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Detection of Virulence Genes of *Salmonella* in Diarrhoeic Ducks by using Polymerase Chain Reaction (PCR)

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SALMONELLA spp. is responsible for various food borne outbreaks. Incidence of *Salmonella* among the examined diarrheic ducks was 5.5%. The occurrence was 6.15% in young ducks which was higher than that in adult duck 4.28%. The total incidence of *Salmonella* in slaughtered ducks was 3.33%. The serovars were *S.*Infants, *S.* Typhimurium , *S.* Virchow from fecal samples of adult ducks and *S.* Agona, *S.* Infants, 2 *S.* Kentucky, 2 *S.* Longhorn, *S.* Typhimurium and *S.* Virchow in fecal samples of young ducks while from slaughtered ducks were (*S.* Kentucky and *S.* Typhimurium from both caeci and intestines and *S.* Typhimurium from livers). Variation in pathogenicity and enterotoxin production of *Salmonella* isolates were observed.

Sensitivity of salmonellae to ciprofloxacin, flumequine were 56.3%, enrofloxacin , gentamicin and norfloxacin were 50% and trimethoprim was 18.8%. All isolates were resistant to doxycycline hydrochloride and penicillin.

All isolates from diarrheic and slaughtered ducks harbor *invA* gene and amplified at 284bp. Sixteen *Salmonella* isolates examined for *pef* gene at 700bp that was present in 2 *S.* Typhimurium, *S.* Agona and *S.* Kentucky isolated from diarrheic ducks and 2 *S.* Kentucky isolated from slaughtered ducks. Finally we investigate the presence of *stn* gene that was encoded on plasmid DNA and amplified a region 617bp in 16 isolates of *Salmonella* 2 *S.* Typhimurium, 2 *S.* Virchow, *S.* Agona, *S.* Infant, 2 *S.* Kentucky and *S.* Longhorn isolated from diarrheic ducks while 2 *S.* Kentucky isolated from slaughtered ducks harbored this gene.

Keywords: *Salmonella*, Ducks, Virulence genes *invA, pef,* PCR.

Introduction

The genus *Salmonella* is Gram negative, Non- spore forming, usually motile, facultative anaerobic bacilli belong to the family of *Enterobacteriaceae* [1] *Salmonella* is identified as one of the major bacterial foodborne pathogen causing human illnesses worldwide [2]. The most characteristic high light of *Salmonella* is the wide host range, which involves most animal species, birds, cold-blooded animals, foods such as meat and dairy products in addition to humans [3]. *Salmonella* virulence genes such as *invA*, and *stn* are related to a combination of chromosomal and plasmid factors, which have been identified as major virulence genes responsible for salmonellosis. *Salmonella* pathogenicity islands (SPIs) are large gene cassettes within the *Salmonella* chromosome that encode determinants responsible for establishing specific interactions with the host, and are required for bacterial virulence in a given animal like other pathogenicity islands, there is more than 20 SPIs have been described [4]. The chromosomally located invasion gene *invA* codes for a protein in the inner membrane of bacteria that is necessary for invasion of epithelial

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cells [5], in addition to that, *invA* gene of *Salmonella* has a unique sequence to this genus so it is a suitable PCR target with potential diagnostic application [6]. Molecular tools were used to detect various gene -encoded virulence factors as Salmonella enterotoxin (*stn*) and plasmid encoded fimbriae (*pef*) genes [7] and [8].

Our study was aimed to cover the following points: Isolation and identification of *Salmonella* spp. from diarrheic ducks, serological identification of these strains, antimicrobial sensitivity test, pathogenicity test in mice, detection of Salmonella enterotoxins and molecular detection of certain Salmonella virulence genes (*stn*, *pef* and *invA*).

Materials and Methods

Samples

A total of 200 fecal samples from diarrheic ducks including (70 from adult and 130 from young ducks) and 150 samples from cecum, intestine and liver of slaughtered ducks (50 samples each) were collected under aseptic condition and transferred directly to the laboratory as soon as possible.

Isolation and identification of Salmonella spp.:

According to ISO6579:2002, the collected samples were inoculated into buffered peptone water at a dilution 1:10 for18 hrs at 37°C then transferred 100 µl into Rappaport Vassiliadis broth and incubated at 41.5°C for 24 hrs . XLD plates were inoculated, and incubated for 24 hrs at 37°C.Non lactose fermented colonies were confirmed biochemically by (Indole, TSI, Methyl red, Voges-Proskauer, Citrate and Urease) according to Akbarmehr [9].

Serological identification

It was carried out using White Kauffmann-Le Minor scheme as described by Grimont and Weill [10]. The typing antisera were obtained from Denka Seiken Co.Ltd,Tokyo, Japan.

Antimicrobial Sensitivity Test

The test was carried out according to Clinical Laboratory Standard Institute [11] method by means of the Kirby-Bauer Disk Diffusion test using Mueller-Hinton agar (Hardy Diagnostics CA, USA).A total of 16 *Salmonella* strains were examined for their susceptibility to eight antimicrobial discs (Oxoid): ciprofloxacin (CF,5 µg), enrofloxacin (ENR,5 µg), doxycycline (DO,30 µg), flumequine (UB,30 µg), gentamicin (G,10 µg), norfloxacin (NX,10 µg), penicillin -G (P,10 µg) and trimethoprim (TR, 5µg). Each isolate was inoculated into Mueller-Hinton broth separately and incubated for 24 hours at 37°C. The broth was streaked using sterile cotton swabs on Mueller-Hinton agar plates. The diffusion discs with antimicrobial drugs were placed on the plates and incubated for 24 hours at 37°C.

Pathogenicity test in mice

A total of 85 albino white mice with average weight of about 19-20 grams and aged 28-30 days old were used to investigate the pathogenicity of sixteen isolates of *Salmonella* (5 mice for each strain) .All mice were inoculated I.P with 0.1ml of $5x10^8$C.F.U/ mouse of the tested strain and kept separately and the last group was kept as control and was injected only with saline .Mice were kept under observation for 7-10days, the number of dead mice was recorded and re-isolation of the inoculated strains was done.

Detection of Salmonella enterotoxins [12]

Each isolate was inoculated into tryptone soya broth and incubated overnight at 37°C .Then 10ml of culture was placed in 200ml of medium containing 2% casamino acid ,1% yeast extract and 0.4% glucose (pH 8.5) in 250ml flask . The inoculated flasks were incubated on a rotator shaker 200rpm at 37°C for 18hours then centrifuged at 12000xg for 10 minutes. The supernatant was filtrated through millipore membrane filter pore 0.45um and stored at -20 ° C until used. A part of sterile medium was used as control. Infant mouse assay 0.1ml of each filtrate was injected through the abdominal wall into milk filled stomach of each 3 mice 2-4 days old for each examined strain

and 3 infant mice were injected by 0.1 ml of sterile medium and were used as negative control. After 4 hours, the mice were killed and the entire intestine was removed. The intestine and remaining body were weight to calculate the ratio of intestine weight / remaining body weight. Ratio greater than (0.083) was recorded as positive test for enterotoxin.

Genomic DNA extraction:

Sixteen Salmonella strains were selected for molecular identification of certain virulence genes (*invA, pef* and *stn*).Genomic DNA of *Salmonella* strains were extracted using an extraction kit (QIAamp mini kit, Qiagen,).

Molecular detection of (invA, pef and stn) genes

DNA amplification and PCR running, the amplified reactions were performed in 50 µl volumes in micro-amplification tubes (PCR tubes).The reaction mixture consisted of 10 µl (200 ng) of extracted DNA template from bacterial cultures, 5µl 10X PCR buffer, 0.5 µl MgCl2 (2 mM), 1µldNTPs (200 µM), 0.1 µl (0.5 Unit) AmpliTaq DNA polymerase, 0.1 µl (0.2 µM) from each primer pairs and the volume of the reaction mixture was completed to 50 µl using DDW,PCR amplifications were performed in thermal cycler(Biometra).Primer sequence, target genes and PCR programs were mentioned in Table 1.

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. Gelpilot 100 bp, 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) and gene ruler 100 bp DNA ladders were used to determine the fragment sizes.

TABLE 1. Primer sequence and PCR Protocols.

Target gene	Primers sequences	Amplified segment (bp)	Amplification (35 cycles)				Final extension	Reference
			Primary denaturation	Secondary denaturation	Annealing	Extension		
Stn	TTG TGT CGC TAT CAC TGG CAA CC	617	94°C 5 min.	94°C 15 sec.	59°C 45 sec.	72°C 45 sec.	72°C 10 min.	**[13]**
	ATT CGT AAC CCG CTC TCG TCC							
Pef	TGT TTC CGG GCT TGT GCT	700	94°C 5 min.	94°C 15 sec.	55°C 45 sec.	72°C 45 sec.	72°C 10 min.	
	CAG GGC ATT TGC TGA TTC TTC C							
invA	GTGAAATTATCGCCACGTTCGGGCAA	284	94°C 5 min.	94°C 15 sec.	55°C 30 sec.	72°C 30 sec.	72°C 7 min.	**[14]**
	TCATCGCACCGTCAAAGGAACC							

Results and Discussion

Salmonella infection is the major bacterial disease in ducks [15]. Ducks play role in transmission and spread of *Salmonella* infections.

The data in Table 2 indicated the total incidence of *Salmonella* in diarrheic ducks was 5.5% with incidence of 6.15% in young ducks which is higher than that in adult duck 4.28% this may be due to low immunity in young ducks. Adzeity et al. [16] recorded an incidence 39%in fecal samples, while Abu-Zaid [17] detected 12% among fecal samples in healthy and diseased ducks.

Table 3 showed the total incidence of *Salmonella* in slaughtered ducks was 3.33% (4% each from caeci and intestines and 2% from livers), these results disagree with Abu-Zaid [17] who reported that the incidences of *Salmonella* were14% from livers and 16% from intestines.

Presented data in Table 4 shows that *S*.Infants, *S*. Typhimurium , *S*. Virchow were detected from fecal samples of adult ducks and *S*. Agona, *S*. Infants, 2 *S*. Kentucky, 2 *S*. Longhorn, *S*. Typhimurium and *S*. Virchow in fecal samples of young ducks .Among slaughtered ducks (*S*. Kentucky , *S*. Typhimurium were identified from cecum and intestines (each) and *S*. Typhimurium from livers) .These results somewhat agree with Abu-Zaid[17] and Ibrahim *et al* [18] who isolated *S*. Typhimurium from ducks.

Table 5 illustrated variation in pathogenicity and enterotoxin production of *Salmonella* according to serovars and age of duck at the time of isolation. *S*. Typhimurium isolated from diarrheic ducks was 60% pathogenic to mice and enterotoxigenic while *S*. Typhimurium isolates from cecum , intestine and liver were 40% pathogenic and were non enterotoxigenic .

Also *S*. Virchow isolated from fecal samples of adult ducks was 40% pathogenic to mice and non enterotoxigenic .While *S*. Virchow isolated from fecal samples of diarrheic young ducks was 60% pathogenic and enterotoxigenic.

This variation may be due to pathogenic nature of examined strains, no clear pattern could be established relative to serovars , age and type of organ of isolation of salmonellae .

As recorded in Table 6 there were variations in sensitivity of isolated salmonellae between different serovars and between the same serovars, as *S*. Typhimurium in adult ducks was resistant to gentamicin &norfloxacin and sensitive to enrofloxacin , while *S*. Typhimurium isolated from young ducks were resistant to enrofloxacin and sensitive to gentamicin and norfloxacin. Generally the sensitivity of salmonellae to ciprofloxacin , flumequine were 56.3% , 50% to enrofloxacin , gentamicin and norfloxacin while trimethoprim was 18.8% and totally resistant to doxycycline hydrochloride and penicillin . These results agree somewhat with Adzeity et al [16], Abu-Zaid [17] and Yhiler and Bassey [19] found *Salmonella* serovars were sensitive to ciprofloxacin, gentamicin and norfloxacin.The expanded usage of antibiotics in both public and veterinary settings has prompted to the rise of antibiotic resistance and as an outcome represents a serious risk to public health safety, However, the use of antibiotics together with the improvement of sanitation and hygiene as well as immunization and proper nutrition has given significant advantages in human life expectancy [20].

The current investigation studied the presence of virulence genes. Table 7 revealed that all isolates from diarrheic and slaughtered ducks harbor *invA* gene and amplified at 284bp fragments. These results were agreed with Borges et al. [21] who found *invA* gene in 100% of *Salmonella* isolates from poultry. Also Mohamed [22] detected *invA* genes in all *Salmonella* isolates from horse.

invA gene is essential for full virulence in *Salmonella* and it thought to trigger the internalization required for invasion of dipper tissues [23].

It is clear that 2 *S*. Typhimurium, *S*. Agona and *S*. Kentucky isolated from diarrheic ducks and 2 *S*. Kentucky isolated from slaughtered ducks had *pef* gene (plasmid encoded fimbriae). Mohamed [22] detected *pef* gene to all *Salmonella* isolates from horse.

Finally we investigate the presence of *stn* gene (heat labile enterotoxin) was encoded on plasmid DNA and amplified a region 617bp in 16 isolates of *Salmonella* 2 *S*. Typhimurium, 2 *S*. Virchow, *S*.Agona, *S*. Infants, 2 *S*. Kentucky and *S*. Longhorn from diarrheic ducks while 2 *S*. Kentucky isolated from slaughtered ducks harbored this gene. This somewhat agree with Ezzat et al. [24] detected *stn* gene in all tested *Salmonella* which isolated from broilers farms in Dakahlia Governorate, Egypt. It is clear that

TABLE 2. The incidence of salmonellae of diarrheic ducks:

Life stage of ducks	No. of examined samples	No. of salmonellae isolates	%
Adult	70	3	4.28
Young	130	8	6.15
Total	200	11	5.5

TABLE 3. The incidence of salmonellae of slaughtered ducks:

Type of samples	No. of examined samples	No. of salmonellae isolates	%
Cecum	50	2	4
Intestine	50	2	4
Liver	50	1	2
Total	150	5	3.33

TABLE 4. Serological identification of isolated salmonellae from ducks:

Source of samples		No. of isolates salmonellae	Serotypes and number
Fecal sample of adult ducks		3	*S.*Infants , *S.*Typhimurium , *S.*Virchow
Fecal sample of young ducks		8	*S.*Agona *S.*Infants 2 *S.*Kentucky 2 *S.*Longhorn *S.*Typhimurium *S.*Virchow
Slaughtered ducks	**Cecum**	2	*S.*Kentucky *S.*Typhimurium
	Intestine	2	*S.*Kentucky *S.*Typhimurium
	Liver	1	*S.*Typhimurium
Total		16	

TABLE 5. Detection of pathogenicity and enterotoxin of salmonellae isolated from ducks:

Source of samples	Serotypes	Pathogenicity		Enterotoxin production
		*No. of dead mice	%	
Fecal samples:adult ducks	*S.*Infants	2	40%	-
	*S.*Typhimurium	3	60%	+
	*S.*Virchow	2	40%	-
young ducks	*S.*Agona	3	60%	+
	*S.*Infants	2	40%	+
	*S.*Kentucky	3	60%	+
	*S.*Kentucky	3	60%	-
	*S.*Longhorn	2	40%	+
	*S.*Longhorn	2	40%	-
	*S.*Typhimurium	3	60%	+
	*S.*Virchow	3	60%	+
Slaughtered duck :Cecum	*S.*Typhimurium	2	40%	-
	S. Kentucky.	3	60%	+
Intestine	*S.*Typhimurium *S.* Kentucky	2	40%	-
		3	60%	+
Liver	*S.*Typhimurium	2	40%	-

No. of inoculated mice 5*.

TABLE 6. Antimicrobial sensitivity among salmonellae isolated from ducks:

Source of samples	Serotypes	CF5	ENR5	DO 30	UB 30	G 10	NX 10	P 10	TR 5
Fecal samples of adult ducks	*S.*Infants	I	S	R	S	S	I	R	R
	*S.*Typhimurium	S	S	R	S	R	R	R	R
	*S.*Virchow	S	S	R	R	R	R	R	R
Fecal samples of young ducks	*S.*Agona	S	S	R	R	S	I	R	R
	*S.*Infants	I	R	R	R	R	S	R	R
	*S.*Kentucky	I	R	R	S	R	S	R	S
	*S.*Kentucky	S	R	R	S	R	S	R	I
	*S.*Longhorn	I	R	R	R	S	S	R	S
	*S.*Longhorn	I	R	R	R	S	S	R	S
	*S.*Typhimurium	S	R	R	R	S	S	R	R
	*S.*Virchow	S	S	R	R	R	I	R	R
Slaughtered duck Caecum	*S.*Typhimurium	I	R	R	S	R	S	R	I
	*S.*Kentucky	S	S	R	S	S	I	R	R
Slaughtered duck	*S.*Typhimurium	I	R	R	S	R	S	R	I
	*S.*Kentucky	S	S	R	S	S	I	R	R
Intestine Slaughtered duck liver	*S.*Typhimurium	S	S	R	S	S	I	R	R
Total of sensitive strains		9	8	0	9	7	8	0	3
% of sensitivity		56.25%	50%	0%	56.25%	43.75%	50%	0%	18.75%

R: resistant, I: intermediate and S: sensitive.

CF 5: ciprofloxacin , UB 30: flumequine, G10: gentamicin, NX 10: norfloxacin, TR 5: trimethoprim, ENR 5: enrofloxacin, DO 30: doxycycline hydrochloride and P 10: penicillin.

TABLE 7. Distribution of virulence genes (*invA*, *pef* and *stn*)

Source of samples	Serotypes	*invA*gene	*pef* gene	*stn* gene
Fecal samples:	*S.*Infants	+	–	-
a- adult ducks	*S.*Typhimurium	+	+	+
	*S.*Virchow	+	-	+
b- young ducks	*S.*Agona	+	+	+
	*S.*Infants	+	-	+
	*S.*Kentucky	+	–	+
	*S.*Kentucky	+	+	+
	*S.*Longhorn	+	-	+
	*S.*Longhorn	+	-	-
	*S.*Typhimurium	+	+	+
	*S.*Virchow	+	-	+
Slaughtered ducks:	*S.* Typhimurium	+	-	–
a- cecum	*S.* Kentucky	+	+	+
b-Intestine	*S.* Typhimurium	+	-	–
	S. Kentucky	+	+	+
c- Liver	*S.*Typhimurium	+	-	-

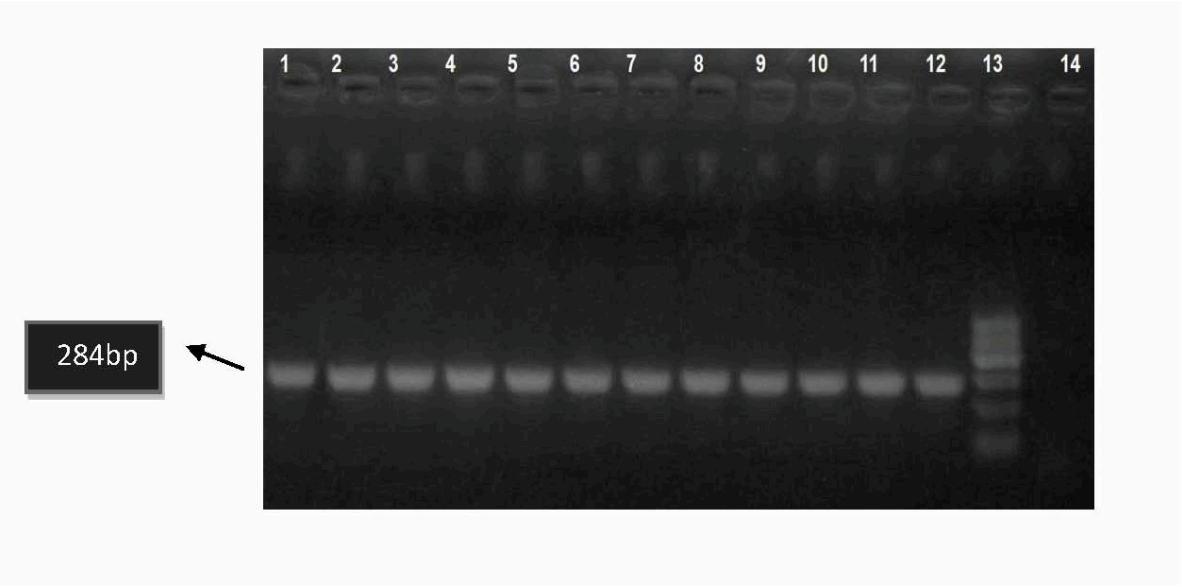

Photo1. invA gene of salmonellae isolated from diarrheic ducks.

Lane1 S.Infants, Lane2 S.Typhimurium and Lane3 S.Virchow isolated from diarrheic adult ducks, Lane4 S.Agona, Lane5 S.Infants, Lane 6 S.Kentucky, Lane7 S.Kentucky, Lane8 S.Longhorn, Lane9 S.Longhorn, Lane10 S.Typhimurium and Lane11 S.Virchow from diarrheic young ducks, all samples carried inv A genes at 284 bp.Lane 12 positive control (salmonella reference strain ATCC 14028), Lane 13 ladder 100 – 600 bp and Lane14 negative control.

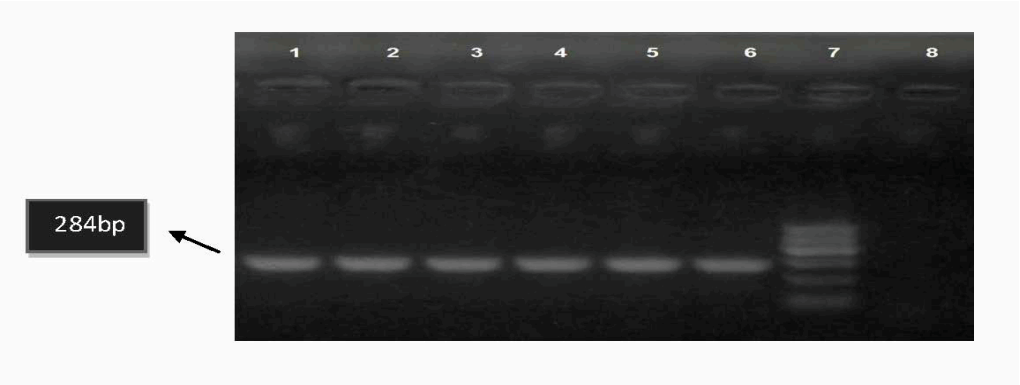

Photo2. *invA* gene of salmonellae isolated from slaughtered ducks.

Lane1 *S*.Typhimurium and Lane2 *S*. Kentucky isolated from cecum, Lane3 *S*.Typhimurium and Lane4 *S*. Kentucky isolated from intestine, Lane5 *S*.Typhimurium isolated from liver .All samples carried *invA* genes at 284 bp .Lane 6 positive control (*salmonella* reference strain ATCC 14028) , Lane 7 ladder 100 - 600bp and Lane 8 negative control.

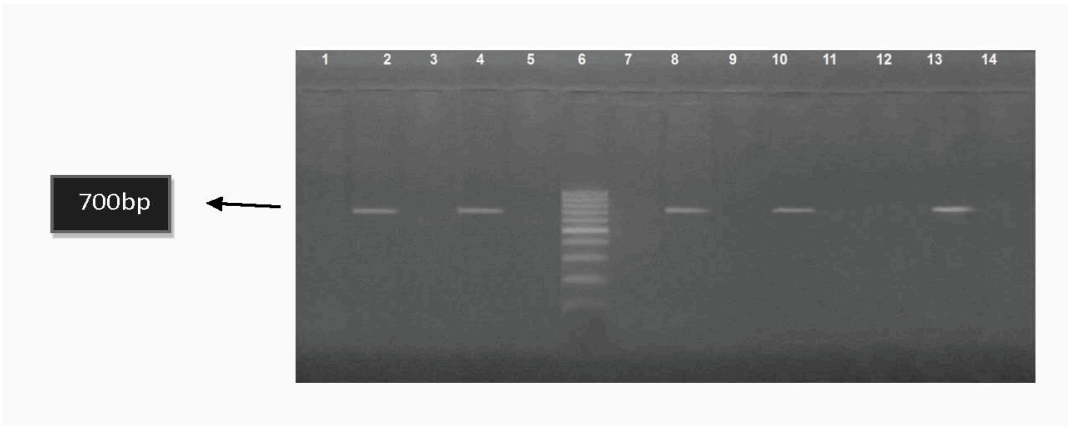

Photo3. *pef* gene of salmonellae isolated from diarrheic ducks.

Lane1 *S*.Infants -ve, Lane 2 *S*.Typhimurium+ve and Lane 3 *S*.Virchow –v isolated from diarrheic adult ducks, Lane 4 *S*.Agona +ve, Lane 5 *S*.infants –ve , Lane 9 *S*.Kentucky -ve , Lane10 *S*.Kentucky +ve , Lane11 *S*.Longhorn -ve , Lane12 *S*. Longhorn -ve , Lane13 *S*.Typhimurium +ve and Lane14 *S*.Virchow –ve isolated from diarrheic young ducks. Lane 6 ladder 100- 1000 bp, Lane 7 negative control and Lane 8 positive control (field strain previously confirmed to be positive for the selected genes by PCR), Positive samples carried *pef* gene at 700 bp.

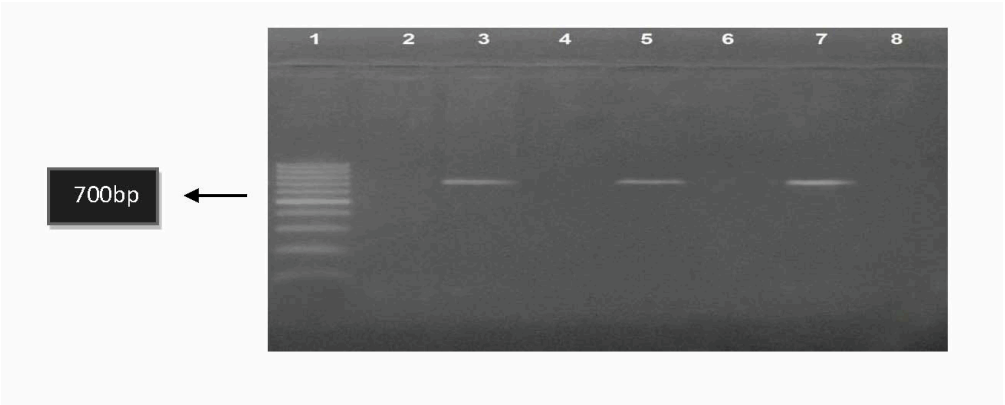

Photo4. *pef* gene of salmonellae isolated from slaughtered ducks.

Lane1 ladder 100 – 1000 bp, Lane 2 negative control ,Lane 3 positive control(field strain previously confirmed to be positive for the selected genes by PCR) ,Lane4 *S*.Typhimurium -ve, Lane 5 *S*. Kentucky +ve isolated from cecum, Lane 6 *S*.Typhimurium -ve and Lane7 *S*. Kentucky +ve isolated from Intestine and Lane8 *S*.Typhimurium –ve isolated from Liver. Positive samples were carried *pef* gene at 700 bp.

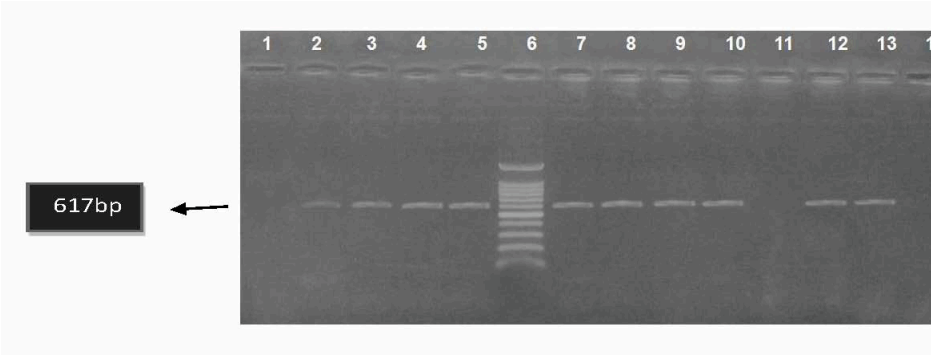

Photo 5. *stn* gene of salmonellae isolated from diarrheic ducks.

Lane1 *S*.Infants -ve, Lane2 *S*.Typhimurium +ve, Lane3 *S*.Virchow +ve isolated from diarrheic adult ducks, Lane4 *S*. Agona +ve, Lane5 *S*.Infants +ve , Lane6 Ladder 100 – 1500 bp, Lane7 positive control (field strain previously confirmed to be positive for the selected genes by PCR) , Lane8 *S*.Kentucky +ve , Lane9 *S*.Kentuckey + ve , Lane10 *S*.Longhorn +ve , Lane11 *S*.Longhorn -ve, Lane12 *S*.Typhimurium +ve, Lane13 *S*.Virchow +ve. Lane14 negative control. Positive samples carried *stn* gene at 617 bp.

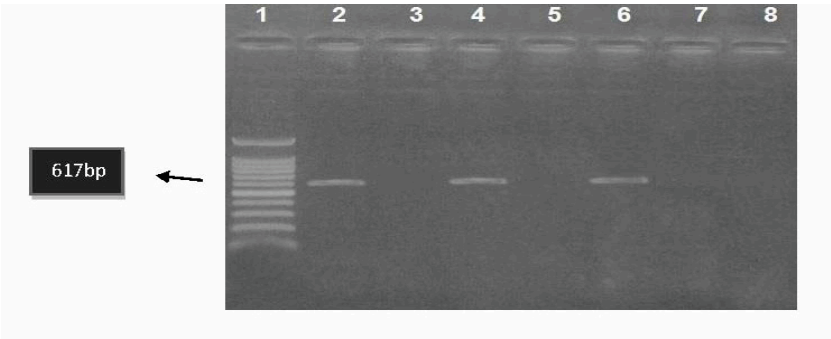

Photo 6. *stn* gene of salmonellae isolated from slaughtered ducks.

S. Typhimurium isolated from diarrheic ducks had *pef* gene and had 60%pathogenicity in mice , but *S*. Typhimurium isolated from slaughtered ducks had no genes and lower in pathogenicity in mice 40%. Also *S*. Virchow isolated from diarrheic ducks contain no *pef* gene although it differ in pathogenicity 40% in adults and 60% in small ducks and also *S*. Kentucky from diarrheic ducks one contain *pef* gene and other had no gene although pathogenicity in both 60% , *S*. Infants had no *stn* gene and not enterotoxigenic , *S*. Virchow had *stn* gene and not enterotoxigenic (this indicated that may gene not expressed). While *S* . Longhorn one contain *stn* gene and enterotoxigenic and the other had no *stn* gene and not enterotoxigenic.

No clear pattern could be established between presence of virulence gene (*invA, pef* and *stn*)and pathogenicity in mice, enterotoxigenicity, isolated serovars and source of isolation.

We concluded that the *Salmonella* present in ducks caused many signs of disease as diarrhea and affect production also may transmit to human so it is necessary to focus on tracing the source of infection. Therefore measures to reduce *Salmonella* transmission and contamination needs to sanitary measures and personal hygiene and to increased implementation of hazard analysis and critical control point (HACCP) to help curb the spread of *Salmonella invA*, *pef* and *stn* virulence genes may be utilized as a gene marker for the fast recognition of the virulent strains of *Salmonella*.

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الكشف عن جينات الضراوة في السالمونيلا في البط المسهل باستخدام تفاعل البلمرة المتسلسل

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انواع السالمونيلا هي المسئولة عن تفشي الامراض التي تنقلها الاغذية , وكانت نسبة حدوث الاصابة بالسالمونيلا في البط المسهل ٥,٥٪ . نسبة وجودة في البط صغير السن كانت ٦,١٥٪ وهي اعلي من ذلك في البط الكبيرالسن وكانت نسبتة ٤,٢٨٪ . وكان مجموع حالات السالمونيلا في البط المذبوح ٣,٣٣٪ . السيروفار التي عزلت هي سالمونيلا انفانتس , سالمونيلا تايفيميوريم و سالمونيلا فيرتشو من عينات البراز للبط كبير السن و سالمونيلا اجونا , سالمونيلا انفانتس ,٢ سالمونيلا كنتاكي, ٢ سالمونيلا لونجهورن , , سالمونيلا تايفيميوريم و سالمونيلا فيرتشو من عينات البراز للبط صغير السن اما بالنسبة للبط المدبوح كانت (سالمونيلا كنتاكي , سالمونيلا تايفيميوريم من الامعاء و سالمونيلا تايفيميوريم من الكبد).

التباين في مرضية و انتاج السموم من عزلات السلامونيلا كانت ملاحظة . و كانت حساسية السلامونيلا الي السبروفلوكساجسين و الفلومكوين ٥٦٪ ، إنروفلوكساسين، الجنتاميسين والنورفلوكساسين ٥٠٪ وكان تريمثوبريم ١٨,٨٪. وكانت جميع العزلات مقاومة للدوكسي هيدروكلوريد و البنسلين. جميع المعزولات من البط المسهل و المذبوح تؤوي جين *invA* الذي يظهر عند ٢٨٤bp وتم فحص ال١٦ عزلة من السالمونيلا لجين *pef* وتكبيرة عند ٧٠٠bp وكان موجودا في ٢ سالمونيلا تيفيموريوم،سالمونيلا أغونا وسالمونيلا كنتاكي المعزولة من البط المسهل و ٢ سالمونيلا كنتاكي معزولة من البط المذبوح . واخيرا تم التحقق من وجود الجين *stn* و تكبيرة عند ٦١٧bp و تم ترميزها في ال١٦ عزلة من السالمونيلا ٢ سالمونيلا تيفيموريوم، ٢ سالمونيلا فيرتشو، سالمونيلا أغونا، سالمونيلا انفانتس، ٢ سالمونيلا كنتاكي و سالمونيلا لونغهورن معزول من البط المسهل بينما ، ٢سالمونيلا كنتاكي معزولة من البط المذبوح.

الكلمات الدالة : سالمونيلا , بط , جينات ضراوة , اي ان في اية , بي اي اف , تفاعل البلمرة المتسلسل.