Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Use of Genetic Method for Investigating of *Salmonella typhimurium* and *Salmonella Dublin* Isolated From Local Cows in Iraq

Halah A. Awadh¹, Hiba Y. Khalaf², Hala M. Majeed³, Nawar A. Jasim² Bashar S. Noomi*²,Nihad A. Jafar², Nawaf N. Dhaher²
¹College of Science, Tikrit University, Iraq.
²College of Veterinary Medicine, Tikrit University, Iraq.
³College of Medicine, Ibin Senaa University, Iraq.

THIS study carried out in Salah aldeen province in period from January to September 2019. The aims of this study were to investigate incidence of *Salmonella typhimurium* and *Salmonella dublin* in local Iraqi cows by using of PCR test.

The results of current study showed that the rate of isolated Salmonella species was 13.3% by culture methods, highest of them from aborted cows in rate of 22.2%.

PCR test detected *Salmonella typhimurium* and *Salmonella dublin* in rate of 55% and 25% respectively, while other Salmonella species has been detected in rate of 20% from total Salmonella isolates.

We can concluded from this result that highly incidence of Salmonella in cow and *Salmonella typhimurium* is most isolated type.

Keywords: *S. typhimurium* , *S. dublin*, local cows , Iraq.

Introduction

Salmonellosis is one of most important zoonotic dangerous diseases, caused by bacteria belongs to genera of Salmonella [1].Salmonella is Gram negative bacilli, non-spore forming ,non-capsulated, motile by Peritrichous Flagella except *Salmonella gallinarum*(*S. gallinarum*) and *Salmonella pullorum (S. pullorum*) . lactose and sucrose non ferment while ferment of glucose, maltose and sorbetoil [2].

Salmonella enterica classified in to six groups which numbering into I, II, IIIa, IIIb, IV , and VI, that's: *Salmonella* Enterica subspecies Enterica, *Salmonella* Enterica subspecies Salamae, *Salmonella* Enterica subspecies Arizonae, *Salmonella* Enterica subspecies Diarizonae, *Salmonella* Enterica subspecies Houtenae and *Salmonella* Enterica subspecies Indica[3].

The main shedding ways of Salmonella is by Feces, abortion discharge, abortion fetus and milk[4].Contamination food and water is main infection methods, when bacteria reach to intestine, it invade mucosal membrane and Lining on Peyer's patches after that reach to blood stream and reach to internal organs by macrophage [4].

Main clinical signs in cow are depression, loss of appetite, decrease in milk production, fever , diarrhea, abortion may be occur in rate of 75%, arthritis and respiratory singes may be appear. All of all the disease characterized by three forms which are septicemic form, acute enteric form and chronic enteric form [5].

Due to close relations among salmonella species in clinical signs even in diagnostic methods, so that therapid, specific and sensitive detection method of Salmonella is by genetic methods like PCR test[6].

The aims of this study were to investigate incidence of *Salmonella typhimurium* and *Salmonella dublin* in local Iraqi cow.

Material and Methods

The current study conducted in Salh aldeen Province In period from January to September 2019 on local breed cows in Iraq.

Sample collection: Samples of Bovine diarrhea collected by sterile swabs from rectum, Samples of aborted cows collected bysterile swabs from vagina in period didn'texceed three days after abortion. Bile of slaughter cow Samples collected bysterile syringes, Whilemilk samples of from aborted cow collected by sterile test tubes. Number of sample as in table (1).

Bacterial culture: all samples cultured in selenite F broth and cultivated at 37°C for 24h, then subcultured on Salmonella – Shigella agar, Xylose Lysine Deoxycholate agar and MacConkey agar. After colony appearance, gram stain and groups of biochemical tests were applied according to Quinn et al.[7].

DNA extraction: One colony were dissolved in Eppendorf tube in 200µl of DNase free water then heated in water bath at 100°C for 10 minutes. Eppendorf tube transmitted to ice then centrifuged at 12000c/m for 20 seconds. supernatant were taken and kept in -20°C. [8].

Primers used: two types of primers were used: type, size and sequence of primers as in Table 2.

- PCR reaction mixture : as in Table 3

Thermocyclar program: 30 cycles, each cycle consists from:
Denaturation step: 95°C for 30 seconds.
Primer-annealing step: 60°C for 1min.
DNA extension step: 72°C for 1min.
Then one cycle of Final DNA extension step at 72°C for 7mins.

TABLE 1. Number and types of samples .

Type of sample	Number of sample
Samples of Bovine diarrhea	42
Samples of aborted cows	18
Bile of slaughter cow Samples	64
Sample of milk from aborted cow	26
Total	150

TABLE 2. Primers used in study .

Gene name		Gene text	product size	Reference
S.Typhimurium	F	CGGTGTTGCCCAGGTTGGTAAT	559	El Jakee et al.[9]
	R	ACTCTTGCTGGCGGTGCGACTT		
S. Dublin	F	ACGCGAAATCTGATGGTCTT	203	Stegniy et al.[10]
	R	GCCCACCAGTTGTGAAAGGC		

TABLE 3. PCR reaction mixture Compounds.

Compound	Amount
Taq PCR Master Mix KIT (Qiagen, Germany	25µl
For word primer	1.4µl
Refers primer	1.4µl
DNA template	3µl
DNA free water	21.4µl
Total	50

Results

Result of bacterial isolation

From Table 4 it shows that's Salmonella isolated in a rate of 13.3% from different samples, highest isolation ratio recorded from aborted case and lowest isolation ratio recorded in diarrhea cases.

The rate of diarrheal salmonella that recorded in current study is more than the rate recorded by Noomi, [11] which is 5.4% and the rate recorded by Habeeb, [12] which 4.8% that may be due to different in zone and time of study, number of samples and diagnosis techniques.

This study showed that Salmonella caused abortion in rate of 22.2% , that referred to high ability of Salmonella to cause abortion. The highly Salmonella distribution may be occurred due to admitting sensitive or infected animal to herd or due to contaminated food, water, instrument and etc. [13]

From Table 5 showed that's domination of *S. typhimuriumin* compare with *S. dublin* in all pathological sample subjected in current study, also no detection of *S. dublin* in diarrhea cases.

The overview of the result sowed that high detection rate of Salmonella from the bile of slaughter cow Samples even that the sample is taken from healthy cows, this confirms its ability to survival in bile salt .

The current study showed that *S.Typhimurium* and *S. dublin* is the most isolation species , this result agreed with study of Wary and Davies [14]. Also in this study showed that *S.Typhimurium* is the dominant isolated type in compare with *S. dublin*, this result agreed with study of some authors [13,14].

TABLE 4. Salmonella isolation ratio from different samples.

Type of sample	Number of sample	No. of Salmonella isolate	Rate of Salmonella isolation
Samples of Bovine diarrhea	42	3	7.1%
Samples of aborted cows	18	4	22.2%
Bile of slaughtered cow Samples	64	11	17.1%
Sample of milk from aborted cow	26	2	7.6%
Total	150	20	13.3

TABLE 5. Isolation ratio of *Salmonella typhimurium* and *Salmonella dublin* from cow .

| Type of sample	No. of Salmonella isolate	No and rate of *S. typhimurium* isolates		No and rate of *S. dublin* isolates		No and rate of other Salmonella isolates	
		No.	Rate	No.	Rate	No.	Rate
Samples of Bovine diarrhea	3	2	66.6%	0	0.0%	1	33.3%
Samples of aborted cows	4	1	75.0%	3	25.0%	1	0.0%
Bile of slaughter cow Samples	11	7	63.6%	1	9.0%.	2	18.1%
Samples of milk from aborted cows	2	1	50%	1	50%	0	0.0%
Total	20	11	55.0%	5	25.0%	4	20.0%
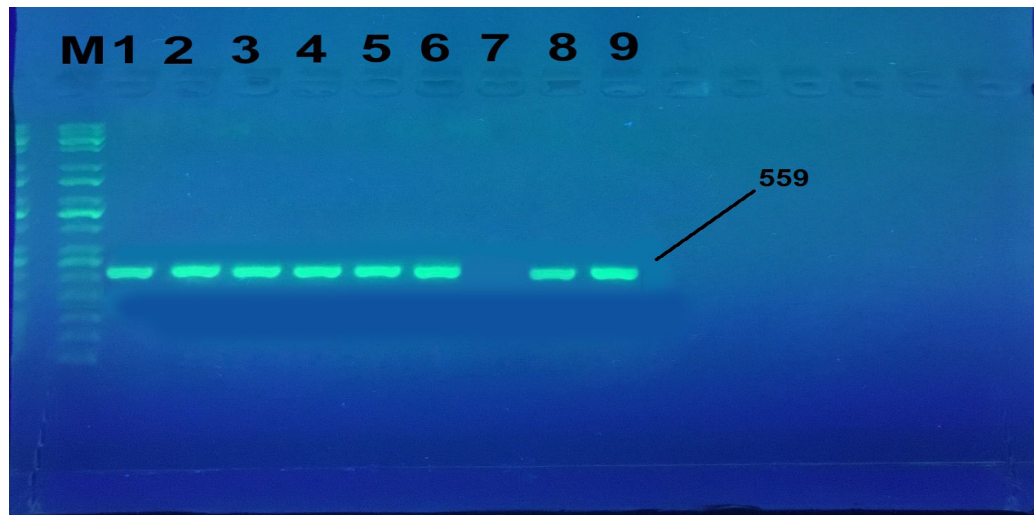
Fig. 1. Electrophoresis on 2 % a garose gel and ethidium bromide staining, showing the results of PCR procedures. M: DNA marker, 2-8 positive results of *Salmonella typhimurium* with band in size 559 bp.
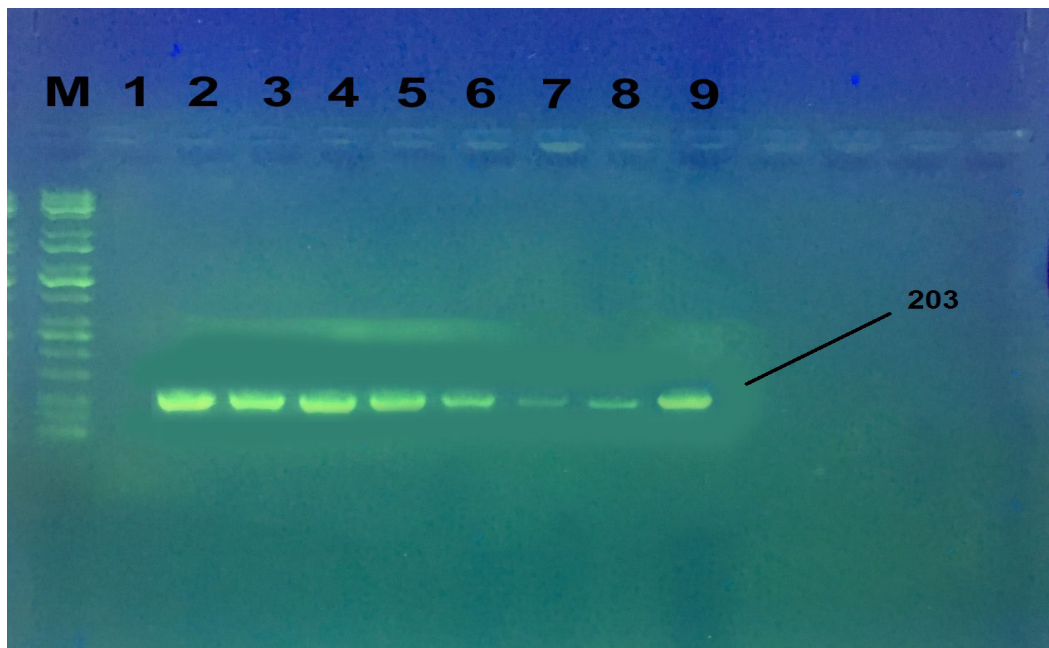

Fig. 2. Electrophoresis on 2 % a garose gel and ethidium bromide staining, showing the results of PCR procedures. M: DNA marker, 2-8 positive results of *Salmonella dublin* with band in size 203 bp.

Conclusion

We can concluded from this result that highly incidence of Salmonella in cow and *Salmonella typhimurium* is most isolated type

Ethical consideration: The study was conducted according to the ethical standards and institutional guides that recorded in Instructions of the Ministry of Agriculture and Animal Health Law

References

1. Chiu, L.H., Chiu, C.H., Horn, Y.M. Shil S.K. and Christensen J.P. Characterization of 13 multi-drug resistant Salmonella serovars from diff erent broiler chickens associated with those of human isolates. *BMC Microbiology*, **10**, 86-93 (2010).

2. Old, D.C. *Salmonella* in: Mackie & McCartney Practical Medical Microbiology. 4th ed. Edited by Collee, J.G., Fraser, A.G., Marmion, B.P., Simmons, A. (Ed.,) Longman Singapore Publishers (Pte) Ltd. PP. 385-403(1996).

3. Battistuzzi, F.U., Feijao, A. and Hedges, S.B. A genomic time scale of prokaryote evolution: insights into the origin of methanogenesis, phototrophy, and the colonization of land. *BMC Evolutionary Biology*, **4**, 44-54 (2004).

4. Ekperigin, H.E. and Nagaraja, K.V. *Salmonella. Vet. Clin. Nor. Ameri.*, **14** (1), 17-29 (1998).

5. Andrew, A.H., Blowey, R.W., Boyd, H. and Eddy, R.G. Bovine Medicine, Diseases and Husbandry of cattle, 7thed. A Black Well publishing company (2004).

6. Mohammed, M., Le Hello, S., Leekitcharoenphon, P., and Hendriksen, R. The invasome of Salmonella Dublin as revealed by whole genome sequencing. *BMC Infectious Diseases,* **17**(1), 544-546(2017).

7. Quinn, P.J. , Carter , M.E. , Markey , B.K. and Carter , G.R. «Clinical Veterinary Microbiology» Mosby . Wolfe , London(1998) .

8. OIE, Terrestrial Manual. Caprine and ovine brucellosis (excluding *Brucellaovis*) Chapter 2.7.2. (2009)

9. El Jakee, J., Khelfa, D.E., El-Safty, M.M., Seida, A.A., Marouf, S., Hahne, J. and Nagy, S.S. Multiplex PCR-based detection of *Salmonella*Typhimurium and *Salmonella* Enteritidis in Specific Pathogen Free (SPF) and Commercial Eggs. *Clinical Microbiology*, **5**(2),1-5(2016). DOI: 10.4172/2327-5073.1000241

10. Stegniy, B., Gerilovych, A., Arefyev, V., Glebova, K. and Potkonjak, A. A method for detecting and typing of Salmonella by multiplex PCR. *Arhiv, Veterinarske Medicine*, **7**(2), 47-56(2014).

11. Noomi, B. S. Distribution of disease caused by of *S.typhimurium* in calves and cattle. Thesis collage of Vet. Medicine . Baghdad (2007)

12. Habeeb, Z. S. diagnostic study of *S.Typhimurium* in patient and cattle. Thesis collage of vet. Medicine . Baghdad University(2003).

13. Dargatz, D.A.; Wells, S.J., and Akkina. J. The Veterinarian's Role in Diagnosis, Treatment and Prevention of Multidrug Resistant *Salmonella typhimurium* DT104. *J. Bovine Practitioner*, **6**,186-211 (1998).

14. Wary, C.; and R.H. Davies. A Veterinary view of *Salmonella* in farm animals. *PHLS Microbiology Digest.*, **13**,44-48 (1996) .

استخدام الطرق الجينية لتميزالسالمونيلا تايفيميوريم والسالمونيلا دبلن المعزولة من الابقار المحلية في العراق

هالة عبد الخالق عوض¹، هبة يونس خلف²، حلا محمد مجيد³، نوار علي جاسم²، بشار صادق نومي²، نهاد عبد الحسين جعفر² و نواف نور الدين ظاهر²

¹ كلية العلوم- جامعة تكريت،العراق ² كلية الطب البيطري- جامعة تكريت،العراق ³ كلية الطب- جامعة ابن سينا- العراق .

أجريت هذه الدراسة في محافظة صلاح الدين/ العراق للمدة من شهر كانون الاول الى شهر ايلول من العام ٢٠١٩، وقد هدفت هذه الراسة الى تميز السالمونيلا تايفيميوريم والسالمونيلا دبلن المعزولة من الابقار باستخدام اختبار تفاعل البوليمرات المتسلسل.

اظهرت نتائج الدراسة الحالية ان نسبة عزل جراثيم السالمونيلا كانت ١٣،٣٪ وقد سجلت اعلى نسبة عزل من عينات الاجهاض بلغت ٢٢،٢٪. اظهرت نتائج اختبار تفاعل البوليمرات المتسلسل ان جراثيم السالمونيلا تايفيميوريم والسالمونيلا دبلن شكلت ٥٥٪ ، ٢٥٪ على التوالي في حين شكلت بقية انواع السالمونيلا نسبة ٢٠٪ من مجموع عزلات السالمونيلا. من خلال النتائج يمكن ان نستنتج ان السالمونيلا منشرة بشكل كبير وان السالمونيلا تايفيميوريم هي الاكثر انواع السالمونيلا انتشاراً.

الكلمات الدالة: السالمونيلا تايفيميوريم ، السالمونيلا دبلن، الابقار المحلية في العراق.