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DIAGNOSTIC VALUE OF PCR OF SALMONELLA ENTERICA SEROVAR ENTERITIDIS IN POULTRY AND HUMAN CONTACTS

(With 3 Tables and One Figure)

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القيمة التشخيصية لتفاعل البلمرة المتسلسل لميكروب السالمونيلا انتريتيدس في مزارع الدواجن والمخالطين لها

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تعتبر السالمونيلا واحدة من أهم المسببات البكتيرية التي تؤثر على صحة الدواجن، وعلى الرغم من التقدم الكبير في مجال الأمن الحيوي والأدوية واللقاحات البيطرية، فأنها لا تزال تشكل تكلفة باهظة لجميع مشاريع الدواجن سواء التسمين أو البياض أو الأمهات ونظرا لخطورة ميكروب السالمونيلا انتريتيدس علي هذه الصناعة بالإضافة إلى انتقاله إلى الإنسان مما قد يتسبب في حدوث تسمم غذائي قد يؤدي إلي الوفاة. وعليه فقد أجريت هذه الدراسة للوقوف علي القيمة التشخيصية لتفاعل البلمرة المتسلسل لميكروب السالمونيلا انتريتيدس في مزارع الدواجن والأشخاص المخالطين لها حيث يعتبر التشخيص السريع الدقيق هو حجر الزاوية في السيطرة على انتشار هذا الميكروب. وبناء على ما تقدم فقد تم إجراء فحص بكتيري لعدد ١١٠ عينة من الدجاج في بعض مزارع الدواجن المتواجدة في مركز الرحمانية التابع لمحافظة البحيرة وكانت أربع عينات ايجابية لعدوي السالمونيلا بنسبة إصابة بلغت ٣,٦٣%. كذلك تم إجراء الفحص البكتيري لعدد ٥٠ عينة من براز بعض الأشخاص المخالطين لتلك المزارع والمترددين على المراكز الطبية في نفس المنطقة ويعانون من بعض الأعراض المرضية مثل ارتفاع الحرارة والحمى والقيء وسجلت حالتين ايجابيتين لعدوي السالمونيلا بنسبة ٤%. كذلك تم تصنيف عدد ٤ معزولات لميكروب السالمونيلا ووجد أن جميعها كانت لميكروب السالمونيلا انتريتيدس، فى نفس الوقت تم استخدام تفاعل البلمرة المتسلسل في الكشف علي عتره السالمونيلا انتريتيدس فى العينات الايجابية وقد أظهرت النتائج أن تفاعل البلمرة المتسلسل قد نجح فى الكشف عن ميكروب السالمونيلا انتربتيدس فى أربع من الست عينات التي خضعت للفحص من كل من الدجاج والحالات البشرية ومن تلك النتائج يتضح أن تفاعل البلمرة المتسلسل أكثر دقة وحساسية وسرعة في اختبار الكشف عن السالمونيلا في الدجاج والأشخاص المخالطين من الطريق التقليدية. هذا وقد تمت مناقشة هذه النتائج مناقشة علمية مستفيضة.

SUMMARY

A total of 110 chicken samples were collected randomly from naturally infected farms in Al-Rahmania district in Behera Province, Egypt where birds suffered from mottled liver with yellowish discoloration, distended gallbladder, slight air sacculitis, congested lungs and enlarged spleens. Liver, heart, spleen and cloacal swabs from chickens were sampled for isolation of *Salmonella enterica serovar Enteritidis* (SE). In addition, 50 human stool samples were collected from persons suffering from pyrexial illness with diarrhea and vomiting for several days hospitalized in areas surrounding examined poultry farms to be investigated for presence of SE by bacteriological examination. The obtained results of bacteriological examination revealed that *Salmonella spp.* were isolated from 4 out of 110 (3.63 %) of examined broiler chicken samples and 2 out of 50 (4 %) of examined stool samples. The serological identification of *Salmonella* isolates by using polyvalent and monovalent (O) and (H) *Salmonella* antisera clarified that 3 isolates from chicken isolates were proved to be SE and one isolate from human isolates was proved to be SE. At the same time, all positive samples were re-examined by using Polymerase chain reaction (PCR) where its result revealed that 4 isolates out of 6 were SE. The obtained results of the current work confirmed the diagnostic value of PCR detection of SE in poultry and surrounding people in which PCR is very sensitive, very specific and relatively rapid test.

Key words: *Salmonella, Poultry, human, PCR.*

INTRODUCTION

Poultry industry plays an important role in offering cheap source of animal protein for human beings in Egypt where prices of red meat, milk products and other sources of animal protein reached very high levels. Classification of *Salmonella* showed that there were more than 2300 serologically identified serotypes but only two serotypes were proved as true poultry pathogens named *S. gallinarum and S. pullorum* (Gast, 1997). On contrary, SE seldom caused disease symptoms in poultry but it was of major concern to public health since it was one from the commonest agents for human food poisoning as it could be transmitted either directly by contact between poultry men and infected birds or indirectly through consumption of poultry meat or egg or

through contamination of poultry meat with *Salmonella* in slaughterhouse (Breytenbach, 2004).

Techniques used for the detection of *Salmonella* in chickens mostly suffer from being either time-consuming, labor intensive, or expensive. The application of the PCR is one approach for the rapid and effective detection and identification of *Salmonella*. Application of PCR for detection of SE was carried out by many authers: Oliveira *et al.* (2002) developed a PCR assay for the generic detection of SE in materials collected from chicken in the field and they found that PCR assay detected more positive field samples than the standard microbiological techniques and results were ready in 48 hrs instead of 7 days, Meran Sleim (2003) compared between bacteriological methods and PCR for diagnosis of SE, and revealed that the bacteriological methods failed to detect the shedding of SE from cloacal swabs collected 8 hrs post infection and incubated for 8 hrs at $37C^0$, while PCR succeeded in detection of *Salmonella* in these incubated samples. Also, Allgayer *et al.* (2008) tested 280 birds samples by PCR using a pair of primers that amplify a 284 base pair fragment of SE then they retested the PCR-positive samples by standard microbiological techniques and discovered that 13 % of the samples were positive by PCR, but negative by microbiological techniques emphasizing the sensitivity and specificity of PCR. Shita (2009) examined 6 serologically identified SE serotypes recovered from feed and litter by PCR and the all of them were positive (100 %). Ayoub (2010) found that the overall occurrence of Salmonella recovered from chicken cloacal swabs and environmental samples under experiment was 6.66 % by PCR and 5.71 % by conventional bacteriological method.

Beside the zoonotic potential of SE transmitted through poultry, the main aim of this study was to evaluate PCR as a rapid accurate diagnostic tool applied for detection of SE in both chicken and human.

MATERIALS and METHODS

- **Collection of samples for surveying *Salmonella*:**
  110 chicken samples were collected randomly from naturally infected farms in Al-Rahmania district in Behera Province, Egypt. Birds suffered from mottled liver with yellowish discoloration, distended gallbladder, slight air sacculitis, congested lungs and enlarged spleens. Liver, heart, spleen and cloacal swabs from

chickens were sampled. In addition, 50 human stool samples were collected from persons suffering from pyrexial illness with diarrhea and vomiting for several days hospitalized in the surrounding areas of examined poultry farms.

- **Isolation of *Salmonella*** was carried out according to Waltman *et al.* (1998).
- **Identification of *Salmonella* isolates** was carried out in the Serology Unit in (Animal Health Research Institute, Dokki Giza).
- **Detection of SE by PCR** was carried out according to Soumet *et al.* (1999):
  The primers were synthesized and supplied by Pharmacia Co. Biolegio BV and were selected to specifically amplify the SefA gene of SE. Two primers were needed:
  Upstream primer with a sequence of 5/AGG TTC AGG CAG GGG TTA CT 3 /.
  Downstream primer with a sequence of: 5 / GGG AGA TTT AGG GTT TCT TG3 /.

RESULTS

Table 1: Percentage of isolation of *Salmonella* from different samples under investigation.

Examined samples	No. of examined samples	Positive samples	
		No.	%
Chickens	110	4	3.63
Human	50	2	4
Total	160	6	3.75

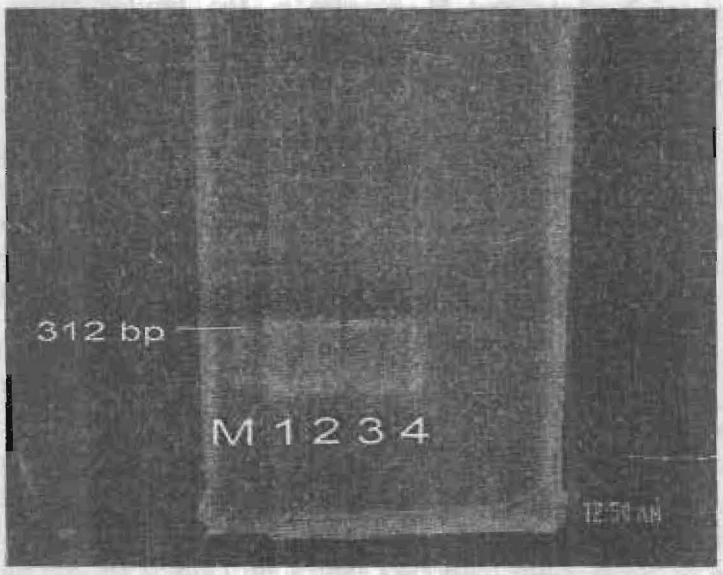
Table 2: Serological identification of *Salmonella* isolates.

No of identified isolates	Antigenic structure			Group	Salmonella serotype
	Somatic (O) antigen	Flageller (H) antigen			
		Phase I	Phase II		
4	1,9,12	g.m	–	O:9 (D1)	*S. Enteritidis*

Table 3: Results of examination of positive samples by PCR using SefA gene of *S. Enteritidis*:

	No of examined samples	Positive	%
Chickens	4	3	75
Human contacts	2	1	50
Total	6	4	6.66

Photo: Sowing the ethidium bromide stained gel of the PCR products of isolates of *S. Enteritidis*.



Lane M: 100 bp DNA ladder. Lanes (1- 4): Positive isolates for *S. Enteritidis*

Lanes 1, 2, and 3 are isolates from chickens, lane 4 human isolate

DISCUSSION

Poultry industry plays an important role in offering cheap source of animal protein for human beings in Egypt where prices of red meat, milk products and other sources of animal protein reached very high levels. At the same time, poultry can be considered a hazardous source

of human infection by many zoonotic diseases including SE infection. Accurate and rapid diagnosis is the corner stone in controlling such zoonosis.

The percentage of isolation of *Salmonella* from 110 chicken samples was tabulated in Table 1 that clarified that the total percentage of Salmonella isolated from organs and cloacal swabs was 3.63 %. This result was found in agreement with that reported by Kim *et al.* (2007); Trawiska *et al.* (2008) who isolated *Salmonella* from broiler chicken farms. The recorded isolation rate of *Salmonella* in the current study was higher that recorded by Hanson *et al.* (2002) (2.25%), while it was lower than that reported by Jafari *et al.* (2007) (5.8 %) and Ayoub (2010) (5.62 %). The isolation of Salmonella from chickens could be an indication of low degree of hygiene and biosecurity in the broiler farms under investigation, state of health, state of immunity and medication during sample collection. Moreover, the variation in isolation rates may be attributed to various methods of isolation and purification. Also, the data presented in Table 1 showed that the percentage of isolation of Salmonella from 50 human stool samples was 4 %. This result was higher than that obtained by Schmid *et al.* (2008) (2.7 %). On contrary, it was lower than that recorded by Heir (2002) (32 %), Arshadab *et al.* (2007) (9.9 %), Toyofuku (2008) (7 %) and Arnold *et al.* (2010) (14 %).

The biochemically identified *Salmonella* isolates were subjected to serological identification using polyvalent and monovalent (O) and (H) *Salmonella* antisera. The results of serological identification illustrated in Table 2 were 3 isolates proved to be SE isolated from chicken and one isolate proved to be SE isolated from human. This finding was in agreement with Sobel *et al.* (2000) who proved that the percentage of *Salmonella* outbreaks that were caused by SE increased from 47% to 55%, and SE was the most common cause of all food borne disease outbreaks in the United States during this same period. Although some serotypes, such as *S. typhimurium,* were common in many different animal species, a number of *Salmonella* serotypes have specific animal reservoirs that are thought to contribute to disease in humans. This might support the higher recovery of SE among the whole isolates.

The present study highlighted that SE was most prevalent Salmonella serotype affects human with history of contact with poultry. It was agreed with the study of Al-Nakhli *et al.* (1999); Carli *et al.* (2001); Abd El-Haleem (2003); Adam (2006); El-Zenky *et al.* (2007); Shita (2009) and Ayoub, (2010).

Because *Salmonella* has veterinary and public health hazards, there is an increasing need for rapid and accurate tool for detection of Salmonella infection in both chicken and human. PCR is more sensitive than conventional methods due to its increased sensitivity compared to culture techniques. Also, PCR has many advantages as it is highly specific and sensitive, results can be obtained within 24 -30 hrs period which could be of critical importance for the food industry (Meran Sleim, 2003; Shita, 2009; Ayoub, 2010). So, depending on above mentioned facts, a PCR based detection assay was employed in this study for detection of SE. From the whole 6 revealed *Salmonella* isolates, SE was represented in 4 out of 6 isolates at a percentage of 6.66 % as shown in Table 3.

The photo displayed PCR products using SefA gene of SE on agarose gel stained with ethidium bromide. The DNA bands of 312 bp were an indication for the presence of SE. Lanes number 1, 2, and 3 represented isolates from chickens while lane number 4 represented human isolate. This result agreed with the finding of Oliveira *et al.* (2002); Eyigor and Carli (2003); Allgayer *et al.* (2008) who found that 312 bp notified the presence of SE DNA. These results indicated that PCR technique is more accurate, rapid and sensitive test than culture method. This result supported by that reported by Soumet *et al.* (1999); Waage *et al.* (1999); Allgayer *et al.* (2008).

The obtained results from this study confirmed the role of chicken in transmitting SE to human contacts that represented a real problem needs to be more investigated and effective control measures to be established in order to prevent human infection.

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