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MICROBIOLOGICAL IDENTIFICATION OF THE TYPES OF BACTERIA FROM WHITE AND BROWN TABLE EGGS CONTENTS WITH BLOOD CLUMPS

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Abstract:
Feeling disgust in the morning from a clump of blood or tissue inside the contents of brown table eggs is also related to an increase in the growth of pathogenic bacteria, albeit slight, compared to white table eggs, where we focused in this study on taking one hundred samples collected from the white and brown table egg contents were taken from Hilla poultry farm and Basmaya poultry fields in Iraq. Evaluation of the extent to which blood and tissue clumps inside brown table eggs encourage the growth of types of bacteria through biological monitoring. In white table egg content, *E.coli* and Gram's positive bacteria were 34 isolates (34%), while *Salmonella spp.* Were 11 isolates (11%) and no growth in 4 samples (4%). *E.coli* and Gram's positive bacteria were in 36 isolates (36%), and *Salmonella spp.* Were 6 isolates (6%), and no growth in 9 samples (9%), In the brown table egg content with blood or tissue clumps. The decimal dilution was applied to measure the concentration of bacterial growth per 1 ml of egg contents, Total count range of bacteria in white egg contents was between 9.2 to 27 $x10^6$ log CFU/ ml. In comparison, the total count range of bacteria in brown egg contents was (10- 39 $x10^6$ log CFU/ ml).

Keywords: *E. coli* and G+ bacteria, *Salmonella spp.*, table egg, blood, and tissue clumps.

1. INTRODUCTION

Egg quality has received more attention due to rising customer demands for safety and high-quality eggs. Internal egg quality includes functional, cosmetic, and nutritional aspects. Egg yolk and albumen microbiological characteristics the egg has internal inclusions (blood and meat mass). That was recognized in 1899 for quality flaws (Stadelman, et al,.2017). Aside from being an aesthetic and ethical issue, there are indications that blood or tissue clots inside the egg may increase the risk of infection such as *salmonella* (YÜCEER,2019).

Blood spots are droplets of blood found usually on the surface of the yolk (Boateng, et al,.2019). Meat spots appear as red, brown, or white spots in the albumen. They are either pieces of tissue from reproductive organs or blood spots that have changed color due to dilution. The factors causing inclusions are unknown. They emerge during the ovulation process in the ovary or later in the oviduct. Blood on the yolk originates from bleeding of the small vessels in the ovary or in the oviduct (Kamanli, et al,.2018). When blood is found adhering to the yolk, bleeding has occurred in the ovary at the time when the yolk was released from the follicle. The follicle has a dense network of blood vessels, aside from an avascular area of the follicular wall, called the stigma. The follicle sac ruptures at the stigma during ovulation. If any blood vessels cross the stigma, a small drop of blood may be deposited on the yolk as it is released from the follicle. Alternatively, bleeding may occur before ovulation on the vitelline membrane, the structure directly adjacent to the outer surface of the yolk. In that case, blood spots are found in the space between the follicular wall and the vitelline membrane (Stadelman, et al,.2017). Blood in the albumen indicates bleeding shortly after the release of the yolk into the oviduct, at the time when the yolk is coated with albumen. Meat spots in the albumen can be formed from a bit of reproductive tissue while the egg is passing through the oviduct. As an egg age, the yolk takes up water from the albumen, which in turn dilutes blood spots and makes them look like meat spots.

In general, the frequency of blood and meat inclusions is less than 1% in all eggs laid in present commercial lines (Li, et al,.2018). However, the incidence of spots varies greatly: is about 18% in brown layers, whereas it is only 0.5% in white egg layers. In some brown layer lines, the frequency can be as high as 30%. The incidence of spots seems to increase when the hen ages (Smythe,2019). Increased frequency also appears at the start of laying. The main isolated food-borne pathogens from table eggs and their contents are Escherichia coli, Salmonella spp., and Staphylococcus aureus (Adesiyun et al., 2005, Gole et al., 2013).

Escherichia coli is a gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms, considered an important pathogen of human diarrheal disease isolated from table eggs. Although most strains of *E. coli* inhabit the normal gut flora of humans and animals (Brooks et al., 1995), *E. coli* had been isolated

from table eggs' shells and their contents (Hope et al., 2002, Adesiyun et al., 2005). *Salmonella species* are considered the most important cause of food-related illness as they lead to more deaths than any other food-borne pathogen. Salmonella can cause illness on the consumption of raw or undercooked eggs (FDA, 2010, USDA, 2011, CDC, 2017).

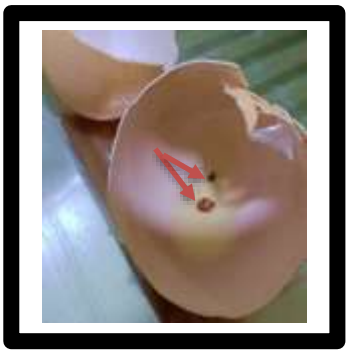
Staph. aureus contaminates different kinds of food. On the other hand, coagulase-positive *Staph. aureus* is considered the most important species of *Staphylococcus spp.* as it evokes a pathogenic effect and produces enterotoxins which cause food toxication (Abeer, 1997). Staph. aureus is transmitted via people-to-food through improper handling and they are mainly found in restaurants or picnics as food is not properly refrigerated or stays out of the refrigerator too long (Songer and Post, 2005) and (Cha et al., 2006) The staphylococcal enterotoxins (SEs) are the products of Staph. aureus and are recognized as the causative agents of classical food poisoning in humans following the consumption of contaminated food (Ikeda et al., 2005). Staph. aureus enterotoxins are of several types; A–E, G, H, I, and R–T, which are commonly produced either singly or combined by most strains of *Staph. aureus* (Argudín et al., 2010). Eggs are involved in outbreaks of Staphylococcal enterotoxication (Yang et al., 2001, Shareef et al., 2009).

Salmonella is a genus of rod-shaped (bacillus) Gram-negative bacteria of the family Enterobacteriaceae. The two species of *Salmonella* are *Salmonella* enterica and Salmonella bongori. *S. enterica* is the type species and is further divided into six subspecies that include over 2,600 serotypes (Gal-Mor, *et al*,.2014). Salmonella species are non-spore-forming, predominantly motile enterobacteria with cell diameters between about 0.7 and 1.5 μm, lengths from 2 to 5 μm, and peritrichous flagella (all around the cell body) (Fàbrega, *et al*,.2013)

2. MATERIALS AND METHODS

2.1. Egg specimen

One hundred specimens (50 white and 50 brown) eggs
Take from table egg content (yolk and albumen),
The brown eggs that contain blood and tissue clump,
Through the period from October to December (2021).
The egg was collected from Fields project
Al_ Hilla and fields project Basmaia**.**



Tissue clump in brown egg
Sabaa H.H 2021

2.2. Preparation of egg Specimen:

At the laboratory, after sterilization of the smaller diameter end of the eggs with 70% alcohol, each egg was broken. eggshells were discarded, and the albumen and the yolk were separated and individually placed in a sterilized container. Samples were individually homogenized and 25mL of yolk and 25mL of albumen were placed in an Erlenmeyer flask containing 225mL of 1% peptone solution (Moraes DMCI Duarte SCII Bastos TSAI Rezende CLGI Leandro NSMI Café MBI Stringhini JHI Andrade MAI, 2016).

2.3. Culturing of bacteria:

Samples in 1% peptone water were incubated at 37°C for 18-20h. After this period, samples were homogenized, and 1mL was transferred to 9mL of brain heart infusion broth followed by incubation at 37°C for 24h. After that, by swab was cultured on XLD agar. The plate was incubated aerobically at 37°C for 24h. The visual examination for growth detection pigmentation and colonial morphology. 3ml of brain heart broth inoculated by swab containing bacteria from XLD agar plate and incubated at 37°C for 24h. The following step is to take 800 micro-letter of broth and 200 micro-letters of glycerol for the preservation of bacteria.

2.4. Total bacterial count determination:

This is the simplest technique for obtaining manageable concentrations of the desired organism and it is complemented by nutritious medium petri dish streaking and spreading (Sanders., E.R. 2012). 1 mL aliquot of the stock solution (solution0) is added to tube 1 which contains 9 mL of 0.45% saline; the product of this mixture is solution1. Repeat by aliquoting 1 mL of the newly created solution1 and adding it to tube 2. Aliquoting and resuspension continue in this fashion until the final tube is reached, diluting the stock concentration by a factor of 10 each with each step (Sanders., E.R. 2012).

2.5. Identification of Salmonella spp. using conventional PCR:

PCR is one of the most widely used molecular tools for the rapid detection of several pathogens, consequently, it was necessary to carry out molecular identification of bacterial isolates, in this regard, molecular identification was carried out: extracted DNA of all isolates with purified using genome DNA purification kit. The present manuscript reports the development of a multiplex qPCR TaqMan assay that allows rapid and accurate detection of Salmonella cells in produce and eggs. The performance of the qPCR assay was comparable to that of the traditional BAM and USDA Salmonella culture methods. The analyses were done in approximately 1 day, in contrast to at least 4 days for traditional microbiological culture methods. It is noteworthy that agreement between the qPCR and the two microbial culture methods was 100% for all artificially inoculated samples

3. STATISTICAL ANALYSIS

The data values were presented as frequency and percentage. Differences in percentage values were analyzed by chi-square test (X2) with the IBM SPSS Statistics 25 software (International Business Machines Corp., Armonk, NY, USA). Values with a $P < 0.05$ were considered to indicate statistical significance

4. RESULTS AND DISCUSSION

In brown egg yolk *E.coli* were in 19 isolates, Salmonella spp. Were 3 isolates, and no growth in 3 isolates. In brown egg albumen *E.coli* were 17 isolates, Salmonella spp. Were 3 isolates, and no growth was in 6 isolates. In white eggs the results, in white egg yolk *E.coli* were 16 isolates, Salmonella spp. were 6 isolates, no growth was 2 isolates, in white egg albumen, *E.coli* was 18 isolates, Salmonella was 5 isolates, and no growth was 2 isolates.

Table (4-1) Show Isolation rates of Salmonella spp. and other enteric bacteria from yolk& albumin table egg content:

Brown egg No:50 (with blood or tissue clumps)		**White egg No:50**	
Yolk no.:25	**Albumen no.:25**	**Yolk no.:25**	**Albumen no.:25**
E.coli & G+ no.:19	E.coli & G+ no.:17	E.coli & G+ no.:16	E.coli & G+ no.:18
Salmonella spp. no.:3	Salmonella spp. no.:3	Salmonella spp. no.:6	Salmonella spp. no.:5
No growth no.:3	No growth no.:6	No growth no.:2	No growth no.:2

Table (4-1) Show Isolation rates of Salmonella spp. and other enteric bacteria from yolk& albumin table egg content: were in brown egg yolk E.coli were 19 isolates, *Salmonella spp.* were 3 isolates, no growth 3 isolate. In brown egg albumen E.coli were 17 isolates, *Salmonella spp.* were 3 isolates, and No growth were 6 isolates. In white eggs the results that, in white egg yolk E.coli were 16 isolates, Salmonella spp. were 6 isolate, no growth were 2 isolates, in white egg albumen, E.coli were 18 isolate, Salmonella were 5 isolate, no growth were 2 isolate, alpha hemolytic Gram positive bacteria, and gamma hemolytic Gram-positive bacteria.

Table (4-2): Show Isolation rates of Salmonella spp. and other enteric bacteria from table egg content:

Bacteria types	**Eggs types**		**Total**
	White	**Brown**	
E. coli	34 (34%)	36 (36(%	70%
Salmonella spp.	11 (11%)	6 (6%)	17%
Gram+ve bacteria	34(34(%	36(36(%	70%
No growth	4 (4%)	9 (9%)	13%

P-value = 0.151

Table displays the estimated percent number of bacteria per egg types under normal condition of preservation. There is a slight increase in the percentage of coliform bacteria in brown eggs with tissue and blood clumps relative to white eggs, but as a total growth of these bacteria, we find that there is a percentage of 70% of them and this is a large percentage and this is found in Prolonging of the storage time of table eggs is expected to result in an increase in the number of illnesses per million servings for uncooked and lightly cooked egg meals but not when eggs are well-cooked. The researcher agrees with this study the greater contamination ($p < 0.05$) of brown eggs in relation to white eggs may be related to genetic heritage (Aarestrup FM, et.al, 2007). While other researchers disagree and found that brown layer strains seem more susceptible to Salmonella infection than white strains, as mentioned by Dunn et al. (2005). Another explanation may be the delay in to start of the classification process of brown eggs, favoring Salmonella multiplication in the eggshell and its penetration into the egg.

Among the common contaminant organisms pathogenic to human beings are Salmonella spp, Staphylococcus spp, Gram-positive Bacillus, and Escherichia coli (Osei-Somuah,2003). (Etches, R.J. 1992), reported that, as eggs stay longer, their resistance is reduced enabling these organisms to penetrate into the egg content. *Escherichia coli* is known to contaminate the surface of eggs while the mechanical process can spread the and bacteria through eggs and meat. Contamination with the pathogen while in the field, occurs through improperly decomposed manure, contaminated water, and poor hygienic practices of the farm workers. *E. coli* causes mastitis, urinary tract infection, meningitis, pneumonia, and peritonitis (Johnson,2006). Researcher found that the major contaminants of eggs were Gram-negative bacteria species as (Methaq G, Noor A, Moutaz A.,2020),(Arabi S, Jafarpour M, Mirinargesi M.,2013) and (Islam S, khondkar P, Islam A.,2010)

E. coli, and . all these agree with this study were found about (17 isolates) 24% from isolates is *E. coli* as Gram-negative bacteria.

Several factors have been implicated in egg contamination, Among these are feces of the birds, litter material, egg crates, packing, and storage. Others are the clothes and hands of poultry workers, dust, the environment, weather conditions, transport, and marketing(I. N. Abdullah, 2010). Gram-negative bacteria are best equipped to overcome the antimicrobial defenses of the egg content. Much of the research on eggshell and egg content contamination focuses on Salmonella, since infection with Salmonella enteritidis, resulting from the consumption of contaminated eggs or egg products, is still a major health problem. Observed Salmonella prevalence on the eggshell and in the egg, the content varies, depending on the fact whether investigations were based on randomly sampled table eggs or on eggs from naturally infected hens(De Reu,2008). Enteric infection with Salmonella spp is an important cause of diarrheal disease worldwide, but the frequency of the infection shows variations between studies. So, the results of this study are more or less similar to the findings of the previous workers who also conducted research investigation on Salmonella from table eggs. For gram-positive bacteria (M.Rad, Z.Esmailnejad, Gh.Keleidari, 2003) show that streptococcus species were isolated from 21 flocks (100% flocks). Staphylococcus species were recovered from 15 flocks (71.41%). Bacillus cereus was isolated from 6 flocks (28.57%), and Clostridium perfringens from 2 flocks (9.52%). Pure culture of streptococcus spp. from 4 flocks (19/04%) isolated. The prevalence of mixed infections were: staphylococcus and streptococcus spp. from 10 flocks (47/61%); staphylococcus, streptococcus, and clostridium spp. from one flock (4/76%); staphylococcus, streptococcus, bacillus and clostridium spp. from one flock (4/76%); staphylococcus, streptococcus and bacillus spp. from 3 flocks (14/28%); streptococcus and bacillus spp. from 2 flocks (9/52%). Isolated streptococcus species included: avium, durans, faecium, faecalis, porcinus and isolated staphylococcus species included: aureus, hycus, gallinarus and epidermidis.

4.1: Identification of the bacteria:

The identification of the bacteria was ensured according to the (standard operating procedures 2007) by cultural characteristics:

The colonies appeared small, smooth, rounded & pale colonies on Nutrient agar, while in Xylose-Lysine Deoxycholate agar *E.coli* appear yellow colonies, *Salmonella spp.* appeared as red small, smooth, rounded colonies with or without a black center., On blood agar, Gram-positive bacteria show two types of hemolysis (alpha hemolysis and gamma hemolysis).

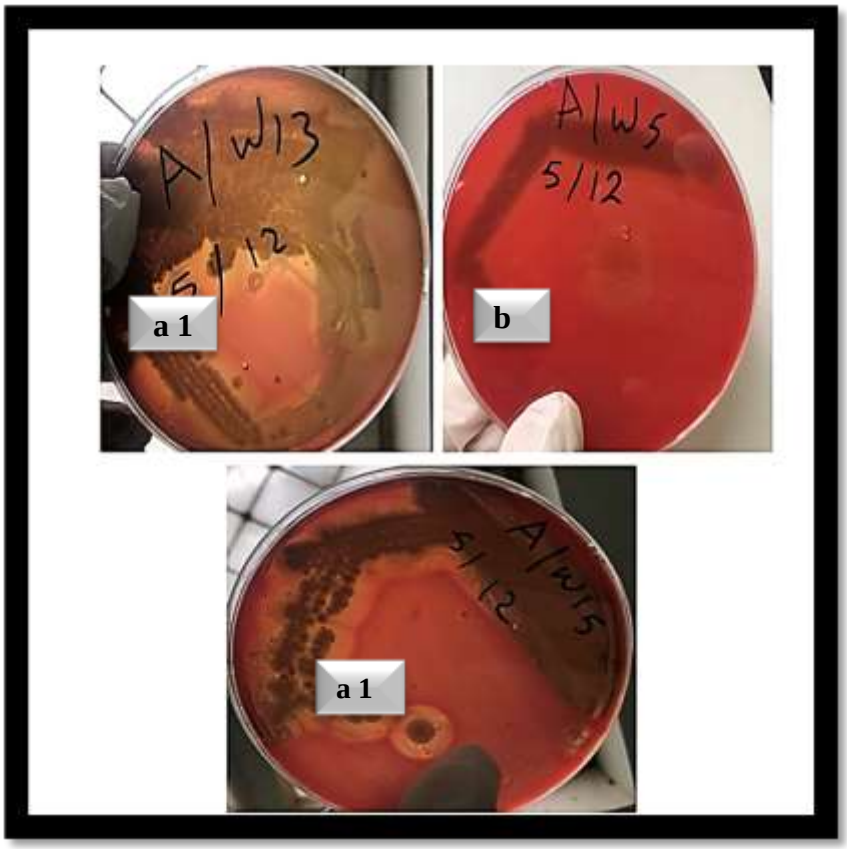
Table (4-3): Morphology, cultural characteristics, and staining characteristics of isolated *E. coli*:

Media used	Colony characteristic	Morphology (staining characteristic)
Nutrient agar	Circular, smooth, colourless colonies	Gram-negative, pink colour, small rod shaped appearance, arranged in single or paired short
Xylose-Lysine Deoxycholate agar	yellow colonies	

Pic. (no.1): show *E.coli* yellow coloniesin XLD agar acidify the medium turning it yellow

Table(4-4): Morphology, cultural and staining characteristics of isolated Gram positive bacteria:

Media used	**Haemolysis**	**Morphology (staining characteristic)**
Blood agar	Alpha haemolysis	Gram's positive, green colored, cocci shaped Non-hemolytic
	Gama haemolysis	



Pic (no.2): Show Gram-positive bacteria with two types of hemolysis (a 1,2 alpha, and b gamma) in blood agar

Table (4-5): Morphology, cultural and staining characteristics of isolated Salmonella spp.

Media used	Colony characteristic	Morphology (staining characteristic)
Nutrient agar	Translucent, opaque, smooth colonies	Gram negative short rod shaped singly arrange
Xylose-Lysine Deoxycholate agar	Red color, small , smooth , rounded some with black center.	

Pic. (no.3): show Salmonella spp. Red colonies In XLD agar with red medium

The study by (MM Islam, 2014) showed that the Identification of E. coli was confirmed by colony characteristics in different bacteriological media, on Salmonella-Shigella agar the colony appeared Slight pink and smooth colony. Welton Taylor created the agar in 1965. Due to the indicator phenol red, which has a pH of about 7.4, it appears brightly pink or red. When sugar is fermented, the pH is lowered, and the phenol red indicator notices this by turning yellow. The majority of gut bacteria, including Salmonella, can break down the sugar xylose to produce acid. Salmonellae also break down thiosulfate to produce hydrogen sulfide, which causes the formation of colonies with black centers and makes it possible to distinguish them from colonies with similar colors like Shigella.

Other Enterobacteria, like E. coli, will ferment the lactose in the medium to the point where decarboxylation prevents pH reversion and acidification turns the media yellow. Gram-negative, pink color, small rod-shaped appearance, arranged in single or paired shorts. on Nutrient agar Translucent, opaque, smooth colonies, on Nutrient broth Turbidity in the broth, the Morphology (staining Characters) Gram-negative short rod-shaped singly arranged. (M. A. Rahman et al., 2018) Salmonella spp. were isolated and identified based on culture, staining, and biochemical characteristics. Salmonella spp. , produced smooth white to the grayish white colony on nutrient agar with peculiar fetid odor, pink colonies on EMB agar, and black centered colonies on SS agar and XLD agar. On Gram staining Salmonella spp., were found Gram-negative, small rod and arranged as single or paired. Among five basic sugars only dextrose, maltose and mannitol were fermented with the production of acid and gas but lactose and sucrose were not fermented by most of the isolates.

In addition to various biochemical tests and the API-20E) Enterobacteriaceae Identification Kit, the morphological and cultural characteristics of the isolates on some selective mediums, such as Brilliant green, XLD, and SS agar, were used in the diagnostic investigation. At the Central Public Health Laboratories, isolates were serotyped (National Center of Salmonellae in Baghdad, Iraq). (Arcan A. and Afaf A. 2013, Sabaa H. H. 2009).

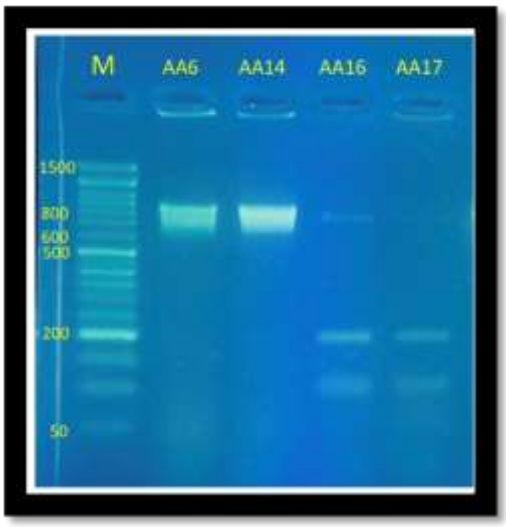
In blood agar gram-positive bacteria show alpha and gama hemolysis, Alpha-hemolysis (α-hemolysis) is a partial or "green" hemolysis associated with the reduction of red cell hemoglobin. Alpha hemolysis is caused by hydrogen peroxide produced by the bacterium, oxidizing hemoglobin to green methemoglobin. It exhibits incomplete hemolysis with 1-2 mm wide. The persistence of some un haemolysed RBC's can be seen microscopically. Gamma-hemolytic (Non-haemolytic) Colonies show neither typical alpha nor beta hemolysis. There may be, however, slight discoloration in the medium (Sagar Aryal, 2018).

The total range of maximum growth of bacteria in white egg contents was: (9.2- 27 x10^6 log CFU/ ml), while the total range of maximum growth of bacteria in brown egg contents was 39 to 10 x10^6 log CFU/ ml) and the minimum concentration of bacteria in white and brown egg contents was: (3- 4 x10^6 log CFU/ ml). there was slight differentiation in the concentration of bacteria according to egg type.

The researcher found that These differences in microbial growth fitness depending on the egg fraction studied have already been demonstrated by numerous authors, such as Kang et al., who reported that Salmonella growth in egg white was slower than that in liquid egg yolk and liquid whole egg Kang et al.,(2011) and kim Y.-j., et al., who demonstrated that there is a difference in Salmonella growth fitness in unpasteurized liquid eggs depending on the type of liquid egg products (liquid whole egg, egg yolk, or egg white) and storage temperature(kim Y.-j., et al., 2018). Salmonella growth fitness is much greater in egg yolk than in egg white because the latter has a high viscosity, an alkaline pH, and a number of antimicrobial components, including lysozyme, ovotransferrin, and several vitamin chelating proteins (Baron F., Gautier M., Brule G. 1997) Since the liquid whole egg is a mix of both fractions, one would expect that Salmonella cells would display intermediate growth fitness.

4.2. Identification of Salmonella spp. using conventional PCR:

Due to the rapidly increasing nature of Salmonella infections, there is a need for the development of fast and suitable techniques for the immediate detection of these infections in order to appropriate control measures. One of the limitations of phenotypic methods for bacterial identification is the inability to identify the bacterium on a species level in cases (Raman et al., 2017). Most biochemical profiles didn't lead to accurate bacterial identification in most cases, and reproducibility of the result is not guaranteed, it depends mainly on the metabolic fingerprint of the isolates that in turn varies based on the physiological status of the isolate at the time of carrying out the assay (Zhang et al., 2011). The present manuscript reports the development of a multiplex qPCR TaqMan assay that allows rapid and accurate detection of Salmonella cells in produce and eggs. The performance of the qPCR assay was comparable to that of the traditional BAM and USDA Salmonella culture methods.



Pic (no.4): PCR products of the amplification of ITR region of Salmonella spp. The size of the PCR product is 855 bp. The gel was 1.5% and the DNA dye is RedSafe (Intron, Korea). V: 90, Time: 45 minutes. M: DNA ladder.

Our study is similar to (Di´essy Kipper, *et.al,* 2019) Showed that All the 63 isolates were submitted to the invA real-time PCR and had positive results for the genus Salmonella.

DNA sequencing is an increasingly affordable tool for Salmonella analysis. Traditional sequencing (with Sanger technology) is still the most used DNA sequencing procedure in veterinary laboratories. Molecular methods are also becoming user-friendly and less labor intensive. Therefore, the analysis of 2 ISRs is a fast and practical way of evaluating Salmonella isolates. It is easier than performing MLST analysis (with the sequencing of 6 to 7 genes) or in silico analysis after WGS (Achtman et al., 2012; Feasey et al., 2016). This analysis could also evaluate the difference among lineages that circulate in the field. The whole methodology is suitable to be used as a diagnostic tool for monitoring Salmonella and the main concerning serotypes in a routine poultry laboratory.

The region of interest spans from the end of a 23S ribosomal gene across a 5S gene and includes the last base pair preceding a tRNA aspU ribosomal gene neighboring dkgB (previously yafB). DNA sequencing analysis of this region made it possible to discriminate Salmonella serotypes are mainly isolated from poultry samples (Guard et al., 2012; Pulido-Land´ınez et al., 2013, 2014).

So, let us conclude by White and brown egg contents are predominantly contaminated with coliform, Gram's positive bacteria, and Salmonella, and rout of microbiological detection, as shown in laboratory methods, and the multiplex qPCR. also, recommend the Selection of poultry breeds with egg production without blood or tissue spots by the laying hens' owners.

THANK everyone for bringing their expertise and experience in the research and constructive interaction to make it a success.

REFERENCES:

1. Stadelman, W. J., Newkirk, D., & Newby, L. (2017). Egg science and technology. CRC Press.
2. YÜCEER, M. (2019). Effect of Ultrasound Processing on the Physico-Functional Characteristics of Liquid Egg Yolk.
3. Boateng, M., Okai, D. B., Amoah, K. O., Boateng, J. O., Frimpong, Y. O., & Atuahene, P. Y. (2019). Occurrence of blood and meat spots in eggs processed and sold by commercial fried egg (Chibom) sellers at three locations in the Ashanti Region of Ghana. Ghanaian Journal of Animal Science, 10(1), 51-57.
4. KAMANLI,S.,& Türkoğlu,M.(2018). Tavukçuluk araştırma enstitüsünde bulunanbeyaz yumurtacı saf hatlar ve melezlerininyumurta iç ve dış kalite özelliklerininbelirlenmesi. Journal of Poultry Research, 15(1), 23-28.
5. Li, P., Wang, R., Jiao, H., Wang, X., Zhao, J., & Lin, H. (2018). Effects of dietary phosphorus level on the expression of calcium and phosphorus transporters in laying hens. Frontiers in physiology, 9, 627.
6. Smythe, V. A. O. (2019). Caracterización de la calidad de los huevos de gallina de campo(Gallus gallus domestica) producidos por la Agricultura Familiar Campesina de la Región de Los Ríos (Doctoral dissertation, Universidad Austral de Chile).
7. Adesiyun, A., Offiah, N., Seepersadsingh, N., Rodrigo, S., Lashley, V., Musai, L. and Georges, K. (2005). Microbial health risk posed by table eggs in Trinidad. Epidemiol. Infect. 133: 1049-1056. Cambridge University Press.
8. Gole, V. C., Chousalkar, K. K. and Roberts, J. R. (2013) Survey of Enterobacteriaceae Contamination of table eggs collected from layer flocks in Australia. Int. J. Food Microbiol, 164: 161-165.
9. Brooks, G. F., Butel, J. S., Nicholas, Ornston L., Jawetz, E., Melnick, J. L. and Adelberg, E. A. (1995). Jawetz, Melnick & Adelberg's Medical Microbiology (20th ed). Practice-Hall International Inc., 206-217.
10. Hope, B. K., Baker, R., Edel, E. D., et al. (2002). An overview of the S. enteritidis risk assessment for shell eggs and egg products. Risk anal. 22: 203-218.
11. CDC, National Center for Infectious Diseases (2017) Content source: Centers for Disease Control and Prevention , National Center for Emerging and Zoonotic Infectious Diseases (NCEZID)
12. FDA, Food and Drug Administeration (2010). New FDA regulation for Salmonella testing in commercial layers. Available from: http://www.vetmed.wsu.edu/deptswaddl/announ cements/FDATesting.pdf. Accessed Jul 9, 2010.
13. USDA, United State Department of Agriculture (2011): Food Safety and Inspection Services. Shell eggs from farm to table. www.fsis.usda.gov, retrieved 12- 04- 2014.
14. Abeer, A.A. (1997). Organoleptic inspection and microbiological quality of different types of fermented milk. M.V.Sc. Thesis, Fac. Vet.Med., Cairo univ.
15. Songer, J. G. and Post, K. W. (2005). Detection of Staphylococcus aureus and Streptococcus Agalactiae: Subclinical Mastitis Causes in Dairy Cow and Dairy Buffalo (Bubalus Bubalis).Veterinary Microbiology Bacterial and Fungal Agents of Animal disease 1st Ed .Elsevier saunder.
16. Cha, J. O., Lee, J. K., Jung, Y. H., Yoo, J. I., Park, Y.K., Kim, B. S. and Lee Y. S. (2006). Molecular analysis of Staphylococcus aureus isolates associated with staphylococcal food poisoning in South Korea. J. Appl. Microbiol.101:864–871.
17. Ikeda, T., Tamate, N., Yamaguchi, K. amd Makino, S. (2005). Mass outbreak of food poisoning disease caused by small amounts of staphylococcal enterotoxins A and H. Appl. Environ.Microbiol. 71: 2793–2795.
18. Argudín, María Á., Mendoza, María C. and Rodicio, María R. (2010). Food Poisoning and Staphylococcus aureus Enterotoxins, Toxins (Basel). Jul; 2(7): 1751–1773, doi: 10.3390/toxins2071751.
19. Yang, S. E., Yu, R. C. and Chou, C. C. (2001). Influence of holding temperature on the growth andsurvival of Salmonella spp. and Staphylococcus aureus and the production of staphylococcal enterotoxin in egg products, Int. J. Food Microbiol. 63 (1–2): 99-107.
20. Shareef, A. M., Mansour, R. S. and Ibrahim, K. K. (2009). Staphylococcus aureus in commercial breeder layer flocks. Iraqi J. Vet. Sci. 23(I): 63-68.
21. Gal-Mor, O., Boyle, E. C., & Grassl, G. A. (2014). Same species, different diseases: how and why typhoidal and non typhoidal Salmonella enterica serovars differ. Frontiers in microbiology, 5, 391.
22. Fàbrega, A., & Vila, J. (2013). Salmonella enterica serovar Typhimurium skills to succeed in the host: virulence and regulation. Clinical microbiology reviews, 26(2), 308-341.
23. Moraes DMCI Duarte SCII Bastos TSAI Rezende CLGI Leandro NSMI Café MBI Stringhini JHI Andrade MAI, (2016). Detection of Salmonella spp. by Conventional Bacteriology and by Quantitative Polymerase-Chain Reaction in Commercial Egg Structures. Mar 2016 / v.18 / n.1 / 117-124.
24. Sanders., E.R. (2012). Aseptic Laboratory Technique: Plating Methods. JoVE 63:e3063.
25. Aarestrup FM, Hendriksen RS, Lockett J, Gay K, Teates K, McDermott PF, White DG, Hasman H, Sorensen G, Bangtrakulnonth A. (2007). International spread of multidrug-resistant Salmonella Schwarzengrund in food products. Emerging Infectious Diseases 2007;13(5):726.
26. Dunn IC, Bain M, Edmond A, Wilson PW, Joseph N, Solomon S, De Ketelaere B, De Baerdemaeker J, Schmutz M, Preisinger R. (2005). Heritability and genetic correlation of measurements derived from acoustic resonance

frequency analysis; a novel method of determining eggshell quality in domestic hens. British Poultry Science 2005;46(3):280-286.
27. Osei-Somuah, A.; Otsyina, H. R.; Arthur, C. T.; Nortey, P. W. K. & Hammond, V. (2003). Microbial Quality Of Table Eggs Sold On Selected Markets In Accra. Animal Research Institute 2006. Published In Ghana Veterinary Medical Association Bi-Annual Newsletter, 6 (314): 18.
28. Etches,R.J.(1992).Theinfluenceofhousingaspectofeggquality.In:ProceedingsOf 19th Worlds Poultry Congress. Amsterdam, Netherlands. Worlds Poultry Congress. Amsterdam, Netherlands. Worlds Poultry Science Association, 3:164-167.
29. Johnson, J.; Kuskowski, M.; Menard, M.; Gajewski, A.; Xercavins, M. & Garau, J. (2006). Similarity between human and chicken escherichia coli isolates in relation to ciprofloxacin resistance status. J. of Infectious Dis., 194 (1): 71-8.
30. Methaq G, Noor A, Moutaz A. (2020). study of bacterial contamination of defected egg shell and egg contents in Baghdad city. Plant Archives. 2020; (20) Supplement 1: 2306-2308.
31. Arabi S, Jafarpour M, Mirinargesi M. (2013). Molecular Characterization of Avian Pathogenic Escherichia coli in Broilers Bred in Northern Iran. Global Veterinaria. 2013; 10(4): 382-386.
32. Islam S, khondkar P, Islam A. (2010). Isolation and In vitro resistannce and suseptability pattern of a strain of E.coli. Bangladish pharmaceutical Journal. 2010; 301: 34-38.
33. I.N. Abdullah. (2010). Isolation and identification of some bacterial isolates from table egg. Al-Anbar J. Vet. Sci., Vol.: 3 No. (2), 2010; ISSN: 1999-6527.
34. De Reu, K.; Messens, W.; Heyndrickx, M.; Rodenburg, T. B.; Uyttendaele, M. & Herman, L. (2008). Bacterial Contamination Of Table Eggs and The Influence of Housing Systems. World's Poult. Sci. J., 64: 5-19.
35. M.Rad, Z.Esmailnejad, Gh.Keleidari. (2003). IDENTIFICATION OF GRAM POSITIVE BACTERIA INVOLVED IN YOLK SAC INFECTION. Acta vet. scand. Suppl. 98 – 2003; P.O.Box: 1793 Mashhad 91775 Iran.
36. MM Islam1*, MN Islam3, Sharifuzzaman2, M Fakhruzzaman1(2014). Isolation and identification of Escherichia coli and Salmonella from poultry litter and feed. International Journal of Natural and Social ISSN: 2313-4461; Sciences 1 (2014) 1-7.
37. Arcan A.N. Al-Zubaid and Afaf Abdulrahman Yousif (2013) Prevalence and antimicrobial susceptibility of salmonella species isolate from slaughtered cows in Iraq. Vol. 37 No. 1 (2013): Iraqi J. Vet. Med.
38. Sabaa, H.H. (2009). "Preparation of Some *Salmonella enteritidis* antigens isolated from Goats & Study the Immune Efficacy of them in Rabbits ". MSC thesis, Vet. Med. College/Baghdad University.
39. Sagar Aryal, (2018). Haemolysis of Streptococci and its types with examples, September 27, microbiologyinfo.com
40. Kang G., Soohyun C., Pilnam S., Beomyoung P., Junsang H., Seokgeun J., Donghun K., Hyunseok C. (2011). Microbial and Physicochemical Properties of Liquid Egg during Cold Storage. Korean J. Food Sci. Anim. 2011;31:557–562. doi: 10.5851/kosfa.2011.31.4.557.
41. Kim Y.-J., Moon H.-J., Lee S.-K., Song B.-R., Lim J.-S., Heo E.-J., Park H.-J., Wee S.-H., Moon J.-S. (2018). Development and Validation of Predictive Model for *Salmonella* Growth in Unpasteurized Liquid Eggs. Korean J. Food Sci. Anim. Resour. 2018;38:442–450. doi: 10.5851/kosfa.2018.38.3.442.
42. Baron F., Gautier M., Brule G.(1997). Factors Involved in the Inhibition of Growth of *Salmonella Enteritidis* in Liquid Egg White. J. Food Prot. 1997;60:1318–1323. doi: 10.4315/0362-028X-60.11.1318.
43. Raman, R.; Colavecchio, A.; Barrere, V.; Levesque, C.; Roger; G.; Lawrence,D. (2017). "Molecular Characterization of bacteria that causefalse-positive and false-negative test results on the VIDAS UP Salmonella (SPT) affinity assay".MSC. Thesis , Faculty of Agriculture and Environmental Sciences; McGill University,Sainte-Anne-de-Bellevue, Québec, Canada.P:1- 76.
44. Zhang, G.;Brown, E. W.; González-Escalona, N. (2011). Comparison of real-timePCR, reverse transcriptase real-time PCR, loop-mediated isothermal amplification, and the FDA conventional microbiological method for the detection of Salmonellaspp. in produce. Applied and Environmental Microbiology, 77(18), 6495-6501.
45. Dí essy Kipper,∗ Rafaella Martins Hellfeldt,† Silvia De Carli,∗,‡ Fernanda Kieling Moreira Lehmann,∗ Andr e Salvador Kazantzi Fonseca,§ Nilo Ikuta,∗,†,§ and Vagner Ricardo Lunge∗,†,§,1, (2019). Salmonella serotype assignment by sequencing analysis of intergenic regions of ribosomal RNA operons. Poultry Science 98:5989–5998.
46. Achtman, M., J. Wain, F. X. Weill, S. Nair, Z. Zhou, V. Sangal, M. G. Krauland, J. L. Hale, H. Harbottle, A. Uesbeck, G. Dougan, L. H. Harrison, S. Brisse, and S. Enterica MLST Study Group. (2012). Multilocus sequence typing as a replacement for serotyping in Salmonella enterica. PLoS Pathog. 8:e1002776.
47. Feasey, N. A., J. Hadfield, K. H. Keddy, T. J. Dallman, J. Jacobs, X. Deng, P. Wigley, L. Barquist, G. C. Langridge, T. Feltwell, S. R. Harris, A. E. Mather, M. Fookes, M. Aslett, C. Msefula, S. Kariuki, C. A. Maclennan, R. S. Onsare, F. X. Weill, S. Le Hello, A. M. Smith, M. McClelland, P. Desai, C. M. Parry, J. Cheesbrough, N. French, J. Campos, J. A. Chabalgoity, L. Be- tancor, K. L. Hopkins, S. Nair, T. J. Humphrey, O. Lunguya, T. A. Cogan, M. D. Tapia, S. O. Sow, S. M. Tennant, K. Bornstein, M. M. Levine, L. Lacharme-Lora, D. B. Everett, R. A. Kings- ley, J. Parkhill, R. S. Heyderman, G. Dougan, M. A. Gordon, and N. R. Thomson.(2016). Distinct Salmonella Enteritidis lin- eages associated with enterocolitis in high-income settings and invasive disease in low-income settings. Nat. Genet. 48:1211– 1217.

48. Guard, J., R. Sanchez-Ingunza, C. Morales, T. Stewart, K. Liljeb- jelke, J. Van Kessel, K. Ingram, D. Jones, C. Jackson, P. Fedorka- Cray, J. Frye, R. Gast, and A. Hinton, Jr. (2012). Comparison of dkgB-linked intergenic sequence ribotyping to DNA microar- ray hybridization for assigning serotype to Salmonella enterica. FEMS Microbiol. Lett. 337:61–72.
49. Pulido-Land´ınez, M., R. Sa´nchez-Ingunza, J. Guard, and V. P. do Nascimento. (2013). Assignment of serotype to Salmonella enterica isolates obtained from poultry and their environment in Southern Brazil. Lett. App. Microbiol. 57:288–295.
50. Pulido-Land´ınez, M., R. Sa´nchez-Ingunza, J. Guard, and V. P. do Nascimento. (2014). Presence of Salmonella enteritidis and Salmonella gallinarum in commercial laying hens diagnosed with fowl typhoid disease in Colombia. Avian Dis. 58:165–170.