did not have statistically significant difference. Bacterial growth in Koekoek's liver was more than 20 (OR=21) times higher than the growth in Local breed's liver. Similarly, bacterial growth in spleen and feces of Koekoek breed was greater than 11 times that of the Local spleen and feces (OR= 11.67) (Table 5). Following the oral inoculation, the highest and lowest bacterial growth was registered at the second and fourth weeks, respectively. At the first week of examination, 44.4% of the chickens were positive for the causative agent which exceeded by 13.6% of the chickens examined at fourth week. Generally bacterial growth had statistically significant difference among the four experimental time (P=0.013). However bacterial growth at weeks one, two and three did not have statistically significant difference (Table 5).

Following the bacterial oral inoculation, there was a slight increment from week one to week two. But it started to decrease from week two to the third and fourth weeks. Although there was a difference in the percent of positives in weeks 1, 2 and 3, their difference was not statistically different however, there was statistically significant variation among week 4 and others (Table 6). The graph in Figure 3 indicates how the bacterial growth

**Table 2.** Bacterial load on organs of the chickens (mean log cfu).

| Organs | Total organs examined | Bacterial load Mean ± SE | 95% Confidence Interval for Mean | | F | P-value |
|---|---|---|---|---|---|---|
| | | | Lower bound | upper bound | | |
| Liver | 24 | 2.01± 0.36 [a] | 1.26 | 2.76 | | |
| Spleen | 24 | 0.69±0.29 [b] | 0.10 | 1.29 | 4.70 | 0.012 |
| Lung | 24 | 0.95± 0.31 [b] | 0.30 | 1.59 | | |
| Total | **72** | **1.22± .20** | **0.83** | **1.61** | | |

* [a b] Bacterial load values with the same letters are not significantly different each other at 0.05 level.

**Table 3.** Bacterial growth on XLD of the three breeds.

| Breed | Organs/feces number examined | Total positives from organs/feces | Positive proportion (%) | OR | CI of OR | $X^2$ | P-value |
|---|---|---|---|---|---|---|---|
| Local (N=8) | 32 | 9 | 28.13 [a] | 1 | | | |
| Fayoumi (N=8) | 32 | 12 | 37.50 [a] | 1.53 | 0.54-4.39 | 8.33 | 0.016 |
| Koekoek(N=8) | 32 | 20 | 62.50 [b] | 4.26 | 1.49-12.19 | | |
| Total (N=24) | **96** | **41** | **42.71** | | | | |

*[a b] Proportions with similar letters are not statistically significant (with p-value = 0.05). N= number, OR= odds ratio, CI= confidence interval, $X^2$= Chi square.

**Table 4.** Bacterial growth on XLD in organs and feces of the three breeds.

| Organs/ feces examined | Total positives | Positive proportion | OR | CI of OR | $X^2$ | P-value |
|---|---|---|---|---|---|---|
| Lung (N=24) | 5 | 20.83 [a] | 1 | | | |
| Spleen (N=24) | 8 | 33.33 [abc] | 1.90 | 0.52-6.97 | 10.69 | **0.014** |
| Liver (N=24) | 13 | 54.17 [bc] | 4.49 | 1.26-16.00 | | |
| Feces (N=24) | 15 | 62.50 [c] | 6.33 | 1.75-22.91 | | |
| Total (N=96) | **41** | **42.71** | | | | |

*Proportions with similar letters are not statistically significant (with p-value = 0.05). N= number, OR= odds ratio, CI= confidence interval, $X^2$= Chi square.

is distributed through the experimental weeks.

### *Polymerase chain reaction (PCR)*

From the 41 culture positive samples, a total of 15 samples (5 from each breed) were randomly taken to the molecular technique (PCR) for confirmation. From the 15 positive samples exactly 14 samples were found to be *S.* Typhimurium. Only 1 from the culture positive samples was negative for the PCR technique. The PCR result is indicated in Figure 4. The 559 bp amplified product from fliC gene specific for *S*. Typhimurium. Lane (L): 100 bp molecular weight marker, Lane (N) negative control, Lane (1 to15 except 9) positive samples for *S*. Typhimurium.

## DISCUSSION

Disease problems are controlled by a variety of means, including the barriers of bio-security, biological methods such as vaccination and competitive exclusion, the use of antibiotics and genetic selection. In reality the combination of these is used, with the combination varying with the prevalent pathogens. The application of prophylactic control is increasingly constrained by the introduction of greater restrictions on the use of medications, in particular antibiotics especially for causative agents like *S.* Typhimurium which are creating a great problem in drug resistance (Doublet et al., 2005). Therefore, the breeding of resistant animals, together with good hygienic practices offers an easy, relatively low risk strategy to control both disease and colonization by *Salmonella*.

Large genetic variation for resistance to *S.* Typhimurium has been confirmed here by comparing the three breeds. This is inconsistence with findings many researchers whose results suggested that susceptibility to diseases of chicken may be genetically related

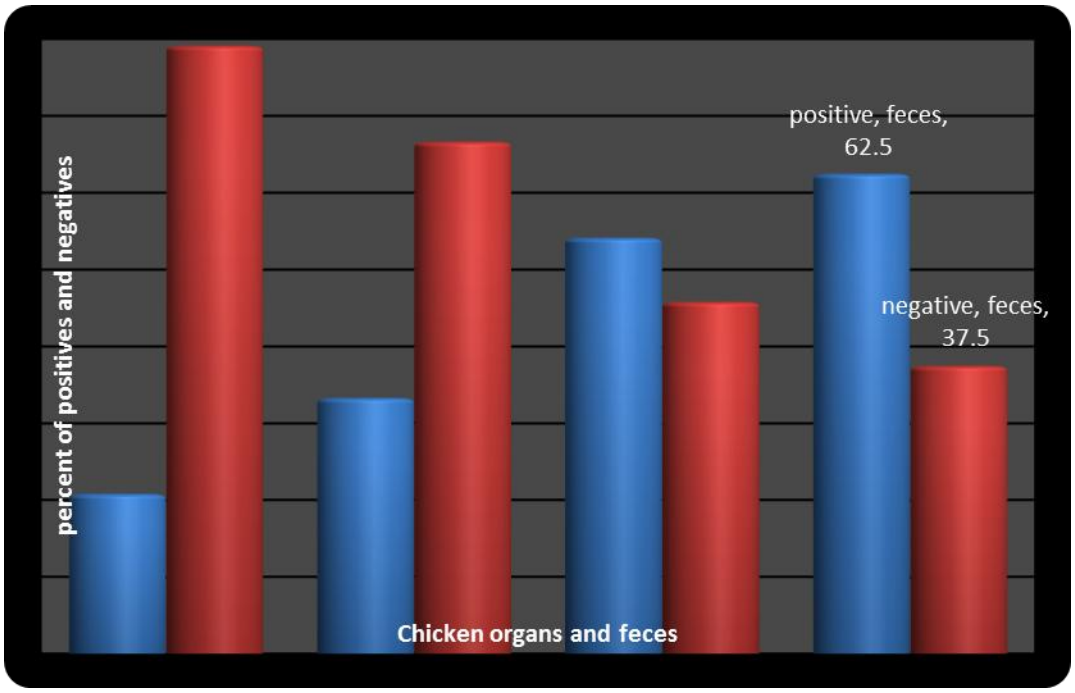


**Figure 2.** Percent of bacterial growth on internal organs and feces of the Local, Fayoumi and Koekoek breeds throughout the four weeks.

**Table 5.** Comparison of bacterial growth among internal organs and feces of the three chicken breeds (independent organ to organ comparison of the breeds).

| Variable | Breeds | Chicken examined | Percent of positives | Odds RATIO | $X^2$ | P-value |
|---|---|---|---|---|---|---|
| | Local | 8 | 25.0 [a] | 1 | | |
| Liver | Fayoumi | 8 | 50.0 [a] | 3 | 6.0 | 0.014 |
| | Koekoek | 8 | 87.5 [b] | 21 | | |
| | Local | 8 | 0.0 [c] | - | | |
| Lung | Fayoumi | 8 | 12.5 [c] | - | 5.81 | 0.016 |
| | Koekoek | 8 | 50.0 [d] | - | | |
| | Local | 8 | 12.5 [e] | 1 | | |
| Spleen | Fayoumi | 8 | 25.0 [e] | 2.33 | 4.31 | 0.038 |
| | Koekoek | 8 | 62.5 [f] | 11.67 | | |
| | Local | 8 | 37.5 [g] | 1 | | |
| Feces | Fayoumi | 8 | 62.5 [g] | 2.78 | 4.09 | 0.043 |
| | Koekoek | 8 | 87.5 [h] | 11.67 | | |

*[a b c d e f g h] Proportions with similar letters are not statistically significant (with p-value = 0.05).

(Bumstead and Barrows, 1993; Wigley et al., 2002). Bacterial load difference of the poultry breeds disclosed susceptibility difference to *S.* Typhimurium infection.

The present study result revealed that differences among the three breeds of chickens were significant to bacterial load on their internal organs and feces. They similarly, had a significant difference on the bacterial culture which was finally confirmed by PCR. Based on the bacterial load determination and culture, Koekoek breeds were found the most susceptible ($P<0.05$).

**Table 6.** Bacterial growth distribution through the four weeks.

| Weeks | Chicken examined | Percent of positives | OR | $X^2$ | P-value |
|---|---|---|---|---|---|
| 1 | 6 | 44.4 [a] | 13.60 | | |
| 2 | 6 | 55.6 [a] | 21.25 | 10.84 | **0.013** |
| 3 | 6 | 38.9 [a] | 10.82 | | |
| 4 | 6 | 5.6 [b] | 1 | | |
| Total | **24** | **36.1** | | | |

* [a b] Proportions with similar letters are not statistically significant (with p-value = 0.05).

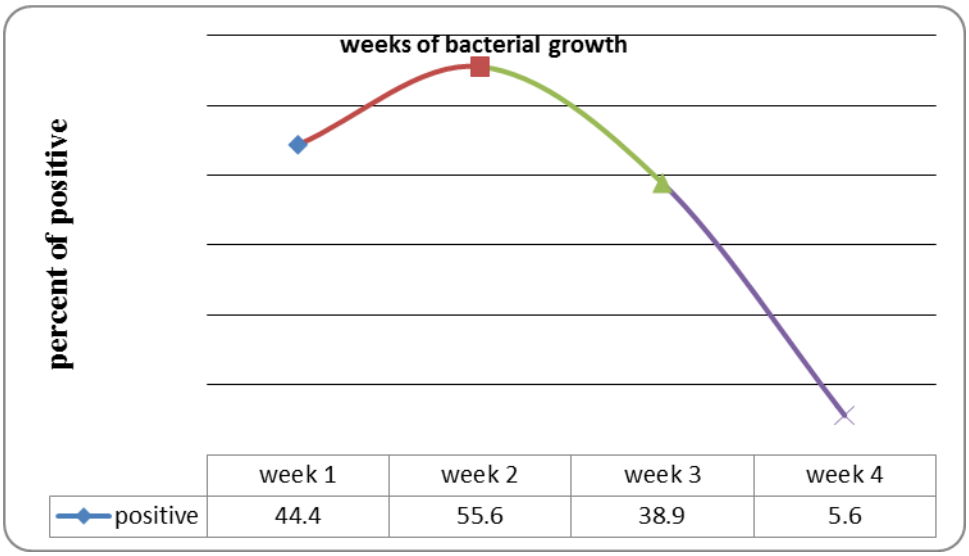


**Figure 3.** Bacterial growth distribution on chicken organs through the four weeks.

However, there was no statistically significant difference between Local and Fayoumi breeds. Considerable researchers explained that the indigenous poultry breeds are better in disease resistance than the exotic breeds (Oluyemi et al*.,* 1979; Okoye and Aba-Adulugba, 1998; Okoye et al*.,* 1999). Although not significant, there was still a difference in the susceptibility to the infection between Local and Fayoumi. This agreed with the result found by Mdegela et al. (1998) demonstrated that of the five ecotypes tested, chickens belonging to the *Kuchi* ecotype were shown to be resistant to fowl typhoid following experimental infection with a virulent *S.* Gallinarum strain.

This study also tried to identify which chicken organ is more infected by *S.* Typhimurium than the others from the lung, spleen and liver. Accordingly, liver was found highly infected followed by spleen which had statistically significant difference (P<0.05). This coincided with result found by Wigley et al. (2002) who conduct their research on in vivo and in vitro studies of genetic resistance to systemic salmonellosis in the chickens in UK. This could be of the fact that *Salmonella* that survive the battle in the intestine are taken to the regional lymph nodes where they are taken up by macrophages, whose actions frequently limit the infection to the intestine. Failure to contain the infection at these sites results in passage to the thoracic duct into the circulation, from which the bacteria are removed by cells of the reticuloendothelial system, notably in the liver and spleen (Libby et al., 2004).

Concerning time, the highest bacterial growth was registered at two weeks of the post infection. About seventy percent (69.2%) of the positives were found within the two weeks of the post infection. This early bacterial infection in the chickens could be due to the invasive property of the causative agent. Kaiser et al. (2000) have reported that *S.* Typhimurium was found more invasive than the common chicken infecting Salmonellae (*S*. Gallinarum and *S*. Enteritidis) which make it early colonies the intestinal mucosa and pass to the systemic infection. During this early period of life, protection against *Salmonella* infection involves activated heterophils, the avian equivalents to mammalian neutrophils, which migrate rapidly to the inflammatory site where they phagocyte and destroy opsonized bacteria through an oxygen-independent mechanism (Kogut et al.,

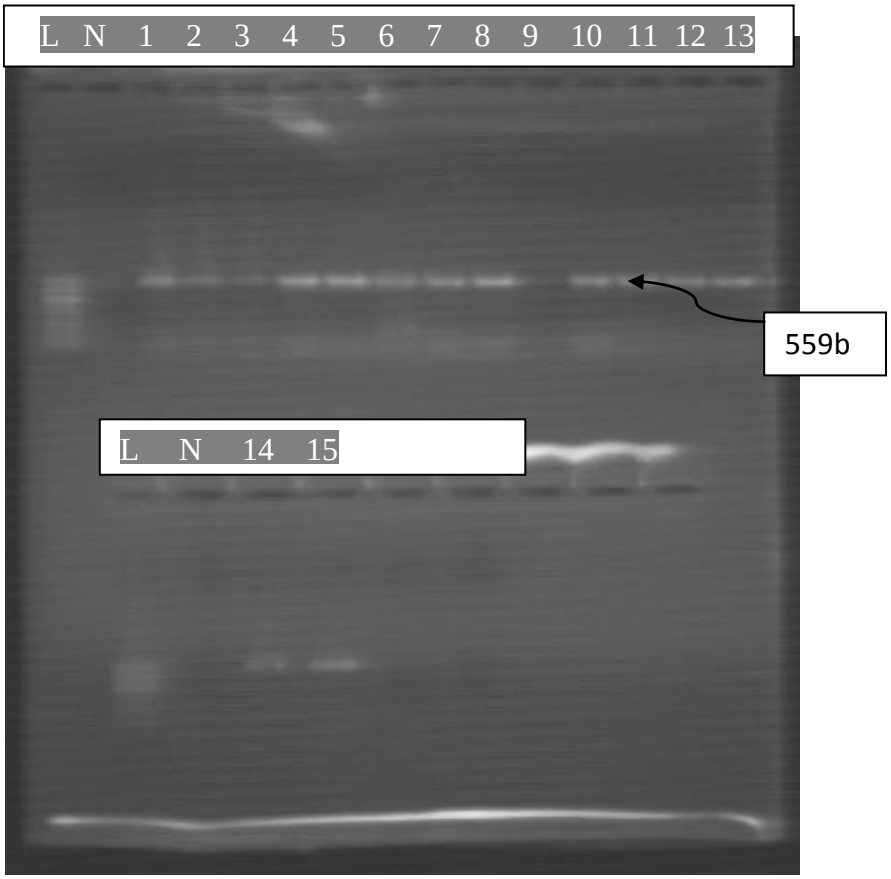


**Figure 4.** Conventional PCR assay.

1995). But at the time goes, resistance of older chickens (4-week-old) to *Salmonella* infection involves both cellular and humoral immune functions as measured by B and T cell proliferation, antibody responses, and cytotoxic activity of cells (Lessard et al., 1995).

## Conclusion

In the present study, both the bacterial load determination and culture method confirmed by the molecular technique showed that there is a relative resistance or susceptibility difference among the three poultry breeds. Koekoek breed is the most susceptible breed of the three indicating that exotic breeds with higher production potential are relatively more susceptible to the causative agent than the Local and Fayoumi breeds. Although there was difference in the relative resistance between the Local and Fayoumi breeds, it was not statistically significant. The study has pointed out that *S.* Typhimurium has the potential as an invasive infection that becomes a systemic. The study has also indicated that there is susceptibility or resistance difference among the internal organs of the chickens showing that liver was the organ where higher bacterial load or count was found. On the other hand lung was found relatively less exposed to the bacterial load of the *S.* Typhimurium.

## Conflict of Interest

The authors have not declared any conflict of interest.

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