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Research Article

Prevalence of Salmonella in Eggs from Modern, Industrial and Semi-Industrial Farms

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Abstract

Bacteria of the genus Salmonella have proven to be one of the leading foodborne illnesses worldwide, with serious consequences for human and animal health. In Mali the spread of this germ from poultry eggs is considered a major source of typhoid fever. Currently, the intensification of animal production has been favored by the use of veterinary drugs, in particular antibiotics. The objective of this study was to evaluate the prevalence of salmonella isolated from egg samples collected at the different study sites. The samples taken numbered one hundred and fifty-eight (158 eggs) in Bamako and in peri-urban areas. Peri-urban areas supply Bamako with foodstuffs of animal origin, particularly eggs. Microbiological analysis of the collected eggs was carried out according to ISO 6579-1:2017 standards using Rappaport Vassiliadis culture medium for enrichment and Salmonella Shigella (SS) agar for identification of salmonella. The density results showed that 8.86% of the eggs floated to the surface of the saline solution and the microbiological analyzes showed that the presence of Salmonella in 08 of the 158 egg samples, giving a prevalence of 5.06%. This low percentage could be explained by the misuse of antibiotics in poultry farming. Studies with more advanced quantitative methods should be conducted to identify the nature of the antibiotics present in these foods, as well as to quantify their contents.

Keywords

Salmonella, Antibiotic Residues, Eggs, Poultry

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1. Introduction

Mali is a vast Sahelo-Saharan country with an agro-pastoral vocation, located in the heart of West Africa with a national herd worth 38,587,450 poultry including 2,757,313 layers based on a forecast of 2,628,000 or 104.91 % of production targets; and 441,079,208 eggs produced out of a forecast of 467,000,000, or 94.43% of production objectives [5], However, this sector is faced with a series of potential contaminants such as microorganisms, pesticide and antibiotic residues. In West Africa, only pathogenic microbial agents, pesticide residues and aflatoxins have been the subject of work or studies as part of an approach to protect the health quality of foods intended for consumption. Intended for human and animal [1-4, 6]. These hazards have been most linked to threatening public health [7]. Foodborne illnesses are a significant cause of morbidity worldwide. Millions of people fall ill and many die after ingesting unsafe food. Deeply concerned about this problem, WHO Member States adopted a resolution in 2000 recognizing that food safety is an essential aspect of public health. In industrialized countries, it has become a demand demanded by consumers, and above all a major health issue, as evidenced by the latest health crisis which occurred in June 2011 in certain European countries. This health alert was linked to the presence of bacteria called Escherichia coli, serotype STEC O104:H4 which caused 31 deaths among the 3000 cases who fell ill across the 14 affected European countries. Among these zoonotic food pathogens, Salmonella is the second cause of these bacterial food toxins in Europe and worldwide. It causes symptoms in humans of a wide range of severity, from mild stomach aches to septicemia, and in some cases leads to death. Indeed, each year, 93.8 million human cases are reported around the world, resulting in 155,000 deaths. A wide range of animals, particularly livestock, have been identified as reservoirs of non-typhoidal Salmonella.

The increase in foodborne illnesses around the world has prompted some scientists to investigate these causes in consumed eggs and poultry meat. Also, the objective of this study is to assess the health risks linked to the presence of salmonella.

2. Material and Methods

2.1. Biological Material

The biological material for this study consists of chicken egg samples.

2.2. Site et échantillonnage

Figure 1. *Fresh egg samples ready for laboratory analysis.*

SAMPLING:

The present study is structured around two parts, a survey carried out among farmers in order to describe the main antibiotic molecules used in poultry farming in the peri-urban areas of Bamako. The surveys were carried out on 22 farms in the communes of: Sanakoroba, Banamba, Safo, Kalaban Coro, Kambila, Commune I in November 2019. For this purpose, we formed a single team which carried out the survey and sampling. Tool training and hygiene measures were followed to avoid any contamination and alteration of products. A total of 158 egg samples were collected from 22 farms. The samples were taken on site in plastic cells, and then covered with aluminum foil to avoid external contamination, sent the same day to the REM Biotech Lab for microbiological analyzes and those aimed at dosing antibiotics were stored at 4 degrees Celsius.

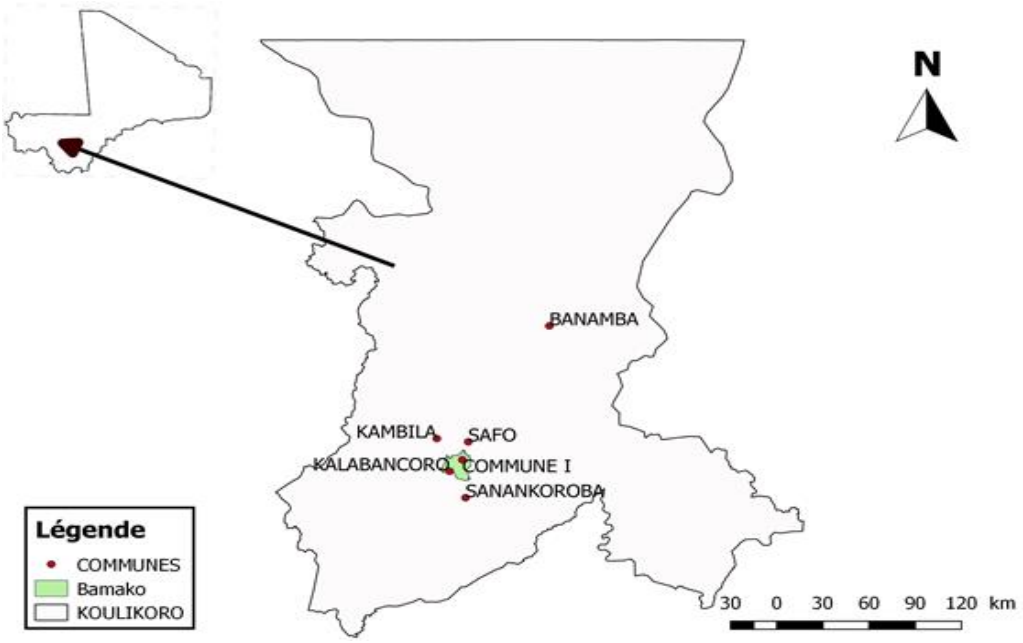

Figure 2. *Study areas and sites.*

2.3. Laboratory Analyzes

2.3.1. Densimetric Control

The eggs were immersed in 12% NaCl salt water [8], in order to see the condition of the eggs for one minute. Floating eggs were excluded from the microbiological analysis.

2.3.2. Sample Preparation and Culturing

Whole eggs that did not float were opened aseptically: after rapid washing with water and alcohol. The shells were opened using the sterile scalpel. The contents of the eggs were collected using a sterile pipette and poured directly into a sterile 50ml Falcon tube and homogenized for 15 minutes next to the Bunsen burner (Solution1);

2.3.3. Pre-Enrichment

Addition of 25 ml of solution 1 with 225 ml of buffered peptone water. Mix well and incubate at 37 ℃ for 24 hours (Solution 2).

2.3.4. Enrichment

1 ml of the pre-enrichment solution (Solution 2) was transferred into the tubes containing the Rappaport Vassiliadis medium and incubated at 37 ℃ for 24 hours (Solution 3).

Isolation and biochemical characterizations:

Analysis of the diversity of bacteria of the genus Salmonella sp. was carried out using the different egg samples contained in the Vassiliadis rappaport broth medium (Solution 3). The different samples were inoculated on Salmonella Shigella (SS) agar, and then incubated at 37 ℃ for 24 hours. SS agar is a selective solid medium for the isolation of *Salmonella* and *Shigella*. After 24 hours of incubation, the presumptive colonies are most often lactose negative and hydrogen sulfur positive (colorless colonies with a black center). These suspicious colonies were subcultured on Trypticase Soya Agar (TSA) to obtain pure cultures.

2.4. Biochemical Characterizations

Salmonella spp was characterized by the use of the reduced Minor rack which includes the media: KLIGER-HAJNA, Simmons Citrate, Iron Lysine, Urea-Indole and the Mannitol-mobility-nitrate medium. Bacteria having presented the following characteristics were retained: fermentation of glucose with production of gas, production of sulphurous hydrogen, use of citrate as the sole source of carbon, presence of a lysine deaminase, absence of an active urease in 24 hours.

3. Statistical Analyzes

SPSS software version 20.0 was used for all analyzes and differences were considered significant when $p < 0,05$.

4. Results

4.1. Densimetric Control

The densitometry applied to the 158 farm eggs gave the results respectively in the table 1.

Table 1. Densimetry of egg samples in 12% saline solution.

Parameters	Eggs Fresh	Eggs not Fresh	Total
Number	144	14	158
Percentage (%)	91,13	8,86	100

The densitometry results show that 8.86% of the eggs float on the surface of the saline solution and therefore are not fresh. These results are much higher than those obtained by [9], who obtained 2.77%. It remains lower than the results of [12] who obtained respectively 91.25% and 100% of the eggs which float on the surface of the saline solution. These differences could be explained on the one hand by the storage time and on the other hand by the increase in the air chamber of the eggs leading to a reduction in their density compared to that of the solution.

Figure 3. Densimeter test results showing non-floating eggs (A).

Figure 4. Densimetric test results showing floating eggs (B).

Table 2. Frequency of antibiotics used in the farms surveyed.

ANTIBIOTICS	NUMBER	FREQUENCE %
Oxyferan	21	52,5
Oxytetracycline	16	40
TTS	16	40
Enrofloxacine	15	37,5
Panteryl	14	35
Tylodox	07	17,5
Enroveto	12	30
Cunicol	07	17,5
Tetracolivite	06	15
Doxycycline	04	10
Aprolium	03	07,05
Aminovit	02	05
Diaziprim	02	05
Norfloxacine	01	02,05
Carnitol	01	02,05
Promotol	01	02,05
TOP Ceryl	01	02,05

The results in Table 2 show that Oxyferan is the most used antibiotic with a percentage of 52.5% followed by Oxytetracycline, TTS, Enrofloxacin, Panteryl and Enroveto respectively 40%, 40%, 37.5%; 35% and 30%. The results also show a significant use of vitamin supplements and some hepatoprotectors.

4.2. Sample Preparation

Whole eggs that did not float were opened aseptically, after a quick wash with water and alcohol. The shells were opened using a sterile scalpel. The contents of the eggs were collected using a sterile pipette and poured directly into a sterile 50ml falcon tube and homogenized for 15 minutes next to the Bunsen burner (Solution 2).

4.3. Pre Enrichment

After 24 hours of incubation at 37 ℃ a change was observed with the increase in turbidity in the vials containing the egg samples, however no phenomenon was observed in the control vial see figure 5 below.

Figure 5. Solution 2 before incubation.

Figure 6. Solution 2 after incubation.

4.4. Enrichment

After 24 hours of incubation at 37 ℃ a change was observed with the increase in turbidity in the tubes containing the samples pre-enriched with eggs, however no phenomenon was observed in the control tube see figure 4 below.

Figure 7. Solution 3 before incubation.

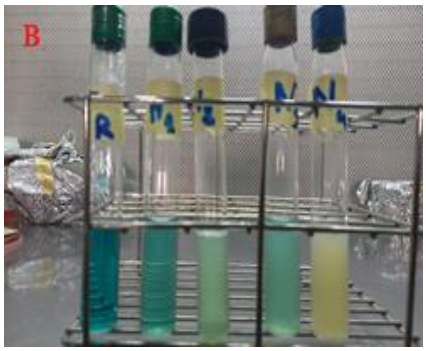

Figure 8. Solution 3 after incubation.

4.5. Isolation and Characterization

Table 3. Prevalence of Salmonella in egg samples analysed.

Nbre of samples analyzed	Salmonella research results			
	Positive		Negative	
	Nber	%	Nber	%
158	08	5,06 %	150	93,94 %

Nber: Number; %: Percentage

In the present study 158 chicken eggs were examined for the isolation and biochemical characterizations of Salmonella sp. Table 2 shows Salmonella prevalence of 5.06% of eggs that are contaminated with Salmonella and 93.94% of eggs tested negative for Salmonella. This prevalence of Salmonella could be interpreted by the abusive use of antibiotics on the one hand and on the other hand by the presence of Salmonella in farms, which could be explained by the non-compliance with good breeding practices. The results of the distribution and conformity of egg samples from the different municipalities and sites are recorded in Table 3.

Table 4. Distribution and compliance of egg samples in the different municipalities and sites.

COMMUNES	SITES	RESEARCH FOR SALMONELLA		
		Number	Positive	Compliance (%)
SANANKOROBA	SANANKOROBA	39	00	100
	ZOUKOUME	14	00	100
	DIALAKOROBA	07	00	100
BANAMBA	TOUMBA COURA	20	04	80
	KOULOUNICO	06	00	100

COMMUNES	SITES	RESEARCH FOR SALMONELLA		
		Number	Positive	Compliance (%)
SAFO	DJAMOUSSABOUGOU	22	00	100
	SERIBALA	04	00	100
	YEDOUMANI	03	00	100
	SAFO	02	00	100
KALABAN CORO	KALABAN CORO	07	04	42,86
KAM BILA	KATI DRALE	04	00	100
	KAMBILA	23	00	100
COMM UNE I	SOTUBA IER	07	00	100
TOTAL		158	08	

The results in Table 4 show a compliance rate of 100% in the communes of SANAKOROBA, SAFO, KAMBILA AND COMMUNE I while in the commune of BANAMBA, the TOUMBA COURA site presented a compliance rate of 80%, or four (04) contaminated samples out of the twenty (20). The municipality of KALABAN CORO presented a compliance rate of 42.86%. These rates could be explained by the misuse of antibiotics on farms and non-compliance with good breeding practices.

5. Discussion

Poultry and eggs are protein-rich foods and constitute an important part of the human diet. In the present study 158 eggs from laying hens were examined for the isolation and characterization of Salmonella sp. The densitometry results show that 8.86% of the eggs float on the surface of the saline solution and are therefore not fresh. These results are significantly higher than those obtained by [9] who obtained 2.77%. They remain lower than the results of [12] who obtained respectively 91.25% and 100% of the eggs which float on the surface of the saline solution. These differences could be explained on the one hand, by the storage time and on the other hand, by the increase in the air chamber of the eggs leading to a reduction in their density compared to that of the solution. The results in Table 2 show a compliance rate of 100% in the communes of SANANKOROBA, SAFO, KAMBILA and COMMUNE I. This result is comparable to those obtained by [13]; while the commune of BANAMBA, the TOUBA COURA site presented a compliance rate of 80%, i.e. four (04) contaminated samples out of twenty (20). In KALABAN CORO four (04) samples out of seven (07) were contaminated by Salmonella, representing a compliance rate of 42.86%. These rates could be explained by the abusive use of antibiotics in livestock farms. Culture of egg samples on Salmonella-Shigella (SS) agar which is a differential and selective medium for the isolation of Shigella and Salmonella species from suspected pathological samples, etc. *Salmonella spp* for cultivation of sodium thiosulfate and ferric citrate allow detection of hydrogen sulfide by producing colonies with black centers and Shigella produced smooth, colorless colonies on SS agar. The present study established a salmonella prevalence of 5.06% in chicken eggs. This prevalence is lower than those obtained by [10] having worked on batches of contaminated eggs from farms close to the landfill; [11] and [14] which had respectively 7.48%; 20.48% and 12.70%. This significant prevalence could be explained by the epizootic situation of Salmonellosis on farms and the misuse of antibiotics on farms. Poultry contaminated during farming are a very important source of dissemination of Salmonella, during the different stages of their processing. The prevalence's observed in chicken and guinea fowl species by [14] were 14.91% and 10.88% respectively. These results could be explained by the poor breeding conditions of the chickens. The microorganisms capable of penetrating into the yolk of the egg are rare and belong mainly to the species of Gram-negative bacteria. This justified the search in eggs for those generally belonging to the *Enterobacteriaceae* family. Among these bacterial species, Salmonella plays an extremely important role, which can cause foodborne illness. Poultry eggs can be one of the sources of contamination of humans by Salmonella. Indeed, Salmonella causes different diseases: typhoid and paratyphoid fevers, and gastroenteritis. The microbiological quality of eggs also depends on the environment in which the hens are raised.

6. Conclusion

This study showed that despite the overuse of antibiotics in eggs, some still remain contaminated with salmonella. Also the present study shows that the Salmonella strains isolated from

these samples are resistant to most commonly used antibiotics. Furthermore, the results obtained confirm that the main factors which promote the contamination of these products are: non-compliance with basic hygiene rules on farms, good breeding practices and the use of rudimentary tools. In order to reduce the level of contamination of poultry products by Salmonella, emphasis must be placed on respecting good breeding practices on farms and controlling Salmonella on poultry farms.

Abbreviations

INSP	National Institute of Public Health
ISA	Institute of Applied Sciences

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Author Contributions

Fanta Kaba Camara: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Ousmane Diarra: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Aisse Bah: Conceptualization, Formal Analysis, Methodology, Visualization

Sognan Dao: Methodology, Resources, Software, Supervision, Visualization, Writing – original draft

Adounignia Kassogue: Data curation, Formal Analysis, Methodology, Visualization

Bakary Diarra: Conceptualization, Supervision

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Boubacar Madio dit Aladiogo Maiga: Data curation, Visualization

Atia Traore: Data curation

Abdoulaye Zie Kone: Data curation, Methodology, Software, Supervision, Visualization

Mamadou Wele: Data curation

Amadou Hamadoun Babana: Data curation, Methodology, Software, Visualization

Conflicts of Interest

The authors declare no conflicts of interest.

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