THE EFFECT OF SALMONELLA SPECIES ON CANNED AND FRESH FOOD

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Abstract :

This study aims to investigate the presence of salmonella in different kinds of food by using culturing techniques in order to highlight the importance of sanitization of food before introducing it to consumers. Culturing techniques have been used in identifying the presence of salmonella in raw meat, raw vegetables, raw egg, and canned food like pineapples, condensed milk, and canned peas. The results revealed that the infection of salmonella is severe due to the contamination by getting in touch with unclean surfaces and many other contamination factors. It also revealed that there is a deep need to take many procedures to prevent the contamination by salmonella and cross-contamination of salmonella to food materials, in order to decrease the number of gastroenteritis cases caused by this bacteria and its effect on the human health and economy.

ملخص البحث

تُعد عدوي السالمونيلا أحد أهم الأسباب للإصابة بالتسمم الغذائي، فطبقاً لإحصائيات الولايات المتحدة الأمريكية ، هناك ١٢٨ ألف حالة من بين ٤٨ مليون حالة تحتاج إلى رعاية طبية في المستشفيات و ٣ آلاف حالة وفاة نتيجة للإصابة بالأمراض التي تنقلها الأغذية كل عام. ومن بين ٣١ مسبب للأمراض مسؤول عن ٤.٩ مليون حالة من الأمراض التي تنقلها الأغذية. كانت السالمونيلا مسؤولة عن ٣٩ ٪ من الحالات. تأتي هذه الدراسة لإلقاء الضوء علي أهمية إتخاذ الإحتياطات اللازمة للحد من إنتشار السالمونيلا عن طريق إستزراع عينات من بعض أنواع الأغذية النيئة والمحفوظة. كشفت النتائج عن وجود بكتيريا السالمونيلا بنسبة أكبر في أطباق زرع الأطعمة النيئة أكثر من نسبة السالمونيلا الموجودة في أطباق زرع الأطعمة المحفوظة وكانت المستعمرات المتكونة من إستزراع الأطعمة النيئة أكثر عدداً من المستعمرات الموجودة في أطباق زرع الأطعمة المحفوظة. تكشف هذه النتائج أهمية إتخاذ التدابير اللازمة للحفاظ علي الأطعمة من التلوث بالسالمونيلا.

English summary

Salmonella infections are one of the main causes of food poisoning. According to US statistics, there are 128,000 cases out of 48 million requiring hospital care and 3,000 deaths due to food-borne diseases each year. Among 31 pathogens were responsible for 9 million food-borne diseases. Salmonella was responsible for 39% of cases. This study is intended to highlight the importance of taking the necessary precautions to reduce the spread of Salmonella by culturing samples of some raw and preserved foods. The results revealed that Salmonella was found to be more in raw food petri dishes than in preserved food petri dishes. The colonies of raw food culture were more numerous than the colonies in the dishes of preserved foods. These findings reveal the importance of taking measures to protect food from salmonella contamination.

1. INTRODUCTION

Salmonella spp. which belongs to the family Enterobacteriaceae is facultative anaerobic gram-negative bacilli bacteria. The identification of this kind of bacteria takes place by the means of complex serological techniques, including the interaction between bacterial surface antigens with the Salmonella antibodies; the antigen-antibody interaction causes an agglutination which is the sign of the presence of the bacteria.
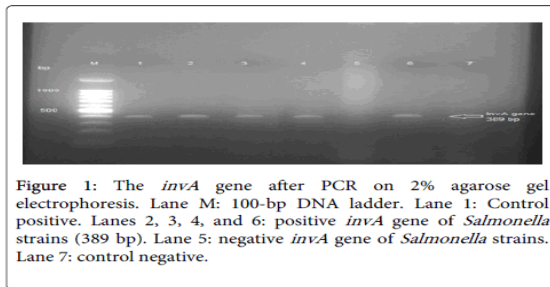
Salmonella spp. have been pointed to as the major cause of foodborne bacterial illnesses in humans, as it is responsible for the highest number of reported clinical foodborne illness cases rather than other pathogenic bacteria, which cause foodborne illnesses.

The interesting in the study of salmonella infections effect on the national level started in 1962, following recognition of the importance of Salmonella organisms as the cause of potentially preventable infectious disease in the United States. The number of reported cases of infection due to Salmonella has been noticed to increase progressively to ~40,000 case per year

The number of reported cases of salmonellosis increased every year, the matter that led to an increase in the interest of studying methods of infection and the procedures to be taken in order to decrease or prevent the infection and raising the awareness towards the correct handling for food.

Determination of the microbial hazards is the target for the validity of the food. It can be determined by knowing the risk and factors that cause the infection. The goal is to determine the pathogenic dose of a certain microorganism that can cause an infection in a given population (Forsythe, S. J., 2008)

For QMRA, there is a need for studying and working with old and new microbiological methods that give reliable quantitative data. For example, PCR techniques are one of the microbiological methods that need to be developed to be able to produce quantitative results.

Figure 1: The *invA* gene after PCR on 2% agarose gel electrophoresis. Lane M: 100-bp DNA ladder. Lane 1: Control positive. Lanes 2, 3, 4, and 6: positive *invA* gene of *Salmonella* strains (389 bp). Lane 5: negative *invA* gene of *Salmonella* strains. Lane 7: control negative.

Salmonellosis is one of the most important foodborne diseases that cause medical and economic encumbrances all over the world (De Jong, B., & Ekdahl, K., 2006). (Voetsch, A. C., Van Gilder, T. J., Angulo, F. J., Farley, M. M., Shallow, S., Marcus, R. ... & Emerging Infections Program FoodNet Working Group, 2004)
The man is infected by salmonellosis mainly by food. *Salmonella* could be found in raw eggs, and raw poultry meat, are the most important sources of human salmonellosis (Berends, B. R., Van Knapen, F., Mossel, D. A. A., Burt, S. A., & Snijders, J. M. A., 1998)

As a result for the infection because of consuming food, scientists have developed many techniques to prevent the contamination of food by salmonella, and determination of the microbial hazards in the slaughters and farms (Malorny, B., Löfström, C., Wagner, M., Krämer, N., & Hoorfar, J., 2008), (E FSA. 2006). Quantitative microbial risk assessment is still unclear because of the lack of quantitative data, and the presence of many assumptions that result in lacking clear information. The availability of quantitative clear data represents a challenge for microbiologists, as the available methods for determining the pathogens are deficient and don't provide a clear data about the methods of determination and ways of preventing the contamination.

Salmonella is a major pathogen that causes foodborne diseases and its presence in the ready-to-eat products including plant-derived food is taking great attention. Because of its high resistance, *salmonella* can survive for a long time in spices and herbs which, if not treated properly before introducing to the consumer it might cause foodborne illness (Zweifel, C., & Stephan, R., 2012)

According to the Canadian surveillance system, each year there are about 1.6 to 2.4 million cases of illness accompanied with known and unknown pathogens. According to the statistics about 4 million cases of foodborne diseases are due to Norovirus, Clostridium prefringens, Campylobacter spp. and non-typhoidal Salmonella spp. which are the leading

pathogens that are responsible for about 90% of foodborne illnesses. Approximately 12.5 % of the population in Canada have suffered at least once of domestically acquired foodborne illness each year in Canada. (Thomas, M. K., Murray, R., Flockhart, L., Pintar, K., Pollari, F., Fazil, A., & Marshall, B., 2013)

In the past, it was shown that the intensity of the infection with salmonella is proportional to the level of contamination (dose-response relationship) (World Health Organization., 2002) Also, the infection with salmonella vary according to the kind of food, the food can catch the contamination at any stage of processing. For example, the most known reason of contamination is the contaminated environment of slaughterhouses for incoming unaffected animals (Boughton, C., Egan, J., Kelly, G., Markey, B., & Leonard, N., 2007).

Afterward, salmonellae can multiply to dangerous levels due to wrong stockpiling conditions. For the most part, Salmonella does not grow at temperatures underneath 6°C for up to 15 days on Chicken meat, while noteworthy growing has been accounted for at 8°C (Pintar, K., Cook, A., Pollari, F., Ravel, A., Lee, S., & Odumeru, J. A., 2007). However, some other reports show that Salmonella may grow at 2 to 7°C (D'Aoust, J. Y., 1991). Furthermore, type of the food plays an important role in the level of infection and its severity in outbreaks.

Fatty foods that carry salmonella may have a privilege during its entrance through the acidic medium of the stomach to the intestine, where bacteria invade the stomach regardless of the damage caused by the acids. Typically, the number of bacteria that are found in food is very low and can't cause infection but when it increases to the pathogenic level it causes the infection (Seo, K. H., Valentin-Bon, I. E., & Brackett, R. E., 2006). Detection and quantization of Salmonella enteritidis in outbreaks caused by homemade ice cream take place by a technique called PCR in which the genetic material of the organism is isolated and proliferated in order to detect its presence in the sample (Boughton, C., Egan, J., Kelly, G., Markey, B., & Leonard, N., 2007), (Fegan, N., Vanderlinde, P., Higgs, G., & Desmarchelier, P., 2004), (Seo, K. H., Valentin-Bon, I. E., & Brackett, R. E., 2006) (Malorny, B., Löfström, C., Wagner, M., Krämer, N., & Hoorfar, J., 2008) .

2.   BACKGROUND LITERATURE

One hundred and fifty-four incidents of food poisoning were due to post-process leakage (PPL). These happened mainly in England (72.7%) and Canada (17.5%) from products exported from South America, Europe, Africa, and Australia.

In the national studies from the different surveillance systems, to estimate the statistics of foodborne illnesses, it was found that, these pathogens are divided into 17 bacterial pathogens, 2 viral pathogens, and 3 protozoan pathogens. Those pathogens are responsible for 2 billion illnesses, more than 1 million deaths, and almost 80 million DALYs in 2010.

By using the available information about the recorded cases, experts found that 30% of the cases that represent 582 million cases that are caused 25 million DALYs were transmitted by contaminated food. Notably, those statistics showed that almost 30% of foodborne illness, deaths from these diseases, happened to children under 5 years old.

Norovirus caused about 125 million cases. While Campylobacter caused about 96 million cases; while non-typhoidal Salmonella enterica is the major pathogen that is responsible for diarrheal and invasive infections. Finally, WHO's African region witnessed the highest recorded cases

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of foodborne diseases (Kirk, M. D., Pires, S. M., Black, R. E., Caipo, M., Crump, J. A., Devleesschauwer, B., & Hall, A. J., 2015)

Around the world, the foodborne pathogen can cause many illness and deaths. Contaminated food and water caused diarrheal diseases that led to the death of 1.4 million people according to the WHO. While in 2012 the number of reported cases due to diarrheal diseases was between 26.000 to 1.565.000 deaths according to the WHO. 125 million cases were due to Norovirus followed by Campylobacter which was the reason of about 96 million deaths.

In Africa, Asia, and South America, 5 million cases of death were reported due to gastroenteritis per year most of them are under the age of five among 1 billion cases every year between children. While in Mexico and Thailand, 50% of the children who are less than 5 years suffer from enteritis that was caused by the Campylobacter.

In Europe, fifty thousand cases / million populations suffered from acute gastroenteritis according to reports. In the Netherlands, the statistics increases as about 30% of the populations suffer from enteritis per year. In Ireland, 3.2 million cases per year. In Australia, 5.4 million gastroenteritis cases are reported each year.

In England, 200.000 cases per million of the population, i.e., 9.4 million people suffered from acute foodborne gastroenteritis each year, and the primary contributing microorganisms are identified as Norovirus, Campylobacter species, rotavirus, and non-typhoidal Salmonella species.

In the USA, 128 thousand cases of 48 million cases needed medical care in the hospitals and 3 thousand deaths as a result of foodborne infections were reported each year. 31 pathogens were responsible for 9.4 million Cases of foodborne diseases. Bacteria were responsible for 39% of the cases; viruses were responsible for 59%, while parasites were responsible for 2% of the cases.

In developing countries, the reported data on the gastrointestinal diseases in Africa are not confirmed, that's why the correct pathogen that causes the disease is not recognized clearly. In a study conducted by Uche, I. V., MacLennan, C. A., & Saul, A, it was found that *Salmonella typhimurium* and *enteritidis* are the major pathogens for about 91% of gastrointestinal diseases reported in Africa. The study confirmed that gastrointestinal diseases are more prevalent between human infected by Immunodeficiency Virus (HIV), infants, and young children who are having malaria, anaemia and malnutrition (Uche, I. V., MacLennan, C. A., & Saul, A., 2017)

58% of cases were caused by norovirus, followed by 11% caused by non-typhoidal Salmonella. It was found that 10% of the cases were caused by Clostridium prefringens and (9%) were caused by Campylobacter spp. 35% of the cases were caused by Salmonella spp. which had obligated the patients to stay in the hospital. Norovirus is taking the second stage as it caused (26%) of the cases, followed by Campylobacter which represents (15%) of the cases, and Toxoplasma gondii which represents (8%) of the cases. Reported cases that led to death were associated with non-typhoidal Salmonella by (28%), Toxoplasma gondii by (24%), Listeria monocytogenes by (19%), and Norovirus by (11%).
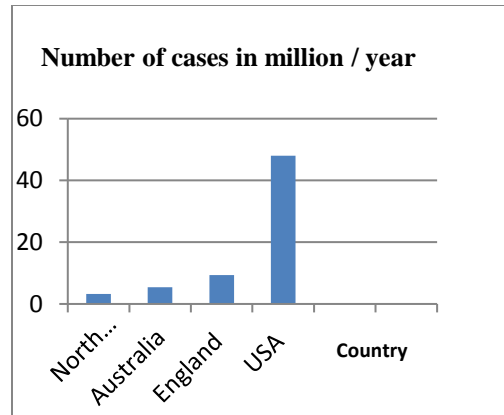
Figure 2: Number of reported gastroenteritis per year in different countries

Meat, milk, or egg products (Ready to eat food- RTE) are the common carriers for many pathogens as those products don't undergo any sterilizing processes before the man consumes it. That's why they may carry a lot of species among them E. coli, Salmonella enterica, Listeria monocytogenes, Campylobacter jejuni, and Yersinia enterocolitica.

Many outbreaks due to foodborne pathogens were due to undercooked Ready to eat meats like salami, hotdogs, and similar products. Dairy products like that were made by unprocessed milk, especially ice cream. Fruits like apple cider, strawberries, and vegetables like sprouts, lettuce, spinach that undergoes minimum stages of processing.

No Doubt that this illness may lead to death or affect the economy due to the cost of the long sick leaves. It was found that the cost of sick leaves in the USA is about 78 billion dollars per year. Besides acute gastroenteritis, there are more complications of foodborne diseases like chronic rheumatoid conditions and some autoimmune diseases, haemolytic uremic syndrome (HUS) which caused by Shigella toxins (Stx). Atherosclerosis happens due to lipid deposition in arteries. Campylobacter infections can also cause Miller Fisher syndrome, reactive arthritis happens due to Salmonella, Shigella, or Campylobacter infections. Foodborne illness could cause autoimmune disease such as allergic encephalitis; and autoimmune polyneuritis. Foodborne infections also vary between countries due to different eating habits of the population. In Japan, high Vibrio parahaemolyticus cases are seen due to the consumption of raw fish.

People from middle/eastern countries and people from middle Europe, sometimes consume improperly cooked food, which lead to the infection with Clostridium botulinum. (Bhunia, A. K., 2018)

The presence of *Salmonella* in foodstuffs attracting great attention on the level of internationally accepted human health concern. There are some evidences that support cross-contamination as a factor of cross -contamination leading to infection with *Salmonella* that causes many foodborne disease outbreaks. Kinds of food that not be treated by kinds of lethal treatments or don't be subjected to proper cooking procedures deserve attention. Food contaminated with *Salmonella* due to poor sterilization practices, poor equipment design, and deficient control of ingredients. Cross-contamination also occurs due to the difficulties of removing the pathogen from the industrial environment which points to the need for improving

the measures of industry preventive control of sanitation. At the consumer level, hygiene education programs should be organized to raise consumer awareness of cross-contamination risks at homes (Carrasco, E., Morales-Rueda, A., & García-Gimeno, R. M., 2012). At the global level, non-typhoidal salmonellosis infected 93.8 million cases and had led to the death of one hundred and fifty five thousands persons each year, among them were 80.3 million cases were due to foodborne diseases (Majowicz, S. E., Musto, J., Scallan, E., Angulo, F. J., Kirk, M., O'Brien, S. J., & International Collaboration on Enteric Disease "Burden of Illness" Studies, 2010).

No specific interventions could be done to raw meat in the first step of production, although the experts showed that the interventions could be done in farms to control salmonella and other foodborne pathogens. Decontamination treatments of livestock can be managed by chemical washes, such as chemicals and organic acids as a preferred chemical intervention to control the contamination of Salmonella. There are two other processes which can be performed as a bio-hazard intervention to control contamination with salmonella which is Scalding and singeing.

Carcass decontamination can be done effectively by washing without water and steam pasteurization that increases the carcass surface temperature to 70°Catleast during washing or steaming. Also washing with organic acid (World Health Organization., 2016)   Defects leading to leakage are due to defective layers and pores during processing; temporary micro leaks during cooling. Staphylococcus aureus (64.9%), Salmonella typhi (3.9%), Other Salmonella spp (5.8%), Clostridium botulinum (2.0%), Clostridium prefringens (2.0%). others and undetermined (21.4%) are the most organisms that contaminate the meat, canned meat, fish and vegetable products.
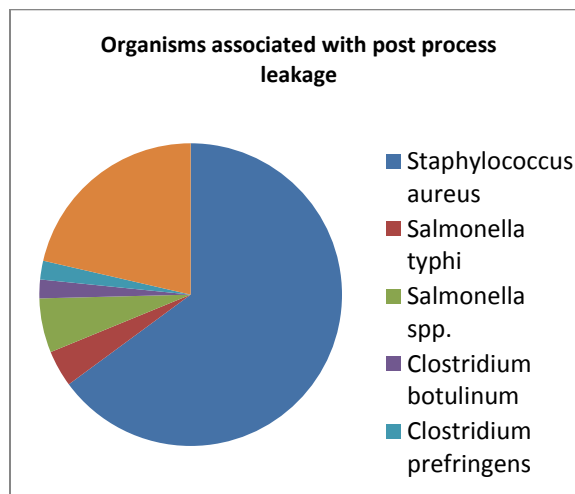

Figure 3: Organisms associated with post-process leakage

In particular, 53 cases happened due to corned beef contaminated with Staphylococcus or Salmonella; 16 cases happened due to pork and ham products contaminated with the same organisms. It was found that the average amount of Salmonella-contaminated meat that may cause illness was 105g. While the amount of Salmonella-contaminated fish that may cause infection, was between 40 and 320 g.

Food can be defined as any substance that man can consume to provide food supplement for the human. Spoilage of the food happens when it reached the point at which the man can't consume

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it anymore or its quality decreases (Brock, T. D., Madigan, M. T., Martinko, J. M., & Parker, J., 1991), (Frazier, W.C. , and Westhoff, D.C.,1994).

 That means that the original nutritional value, physical and chemical properties of the food was changed so the food is not edible anymore. Spoilage of the food may occur due to the following reasons:

(1) Physical changes: happens due to freezing, pressure and chemical reactions, burning, drying, which are not catalysed by tissue enzymes or those happens due to microorganisms or due to biological factors (Springer, 2002).

(2) Spoilage of food may occur due to decay by putrefaction, fermentation, and rancidity. There are many methods to preserve food such as freezing, pickling, canning, treatment and drying (Frazier and Westhoff, 1994).

(3) And other preservation methods like using high pressure, radiation and ionizing, also the use of chemical preservatives and removal of microorganisms.

Figure 4: Salmonella under the microscope

Controlling the growth of certain bacteria is a method that is used to stop decaying caused by microorganisms. Canning and bottling help sustain food taste and flavor (Desrosier, N.W., 2004)

Canned foods are sterilized before being sold but unsuccessful sterilization may cause contamination and food spoilage (Desrosier, 2004). Swollen can often contain gas produced by Clostridium. Lactobacilli which break down the carbohydrates are responsible for acid spoilage fruits and other. Vegetables, fruits, condensed milk, meat and other kinds of food can be preserved by canning

According to the agriculture department in the USA, water-bath canning and pressure canning are the only two accepted methods according to its standards for canning. Canning helps to preserve the food ready to eat for long after the processing time. (Springer, 2002)

To improve food safety for the consumers of canned food, governments have made some standards that should be available on the cans like alphanumeric codes to indicate health information, such as manufacturing date to help the store knows the suitable period for putting the product for sale. And also help the buyer to know expire date. On the other hand, foods that get offensive odour or bad flavor or different appearance should not be used, as it will be affected by any of the organisms that may cause illnesses like Clostridium botulinum, Salmonella, Listeria, etc. (Dainty, 2004)

In some developing countries, the products are kept on the shelves for a long time as the supermarkets leave them often beyond the expiration date. Some of the sellers don't follow the instructions of storage and leave the canned food on the shelves exposed to the sun during the sale which leads to the decomposition of the products due to environmental conditions that can enhance bacterial growth. (Ogbulie, T. E., Uzomah, A., & Agbugba, M. N., 2014)

High nutritive value of meat is due to containing macro and micronutrients that consider the meat one of the most widely used animal food, as it is an important part of the balanced diet for most of most people. Meat is also considered as a rich media for different micro-organisms to grow. Salmonellosis is considered a major cause of bacterial gastroenteritis for man.

Worldwide, salmonella is the main infectious bacteria that cause enteric infectious diseases due to consuming foods. *Salmonella (S.)* is one of the major bacterial pathogen caused by meat and meat product which led to an increase in the number of salmonellosis cases in the world during the last 2 decades.

Insufficient cooking for chicken, chicken products, egg, and other animal products are the main cause of 50 % of Salmonellosis. Improperly cooked meats like pork and beef are responsible for about 13% of salmonellosis cases (Centers for Disease Control and Prevention (CDC. 1999)

Healthy animals are sterile but in some cases, some bacteria may exist in muscle tissue, the unusual source of contamination. The external factors are the most common causes of contamination, as the unclean slaughtering facilities and wrong handling of the meat contribute to contamination. Meat may be contaminated with feces, paunch content, and from the hide (Lahr, J. A., 1996)

Another contamination factors may be due to getting in contact with unclean slaughtering tools, equipment, contaminated to carcass contact and human contact. (Huffman, R. D., 2002)

Each time the carcass is cut, the percentage of contamination increases, as there are fresh new surfaces are exposed to contamination, immediately after slaughtering, major types of bacteria that are found on carcasses are animal strains; as soon as the final cuts reach the retail consumer level, the human strains become prevalent. (Satin, M., 2002)

It was found that the major group of food that can cause salmonellosis to human, are Eggs, chicken, and meat products. In America and Europe, *Salmonella enteritidis* has become the major strain that can be colonized in the ovaries of the hens leading to the infection of its flesh and its eggs causing outbreaks.

In Nepal, buffalo cause salmonellosis by about 64% of the used meat, while goat resembles 20%, pork resembles 7%, and chicken resembles 6% (Joshi, D. D., Maharjan, M., Johansen, M. V., Willingham, A. L., & Sharma, M., 2003) The warm environment, unsterile conditions, unclean storage places, poor practices for food safety, and lack of strategy for preventing diseases have all became number of causes of diseases caused by meat.

Due to the absence of sufficiently well-organized slaughters, un-cleaning meat shops and shortage of clean water, meat becomes exposed for contamination, and this may lead to epidemics from time to time (Maharjan, M., Joshi, V., Joshi, D. D., & Manandhar, P., 2006)

3. Characterization of Salmonella species by different methods:

- Culture-based identification

Salmonella species are gram-negative rod bacteria that when cultured on Salmonella-Shigella agar "a suitable media for culturing salmonella" black pigmented non-lactose fermenting colonies are formed.

Figure 5: Salmonella on Salmonella-Shigella agar

- Gram Stain based identification *Salmonella* species are seen under the microscope as rod shaped bacteria. And when gram stain is applied to the bacteria it refuses the stain which indicates that it is gram positive bacteria.

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Figure 6: Gram negative rod shaped *Salmonella* *typhi*

4. Methods

Collecting the samples and isolation of Salmonella

*Collecting the samples: 120 samples were purchased from 20 markets in Mosul city, Iraq in the period between September and November of 2018. The samples divided into fresh samples, and canned food. The fresh samples were raw meat (n=20), raw egg (n=20), freshly chopped vegetables (n=20), while the canned food was canned pineapples (n=20), canned peas (n=20), and condensed milk (n=20).

*Isolation of salmonella took place by grinding 25gm of the components of each sample with 225 ml buffered peptone water (BPW) and filtrated with a specific filtration bag, and incubated at 37ºC and put on a rotator which rotates by 100 rpm.

After 24 hours, the precipitant was cultured on salmonella Shigella agar and incubated for 48 hours at 37˚C. The reason for using this media is to prevent growing of other undesired microbes and encourage the wanted microbes to grow and form the characteristic colonies.

Each sample was marked and arranged in a specific order. Each sample was cultured on one petri dish, the number written on the dish is the same written on the sample. The culturing takes place according to the standard methods of culturing.

After 48 hours, the Petri dishes were removed from the incubator and examined for the presence of salmonella colonies.

Formation of black pigmented non-lactose fermenting colonies is an indication for the presence of Salmonella in the sample.

5. <u>Results</u>

The results were as follow, from the 20 dishes of the raw meat, there were 10 plates on which the unique colonies of Salmonella were formed with 50% positivity. Number of colonies on the positive petri dishes was between 20 and 25 colonies per dish,

While from the petri dishes of raw eggs, 8 Petri dishes formed the black colonies with 40 % positivity for the presence of salmonella. The number of colonies of positive petri dishes of raw egg was between 15 and 18 colonies per dish.

In the last place, come the petri dishes of chopped vegetables by 0.8% formation of black colonies as the colonies appeared only on 2 petri dishes. The number of colonies on the petri dishes of raw vegetables showed the lowest number of colonies as the number of colonies found on that petri dishes was between 5 to 8 colonies.

Canned food showed less positivity for the presence of Salmonella as from the 20 cans of pineapple, 10 percent only formed the black colonies of salmonella on Salmonella-Shigella agar. The number of colonies was between 10 to 12 colonies. While canned peas showed only 15 % positivity for Salmonella as 3 petri dishes had the black colonies of salmonella with number of colonies ranged from 7 to 10 colonies per petri dish. 1 Sample of the condensed milk gave black colonies on the petri dishes with positivity 5% percent for the presence of salmonella, the number of colonies was between 4 to 6 colonies.

6. <u>Discussion</u>

Raw samples show high level of positivity for salmonella as those samples were not subjected to any factors that prevent the growth of salmonella spp. Un-clean slaughters and equipments are the basic factors of contamination of raw meat, while the reasons of contamination of raw egg were the unclean farms and wrong handling of the egg.

Canned samples show a low level of positivity for salmonella as those samples were subjected to many sterilizing factors that prevent the growth of salmonella and other bacterial species. These factors such as high PH, high temperature, and pasteurization decrease the chances of infection. Usually, the contamination happens due to post-process leakage and bad storage conditions.

Main causes of infection by salmonella in the previous fresh samples were due to getting in touch with contaminated surfaces, unclean handling of the samples. While the main causes of infection in the canned samples were due getting in touch with unclean surfaces during processing, post-processing infection, and leakage during the storage due to some cracks in the can.
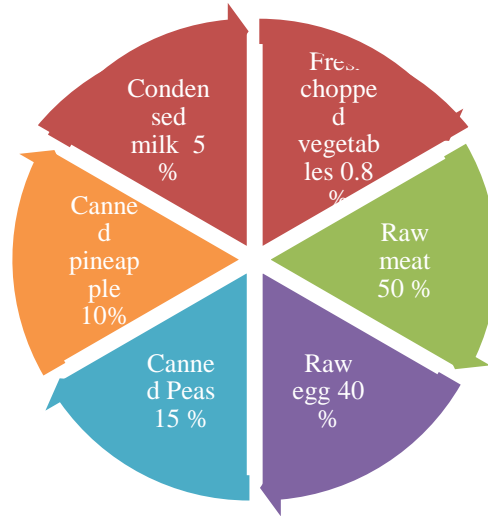
Figure 7: Percent of Positive samples.

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