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Detection of microbial contamination of Salmonella in fodder and imported food that available in poultry fields and local markets

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ABSTRACT
This research was conducted to investigate the presence of Salmonella bacteria in animal production foods circulating in Baghdad markets, fish, imported feeds, and local meat chicken fields, A total of 270 food samples were collected, bacteriological and serological tests were conducted to diagnose salmonella and the results showed that 79 Salmonella isolates were isolated from a total of 270 chicken meat and feed samples, 37 isolates from chicken meat and its products, at a rate of 24.6%, in which eleven species of Salmonella bacteria were diagnosed, as they formed S.typhi of (13.51%), the percentage of S. typhimurium, S. anatum, S. gallinarinm and S. living stone was 8.1%, while the percentage of S. enteritidis, S. menston, S. menchen and S . ohio was 10.81%, the percentage of S. blokly and S. thompson was 5.4%. and 16 isolates of Salmonella bacteria were isolated, at a rate of 24.6%, out of the total of 60 samples obtained from fish and poultry feed in which eight species of Salmonella bacteria were diagnosed, as they formed S. ohio of (25%) while the other salmonella species of S. Dublin and S. thompson of (18.75%), and the percentage of other species of S. typhimurium, S. enteritidis, S. menston and S . hadar was 6.25%, while the percentage of S. braendrup was 12.5%. This confirms the existence of risks to public health and animal health as a result of the consumption of contaminated food and the use of contaminated feed in chicken farming fields.

Keywords: chicken meat, poultry feed, fish, Salmonella bacteria

INTRODUCTION

With a population of more than 30,000 known species, Poultry meat and fish forms the most important group in the animal kingdom that necessary for human health, safety, and growth, as it contains most of the essential amino acids, easily digestible vitamins, and essential micronutrients in energy production (Eze et al., 2017).

The demand for poultry meat and fishery product is increasing due to its ease of storage and palatable taste, as well as its high percentage of animal protein and vitamins and mineral salts (De Smet and Vossen, 2016).

However, some of the Poultry and fish products that are processed in complex and a modern technologically advanced that is on par with any other food industry are exposed to contaminated with pathogenic organisms during slaughtering, processing and transportation that lead to spoilage and some chemical changes such as protein decomposition and rancidity, which poses a threat to the safety of poultry and fish products and human health (Scallan et al., 2011, Mpundu et al., 2019).

Therefore, the world's attention has been directed to improving fish and poultry production and the arrival of white meat to the consumer in good condition to obtain a product High quality (Worley et al., 2018 and Jibril et al., 2020).

Salmonella infection is a very important health problem all over the world, where there are more than 2300 strains of Salmonella strains that can be classified separately depending for their serotypes (Smith et al., 2019 and Song et al., 2020).

Salmonella bacteria is a unicellular, microscopic microorganism found in the intestines of animals and humans. It is transmitted from the feces of infected humans or animals to humans or other healthy animals, but the common ones as a cause of the disease are confined to a number of strains, the most important of which are Salmonella typhimurium and Salmonella Enteritidis (Bucher et al., 2008 and Rasamsetti et al., 2022) which constitute almost half of all human infections (Pinedo et al., 2022). Salmonella infection, and strains that show symptoms of disease in animals can cause disease in humans, and vice versa. Salmonella disease or infection has been known for 100 years or more, and it was discovered by an American scientist called Dr. Daniel Salmon (Gut et al., 2018).

The presence of Salmonella bacteria in the food of animal production causes critical risks and hinders the trade of this type of food. Imported food, feed, and polluted poultry fields are a source of transmission and spread of Salmonella species (El-Sharkawy et al., 2017).
In view of the great role that fish and poultry meat plays as a source of proteins in our country, where the Iraqi per capita consumption rate reached 22 kg of this meat in 2020, and the actual production of poultry meat reached 17000 tons in the same year, this study aims to investigate the presence of genera Which contaminate broiler carcasses and feed available in poultry fields and fish in local markets in order to contribute to reaching a healthy food product.

MATERIALS AND METHODS

SAMPLING

Random collected of 270 samples, including 50 whole chicken carcasses, 50 liver samples, 50 gizzard samples, 60 samples for fish including (carb fish (cat fish or silurus triostegus), pomfrete fish (pampus argenteus), and Tuna fish (cans)) and 60 sample for each of green fodder and dry fodder from several sources, and from various small and large shops inside Baghdad (a few samples were purchased from outside the city Baghdad). The period of collecting samples was six months from May 2022 to October 2022. The collected samples were immediately transported to the Microbiology Laboratory for market research and consumer protection center / the university of Baghdad/Iraq for bacteriological analysis.

PREPARING MEAT AND FODDER SAMPLES AND POULTRY FIELD SAMPLES:

From each sample of fish and chicken meat and its products, feed and animal protein present in poultry fields, 25 gm was weighed and grinded using mortar and pestle, 225 ml of Tryptic Soy Broth (TSB solution) was added to each sample, then the sample was mixed for 2 minutes and the pH was equalized to 6.8. The solution was left for 60 minutes at room temperature and the samples were incubated at 37°C for 24 hours (Wattiau et al., 2011).

SALMONELLA BACTERIA ISOLATION AND CONFIRMATIVE BIOCHEMICAL TESTS:

The grinded samples were performed 10 fold serial dilution using 0.1% peptone water and diluted samples were inoculated on selective liquid tetrathionate broth (TTB) and selenite cystine broth (SCB) to isolate Salmonella bacteria, the tubes were incubated at 37°C for 24 hours. After the growth of the bacteria on the liquid media, it was planted on selective solid media, Xylose lysine descoxycholate agar (XLD agar) and Bismuth sulphite agar (HiMedia®, India) and the plates were incubated at a temperature of 37°C for 24 hours. The ideal colonies of bacteria were taken and grown in test tubes such as urea broth and triple sugar iron agar and incubated at a temperature of 37°C for 24 hours, and for the cultured purified the positive colonies were sub-cultured on solid culture media SS agar and XLD agar and the plates were incubated at a temperature of 37 for 24 hours.
The initial isolation of Salmonella bacteria was confirmed by biochemical tests, and these tests were confirmed using the API 20E diagnostic kit, and then the Vitek 2 system (Vandepitte et al., 2003)

SEROLOGICAL DIAGNOSIS OF BACTERIA

The agglutination test was performed on a glass slide using standard antiserum as follows: a clean glass slide is taken and two drops of physiological saline solution are placed then a portion of the colonies is taken by the loop of the sterile metal carrier and added to each of the two drops and mixed well with the solution then one drop of antiserum is added, the standard polyvalent antigen is added to one of the two drops and mixed well for 30 seconds. The second drop is left without adding the antiserum as a control. The occurrence of clumping within one minute is evidence of the positive result of the test (Zaiko et al., 2021)

RESULTS AND DISCUSSION

A total of 79 Salmonella isolates were isolated from 270 samples, in which 37 Salmonella bacteria were isolated from 150 sample of chicken meat and its products, at a rate of 24.6%, where the percentage of isolation of salmonella bacteria in whole chicken meat was 17 positive isolates, at a rate of 34%, while the percentage of isolation in chicken livers was 13 isolates, at a rate of 26%, while the positive result of gizzard samples was 7 bacteria isolates, at a rate of 14%, As shown in (Table 1).
Eleven species of Salmonella bacteria were diagnosed, as they formed S.typhi of (13.51%), and the percentage of other species ranged between (10.81% to 5.4%), the percentage of S. typhimurium, S. anatum, S. gallinarinm and S. living stone was 8.1%, while the percentage of S. enteritidis, S. menston, S. menchen and S. ohio was 10.81%, the percentage of S. blokly and S. thompson was 5.4%, As shown in (Table 2).
From the 60 collected fish samples including (carb fish (cat fish or silurus triostegus), pomfrete fish (pampus argenteus), and Tuna fish (cans)), 26 salmonella bacterial isolates were isolated in rate 43.3%, as shown in (Table 3).

Nine species of Salmonella bacteria were diagnosed, as they formed S.typhimurium and S. ohio of (15.38%), and the percentage of other species ranged between (3.84% to 11.53), the percentage of S. enteritidis, S. menston, S. thompson, S. hadar and S. anatum was 11.53%, while the percentage of S. braendrup and S.dublin was 7.69% and 3.84% respectively, as in (Table 4).

While From animal feed 60 samples were collected from meat and fish protein powder, imported soybean meal, wheat grain, barley, yellow corn, damaged flour, fish feed, poultry feed, pelleted feed and feed additives, 16 Salmonella isolates were isolated, with a rate of 26.6%, As shown in (Table 5).

Eight species of Salmonella bacteria were diagnosed, as they formed S. ohio of (25%) while the other salmonella species of S. Dublin and S. thompson of (18.75%), and the percentage of other species of S. typhimurium, S. enteritidis, S. menston and S. hadar was 6.25%, while the percentage of S. braendrup was 12.5%. as shown in (Table 6).

Table 1: Number of chicken meat and its products sample with number of positive results

Chicken meat and its products	Sample number	Number of positive results	Percentage
Whole chicken meat	50	17	34
Liver	50	13	26
gizzard samples	50	7	14
total	150	37	24.6

Table 2: Types of salmonella diagnosed in chicken meat and its products traded in Baghdad local markets

Number	Types of salmonella isolates	Number of isolates	Isolation ratio for total isolation
1	S. typhi	5	13.51%
2	S. typhimurium	3	8.1%
3	S. enteritidis	4	10.81%
4	S. anatum	3	8.1%
5	S. menston	4	10.81%
6	S. blokly	2	5.4%
7	S. gallinarinm	3	8.1%
8	S. thompson	2	5.4%
9	S. menchen	4	10.81%
10	S. ohio	4	10.81%
11	S. living stone	3	8.1%
Total of salmonella isolates		37	99.95

Table 3: Number of fish sample with number of positive results

Fish sample	Sample number	Number of positive results	Percentage
carb fish (cat fish or silurus triostegus),	20	11	55
pomfrete fish (pampus argenteus)	20	9	45
Tuna fish (cans)	20	6	30
total	60	26	43.3

Table 4: Types of salmonella diagnosed in fish sample traded in Baghdad local markets

Number	Types of salmonella isolates	Number of isolates	Isolation ratio for total isolation
1	S. Dublin	1	3.84%
2	S.typhimurium	4	15.38%
3	S. enteritidis	3	11.53%
4	S . menston	3	11.53%

5	S. braendrup	2	7.69%
6	S. thompson	3	11.53%
7	S. hadar	3	11.53%
8	S. ohio	4	15.38%
9	S. anatum	3	11.53
Total of salmonella isolates		26	99.94%

Table 5: Number of feed sample with number of positive results

Feed type	Sample number	Number of positive results	Percentage
Wheat, barley and yellow corn	12	6	30
Fish and protein powder	8	2	15
Soybean meal	10	1	6
Ready Poultry feed	9	3	20
Pressed feed and fish feed	21	4	11.42
total	60	16	26.6

Table 6: Types of salmonella diagnosed in feed sample traded in Baghdad local markets

Number	Types of salmonella isolates	Number of isolates	Isolation ratio for total isolation
1	S. Dublin	3	18.75%
2	S. typhimurium	1	6.25%
3	S. enteritidis	1	6.25%
4	S. menston	1	6.25%
5	S. braendrup	2	12.5%
6	S. thompson	3	18.75%
7	S. hadar	1	6.25%
8	S. ohio	4	25%
Total of salmonella isolates		16	100

Salmonella colonies showed ideal traits on selective solid plates, they were pink with a black center in XLD agar plates, blackish-brown in BS agar plates, green with a black center in HE agar, and biochemical assays on API 20E were negative for ONPG, urease, esculin and indole, and positive for examination Lysine and H2S production. The carbohydrate assays were glycolysis and positive for Arabinos, Xylose, Rhamnose, Melibiose, Glucose and negative for Malonate, Adonitol, Cellobiose, Saccharose Raffinose. The colonies were positive in the serological tests for the autosomal antigen O and the flagellum antigen H.

Salmonellosis remains a global public health challenge (World Health Organization, 2010). Salmonellosis is a worldwide foodborne infectious disease that often occurs as sporadic cases in families or as outbreaks. Nowadays, poultry meat and its products are among the most infectious foods for humans (Akbar and Anal, 2015 and Ramtahal et al., 2022).

Chicken meat and its products and fish are a good medium for the growth and transmission of Salmonella and causing cases of food poisoning, and that its presence in fresh, chilled and undercooked chicken meat constitutes a threat to public health and a source of food contamination during the stages of food preparation (Ansari-Lari et al., 2022).

A number of studies have shown different percentages of contamination of chicken meat with salmonella. In Iraq, it was observed that the percentage of contamination of chicken meat and its products ranged between 8-17.5% (Taib et al., 2019). In a field study of the Ministry of Health, Nutrition Research Institute (Talebi et al., 2019), the percentage of contamination in chicken meat was 36.4%, and more than one isolate was isolated from the model. The isolate of a new species of Salmonella not previously isolated in a research conducted in Iraq in 2015 (Kamil et al., 2015), it was noted that the contamination rate was 4.5%, and 32 isolates of salmonella bacteria were isolated. Asian countries are among the largest and most productive countries interested in

aquaculture (Cabral, 2010) (Al Bulushi et al 2010). The fish product is less processed and more susceptible to microbiological contamination. It was noted that most of the samples contaminated with salmonella bacteria came from ponds and markets interested in aquaculture. Studies and research were interested in analyzing several types of meat samples, but fish were the most sampled species, as salmonella bacteria were discovered in different types of fish and analytical samples were taken from different anatomical parts of the fish, which indicates that the pathogen adapts to many environments and different animals, and thus pollution occurs and the transmission of diseases from animals to humans (Silla-Santos et al., 2018 and Adesiyun et al., 2020).

The percentage of contamination in sheep feed, local protein, poultry feed, and cow feed was 24.4, 6.6, 13.3, and 15.5%, respectively. In another study, the percentage of contamination was in protein powder was 86%, feather protein powder was 57%, fish meal was 18%, poultry feed was 5%, cow feed was 1%, and grains was 1%. In research in Niger (Sanda Abdelkader et al., 2019), the percentage of positive samples was 12.13%, and 19 isolates formed S. lille, S. newhaw and S. livingstone high percentages and more than one isolate was found in the model, and in another research (Terentjeva et al.,2017) conducted on poultry feed, the contamination rate was 2.91%.

In a study conducted (Pomianowski et al., 2011) on 10 factories for the manufacture of pellet feed, it was noted that the percentage of pollution ranged between 10-45.5%. In corn and 100% in cottonseed meal, fishmeal and soybean 10%. Salmonella was not isolated from meat protein, bones, wheat grain, barley, wheatgrass powder, and minerals. In a survey conducted (Stanaway et al., 2019), it was noted that the percentage of contamination in feed was 0.7%.

CONCLUSION AND RECOMMENDATION

It is clear from the comparison of the results of this research and different researches and studies that there is a difference in the percentage of contamination in the feed materials used in the feed industry and the results of the study conducted in Iraq in previous years. The percentage of salmonella contamination in ready-made feed and primary feed materials that make up the final production of feed, which poses a threat to the health of poultry fields, animal husbandry and public health.

Contamination with pathogenic salmonella bacteria at a high rate poses a threat to public health, so the percentage of pollution can be reduced by controlling the presence of salmonella in the feed, ensuring the safety of hatcheries and fields, and protecting laboratories in slaughterhouses and fields from side pollution.

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