Direct Research Journal of Veterinary Medicine and Animal Science
Vol. 9(1), Pp. 21-28, July 2024
ISSN: 2734-2166
DOI: https://doi.org/10.26765/DRJVMAS3418910945
Article Number: DRJVMAS3418910945

https://journals.directresearchpublisher.org/index.php/drjvmas/issue/archive

Survey for the isolation of *Escherichia* coli and salmonella organisms from Japanese quail (*coturnix cortonix japonica*) raw eggs from selected farms in Sokoto metropolis, Sokoto State, Nigeria

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Received 11 May 2024; Accepted 10 June 2024; Published 28 July 2024

ABSTRACT: *This research work was conducted to survey and isolate Escherichia coli and Salmonella organisms from Japanese raw quail eggs from 10 selected farms within Sokoto metropolis. A total of 50 Japanese raw quail eggs were used for this study, 5 eggs from each of the selected farms. The eggs were collected and transported aseptically in sterile polythene bags to the Usmanu Danfodiyo University, Sokoto Department of Public health Laboratory for processing. The external surface of each egg sample was swabbed using sterile swab stick pre-enriched in peptone water and selenite F broth separately while the internal content was homogenized and pre-enriched in peptone water and selenite F broth separately incubated at 37°C for 24 hours. The culture was streaked on Mac Conkey agar separately and incubated at 37°C for 24 hours. Plates were then examined for the presence of small circular pale and pinkish colonies; suggestive of non-lactose fermenters and lactose fermenters respectively. Discrete colonies of pale and pinkish colonies were gram stained and observed under a light microscope for cellular characterization. Biochemical tests comprising of Triple sugar ion (TSI) test was conducted on each Salmonella isolate and Indole production on each E. coli isolate. From the samples processed, 21 of the isolates could be identified as E. coli base on their biochemical characteristics of which 11 were from egg external swab and 13 from egg content swab while 19 could be identified as salmonella base on their biochemical reactions, of which 8 were from egg external swab and 11 from egg content swab. Findings from this study shows that raw Japanese quail eggs from 9 farms out of the 10 selected farms within Sokoto metropolis sold to public for consumption were contaminated and this may be partly due to unhygienic practices within farms. To reduce the E. coli and salmonella contamination on quail eggs strategies should be employed which include hygienic maintenance of flock, implementation of hazard analysis for critical control point (HACCP) systems, and increased consumer education through media and public enlightenment. In advance consumption of raw and undercooked quail eggs should be avoided and washed adequately before use.*

Keywords: *E. coli, Eggs, Japanese, Salmonella spp, Sokoto, Survey, quail*
Citation: Alhaji, U. A., Shehu, H. N., Ahmed, G., Mahmud, S., Shehu,S., and Aliyu, S., Yusuf, D. (2024). Survey for the isolation of *escherichia coli* and salmonella organisms from *japanese quail (coturnix cortonix japonica*) raw eggs from selected farms in Sokoto metropolis, Sokoto State, Nigeria Direct Research Journal of Veterinary Medicine and Animal Science. Vol. 9(1), Pp. 21-28. https://doi.org/10.26765/DRJVMAS3418910945. This article is published under the terms of the Creative Commons Attribution License 4.0.

INTRODUCTION

Poultry is any bird reared or hunted for a useful purpose (Oluyimi and Robert 2007). Poultry belong to three others of the avian class, which include *Galliform*, *Anseriforms* and *Columbriform*. Most birds are domesticated and are managed on the same basic principles as domestic fowl. Quails belong to the Order *Galliform* in the family *Odontopharinae* (Oluyimi and Robert, 2007). Egg is one of the important reasons for keeping poultry. Egg is a very important source of nutrition to Humans also eggs are ingredients in a variety of different formulated food production (Froning, 1998). The Formulated food product such as cake mixes, salad

dressing, noodles, confectionary products, e.t.c (Froning, 1998). Marketing of fresh pickled quail eggs and frozen carcass was limited, but expanding commercially. Increase demand for poultry products (meat and eggs) attracted establishment of new farms and slaughter houses away from their respective places of production thus a considerable increase in transportation of poultry (Minka and Ayo 2011). Reasons for egg consumption decrease during these years include; concern over high content of cholesterol, food safety issues predominantly associated with salmonella and replacing traditional breakfast with cereal. The recently estimated level of egg consumption in Nigeria per annum is 20-25 eggs, compared with 250-300 eggs per head per annum in the European countries and united State of America (Oluyemi and Robert 2007).

Cholesterol is an important component of egg which also has the ability to increase the chances of artheroma development, thus predisposing to heart attack. This is why many scientists are now conducting researches to see how they can reduce the level of cholesterol in the egg and thus making it safer for consumption without much effect on the heart and also in Sokoto (Habibullah *et al*., 2007) conducted research using feed supplementation techniques on laying hens to see how their egg cholesterol can be reduced. Recently quail eggs and meat are gradually gaining popularity in Sokoto. However little or no information exist on the surface contamination of quails' eggs by *salmonella* and *E. coli.*

Organisms that cause diseases in humans are transmitted through various food items, leading to increase morbidity and mortality (Zhao *et al* 2001). Meat and eggs are important source of high-quality protein, fats and calories, vitamins and minerals. In many developing countries including Nigeria, lack of appropriate processing facilities as well as unsanitary slaughtering methods are the causes of unnecessary contaminations of meat as well as other animal resources such as eggs and poultry meat. Meat and egg products coming from such conditions are often deteriorated due to bacterial infection which may expose consumers to food poisoning (Joshi *et al.,* 2003). Common pathogenic bacteria that are associated with food borne diseases include strains of *salmonellae and Escherichia coli* (Clarence *et al.,* 2009). These pathogenic organisms (*salmonella species and Escherichia coli*) are gram negative, rod shaped, facultative anaerobes; belonging to the large bacteria family Enterobacteriacae*. Escherichia coli* may cause acute enteritis in humans and young animals; including lambs, calves and piglets as well as colibacillosis in poultry. Over the last two decades, bacterial infections caused by Enterohaemorrhagic *E. coli* have emerged as an important food-borne disease. A multistate outbreak of *E. coli* 0157:H7 infections have been reported. According to the number of cases reported, and the opinion of my public health authorities, Salmonellosis is one of the most common infectious diseases transmitted by contaminated food products including meat and eggs (Zhao *et al* 2001). Studies worldwide have shown that Salmonellae and E. coli are often present and can be isolated from raw meat and eggs (CDC, 2005). There are little or no documented reports on the isolations of *salmonellae species* and *E. coli* from raw quail eggs in Sokoto metropolis. Okonko *et al.,* (2010) isolated salmonella and *E. coli* from raw meat in Calabar, Nigeria. These animal products are consumed by people in many parts of the country, Sokoto inclusive.

Sokoto is one of the major livestock producing area in Nigeria with an estimated number of 3 million cattle, 3.85 sheep, 4 million goat, 0.8 million camels, 2 million chickens and 1million poultry (Kware, 2009). However, not much concern is given to hygienic practices during handling and processing of raw meat and eggs by retailers. These pathways may expose the consumers to increase risk of acquiring food-borne disease-causing agent such as *E. coli* and *salmonella*. Considering the importance of eggs as good source of protein to the populace and their position as vehicle of food borne pathogens to humans, the public health and food safety implication association with the presence of *Salmonella* and *E. coli* in these food items need to be investigated.

The aims and objectives of this research study are; to isolate *Salmonella* and *Escherichia coli* from surface and internal content of raw Japanese quail egg from 10 farms within Sokoto metropolis using bacteriological procedures of isolation and to give advice on possible ways of preventing the contamination of raw quail eggs for the purpose of safety consumption. Since eggs are one of the major sources of proteins to the indigenes of Sokoto state and the Country at large, and with *salmonella* and *E. coli* being their major contaminants, there is need to investigate raw Japanese quail eggs for the presence of bacterial contamination. The result of this research work is expected to provide baseline data on the degree of raw Japanese quail egg contamination by these organisms, which will help in designing an effective preventive measure during their handling and processing.

MATERIALS AND METHODS

Study area

Sokoto is the capital of Sokoto state which is located in the northwestern part of Nigeria between longitudes 4^0 8^l E and 6^0 54^l E and latitudes 12^0 N and 13^0 58^l N. It forms borders with Republic of Niger to the north; Kebbi State to the west and southwest, and Zamfara State to the east. Sokoto metropolis constitutes (Sokoto north, Sokoto south, Wamakko, Kware and Dange shuni) the state is divided into 23 local government areas (LGAs). Farming is the mainstay of the people's economy accounting for a

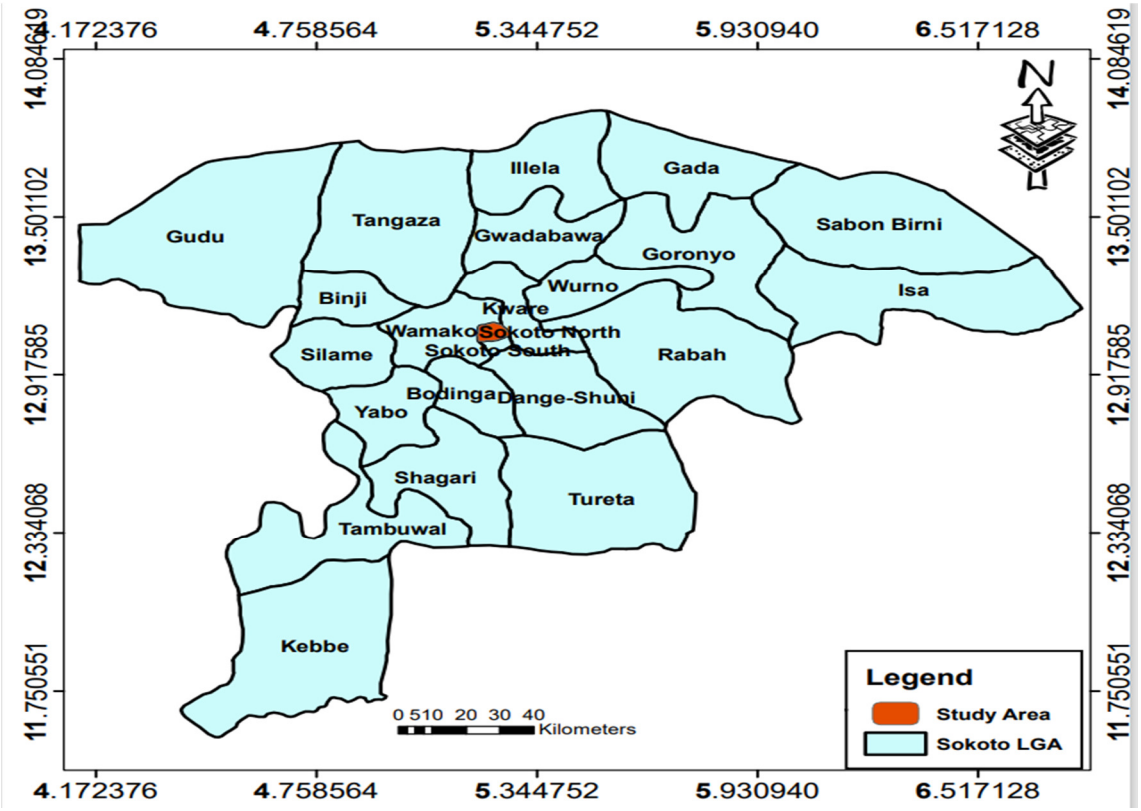

Figure. 1: Map of Sokoto, Sokoto State, Nigeria.

significant proportion of the Gross Domestic Product (GDP) and responsible for about 70% of the total employment (NPC, 1991) (Figure 1). Sokoto state ranks second in the nation in livestock population with estimated 3 million cattle, 3 million sheep, 5 million goats, 4,600 camels (Mocit, 2002) and 2 million chickens (NRDP, 2003).

Sample collection

Quails eggs were collected from 10 farms within Sokoto metropolis, Sokoto State, Nigeria. A total of 50 eggs (5 from each farm) were collected, aseptically placed in sterile polythene bags and transported to the Usmanu Danfodiyo University, Sokoto Veterinary Public health laboratory for processing.

Sample inoculation and processing

The external surface of the eggs was swabbed using sterile swab stick pre-enriched in peptone water and selenite F broth separately while the internal content was homogenized and pre-enriched in peptone water and selenite F broth separately incubated at 37^{o}c for 24hours. After the enrichment a loop-full of the peptone water culture and selenite F broth was streaked on Mac Conkey agar separately and incubated at 37^{o}c for 24hours. Plates were then examined for the presence of small circular pale and pinkish colonies; suggestive of non-lactose fermenters and lactose fermenters respectively. Discrete colonies of pale and pinkish colonies were gram stained and observed under a light microscope for cellular characterization (Zhao *et al.,* 2001; Chauhan *and* Roy, 2008).

Sample preservation

Suspected *Salmonella* and *E. coli* colonies were sub-cultured onto non-selective nutrient agar slant for biochemical characterization.

Biochemical characterization

Biochemical tests comprising of Triple sugar ion (TSI) test was conducted on each Salmonella isolate and Indole production on each *E. coli* isolate based on procedures described by Quinn *et al.,* (2002).

Indole test

A loop-full of the isolate was inoculated from the nutrient agar slant into peptone broth and incubated for 48 hours

at 37°C. After incubation, 2-3 drops of Kovacs reagent were added into the broth culture and allowed to settle. A positive result will show red layer at the top of the culture.

H_2S Production in TSI agar

Triple sugar iron agar contains 0.1% glucose, 1% lactose and 1% sucrose and chemicals to indicate hydrogen sulphide production. Phenol red is used as indicator for pH change (red at ph 8.2, yellow at pH 6.4). A black precipitate of ferrous sulphide is indicative of H_2S production (Table 1). An inoculum from a single isolated colony of the organism under test is stab-inoculated with a wire loop into the butt of the TSI agar and, on withdrawal, the slant surface is inoculated. The loosely-capped tube is incubated for 18 hours at 37°C.

Table 1: Normal Biochemical Reactions of *E.coli* and *salmonella* in Indole and Triple sugar ion test.

Bacteria species	Indole test	H_2S test
E. coli Salmonella	±	±

(Quinn, 2002).

RESULTS

From the total of 50 examined samples (50 egg external swabs which were pre- enriched in peptone and selenite F broth separately and the corresponding egg internal content pre-enriched in peptone and selenite F broth). A total of 24(24%) swabs expressed lactose fermenters on Mac Conkey agar (presumed *E. coli*) of which 11(45.8%) were from eggs external swab and 13 (54.1%) from the eggs content swab. As shown on the (Table 2, Figure 2, Plates 1 and 2). From the total of 50 examined samples (50 egg external swabs which were pre-enriched in peptone and selenite F broth separately and the corresponding egg content swab samples pre-enriched in peptone and selenite F broth). A total of 51(51%) swabs expressed non lactose fermenters of which 24(47.5%) were from egg external swab and 27(52.9%) from egg content swabs. As shown on the (Table 3, Figure 3, Plates 3 and 4).

DISCUSSION

The aim of this study was to isolate *E. coli* and *salmonella* from raw Japanese quail eggs within Sokoto metropolis from ten selected farms by bacteriological isolation and also to give possible advice on how to reduce contamination of the raw eggs which are often associated with unhygienic handling and processing. In this study 24 of the total 200 swabs that were taken from the 50 eggs expressed lactose fermenters on Mac Conkey agar (presumed *E. coli*). However, 21 of the isolates could be identified as *E. coli* base on their biochemical characteristics of which 11 were from egg external swab and 13 from egg content swab. Of the 51 (presumed salmonella) 19 could be identified as salmonella base on their biochemical reactions, of which 8 were from egg external swab and 11 from egg content swab. The tables 4 and 5 show the results of *salmonella* and *E. coli* from a total of 50 examined samples (Tables 4 and 5 and Figure 4). In addition of the ten selected farms 9 were positive for the *E. coli* and *Salmonella* while one of the farms was negative. The 1st sample that was processed comprise of 5 eggs, 9(45%) swabs yielded 7(77.7%) lactose fermenters of which 4 were from egg external swab and 3from egg content swab while the 2(22.2%) swabs yielded non lactose fermenters of which 1(50%) swab was from egg external content and the other from egg content swab. The 2nd sample which comprises of 5eggs, 12(60%) swabs yielded 3(25%) lactose fermenters of which 1 was from egg external swab while the 2 were from egg content swab and the 9(75%) swabs yielded non lactose fermenters of which 4 were from egg external swab and 5 from egg content swab. The 3rd sample comprise of 5 eggs, 6(30%) swabs yielded non lactose fermenters of which 4 were from egg external swab and the others from egg content swab. The 4th sample comprise of 5 eggs, 8(40%) swabs yielded 2(25%) lactose fermenters which were from egg content swab and the 6(75%) swabs yielded non lactose fermenters of which 4 were from egg external swab and the others from egg content swab. The 5th sample comprise of 5 eggs, 11(55%) swabs yielded 3(27.2%) lactose fermenters of which 1 was from egg external swab and the 2 from egg content swab while 8(72.7%) swabs yielded non lactose fermenters of which 4 were from egg external swabs and the other from egg content swabs. The 6th sample comprise of 5 eggs, 8(40%) swabs yielded 2(25%) lactose fermenters which were all from egg external swab while 6(75%) were non lactose fermenters of which 3 were from egg external swab while the others were from egg content swab. The 7th sample comprise of 5 eggs, 8(40%) swabs yielded 3(37.5%) lactose fermenters of which 2 were from egg external swab and 1 from egg content swab while 5962.5%) were non lactose fermenters of which 2 were from egg external swab and 3from egg content swab. The 8th sample comprise of 5 eggs, 7(35%) swabs yielded 1(14.3%) lactose fermenter which was from egg content swab while the 6(85.7%) yielded non lactose fermenters of which only 1 was from egg external swab and 5 from egg content swab. The 9th sample comprise of 5 eggs, 5(25%) swabs yielded 2(40%) lactose fermenters which were from egg content swab while 3 (60%) swabs yielded non lactose fermenters which were all from egg content swab. The 10th sample comprise of 5eggs, 3(15%) yielded 1(33.3%) lactose fermenter which was from egg content swab while 2(66.6%) yielded non lactose fermenters which were all from egg content swab.

Table 2: Total number of lactose fermenters isolated from the corresponding samples.

Sample swab	Number tested	Lactose fermenters
Egg external swab	100	11(11%)
Egg content swab	100	13(13%)
Total	200	24(12%)

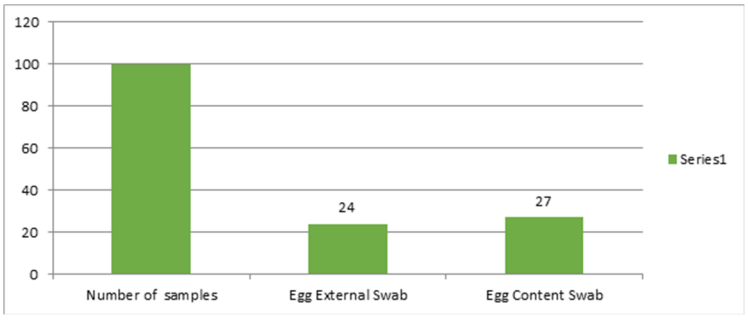

Figure 2: Non-lactose fermenters isolated from the corresponding samples. (Presumed *salmonella*)

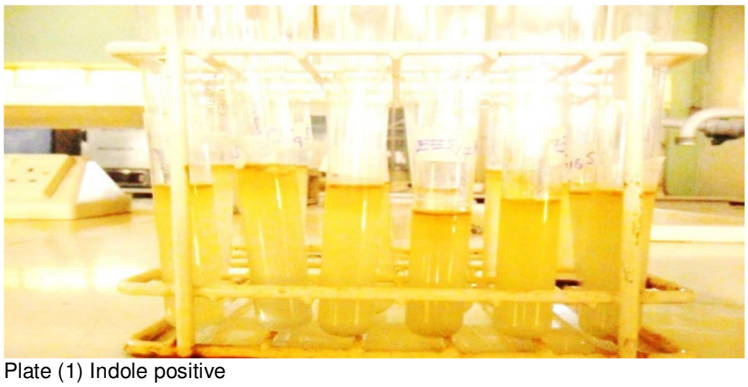

Plate (1) Indole positive

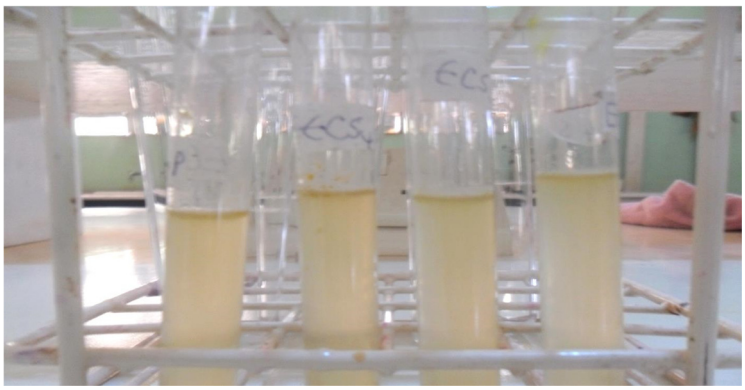

Plate (2) Indole negative

Table 3: Total number of non-lactose fermenters isolated from the corresponding samples.

Sample swabs	Number tested	Non-lactose fermenters
Egg external swab	100	24(24%)
Egg content swab	100	27(24%)
Total	200	51(25.5%)

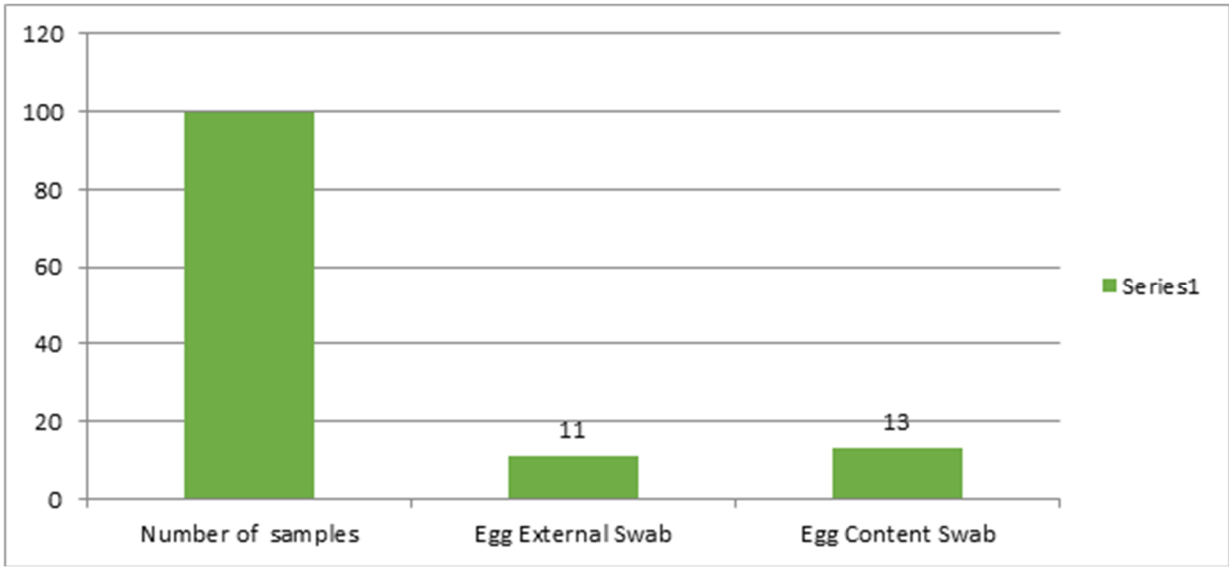

Figure 3: Lactose fermenters isolated from the corresponding samples (Presumed *E. coli*).

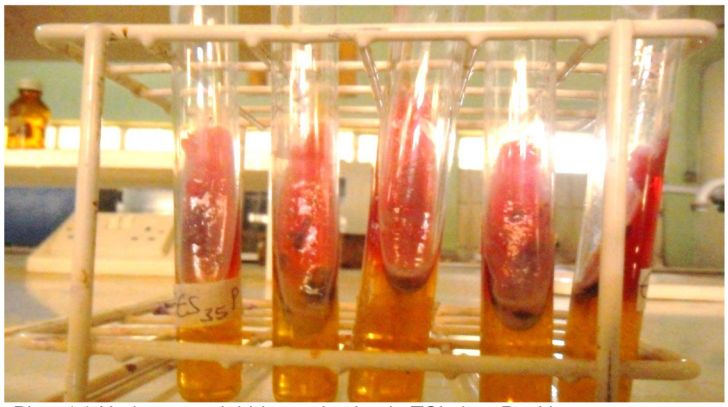

Plate (3) Hydrogen sulphide production in TSI slant Positive

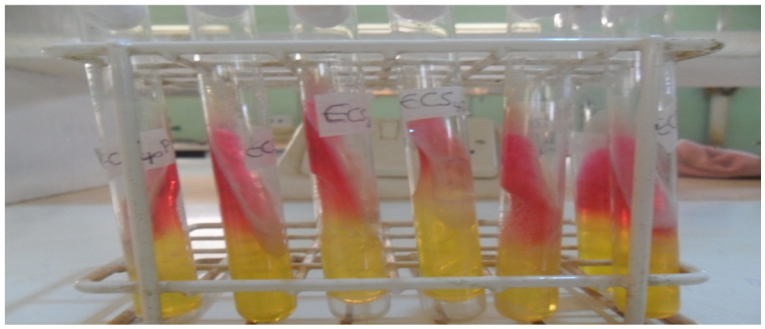

Plate (4) Hydrogen sulphide production in TSI slant Negative

Table 4: Total Number of Biochemically tested *E. coli* and *Salmonella*.

Sample swabs	No. tested	No of samples with biochemically identified *E. coli*	No of samples with biochemically Identified *Salmonella*
Egg external swab	100	11(11%)	8(8%)
Egg content swab	100	10(10%)	11(11%)
Total	200	21(10.5%)	19(9.5%)

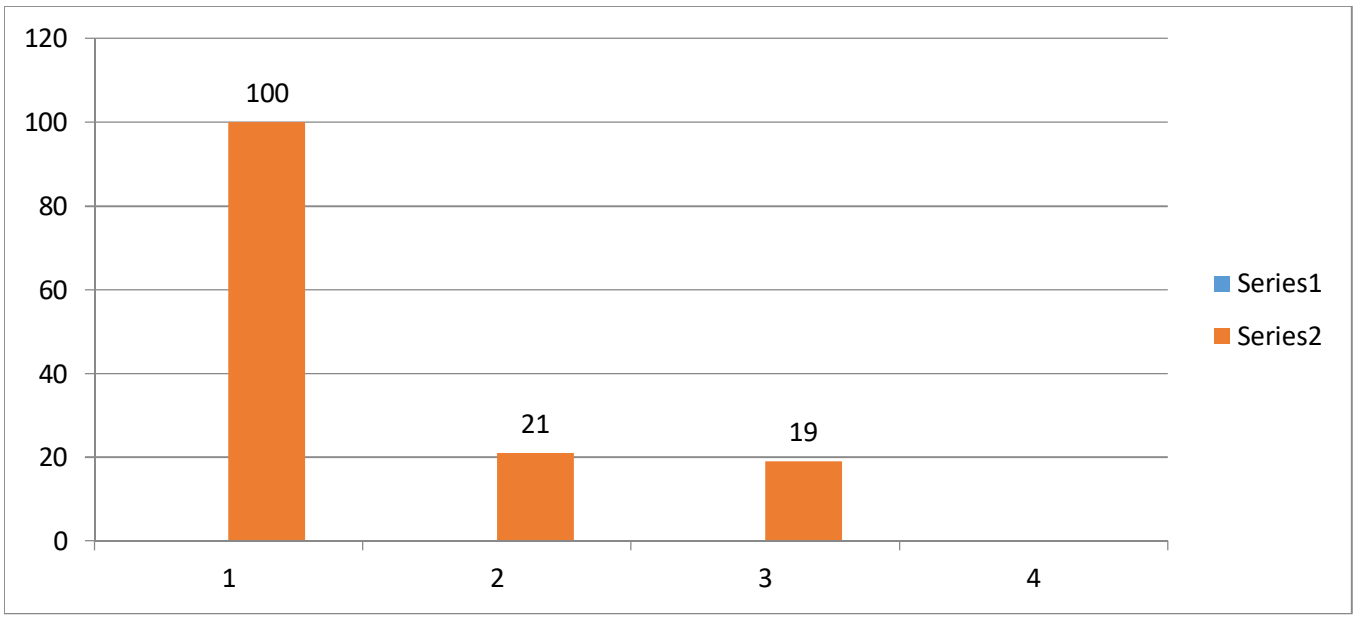

Figure 4: Biochemically identified *E. coli* and *Salmonella*

Table 5: Relationship between Age of laying quails and biochemically isolated *Salmonella*

Sample no.	Age (month)	*Salmonella*
1	3	2
2	6	9
3	2	6
4	8	6
5	5	8
6	5	6
7	4	5
8	7	6
9	4	3
10	5	2

There was no significant association between age and *E.coli* X^2-test=16.919, df = 9, P>0.05

Finally base on this study conducted from the above results the egg content had much contamination than the egg surface.

Conclusion

Findings from this study shows that raw Japanese quail eggs from 9 farms out of the 10 selected farms within Sokoto metropolis sold to public for consumption were contaminated, and this may be partly due to unhygienic practices within farms. To reduce the *E. coli* and *salmonella* contamination on quail eggs strategies should be employed which include hygienic maintenance of flock, implementation of hazard analysis for critical control point (HACCP) systems, and increased consumer education through media and public enlightenment. In advance consumption of raw and undercooked quail eggs should be avoided.

Recommendation

Base on this research study conducted, the following recommendations were made; Ensure good hygienic standard at farms, consumption of raw eggs should be discouraged to reduce the risk of infection, eggs meant for consumption should be washed and cooked adequately.

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