Rapid Detection of *Salmonella* in Dairy Cows Using Polymerase Chain Reaction

Hamza Mohamed Ibrahim Eid
Department of Bacteriology, College of Veterinary Medicine.Suez Canal University, Egypt.

Abstract: Salmonella is widely distributed in cattle in different countries and it is considered the most important related zoontic diseases today which have a public health and economic importance. A total of 298 faecal samples were collected from dairy cows and calves from suez canal area. All samples were subjected to bacteriological examination. Eighteen Salmonella strains were serologically identified using specific antisera. All Salmonella strains were examined using polymerase chain reaction DNA was extracted from Salmonella strains , Two oligonucleotide primers were used for detection of Salmonella invA gene .Thirty PCR cycles were performed with 56oC annealing temperature .PCR products were examined using Agarose gel electrophoresis 2% Agarose in TBE which revealed single 243 base pair amplified DNA fragment. [Journal of American Science. 2010;6(10):31-37]. (ISSN: 1545-1003).

Keywords: Detection; *Salmonella;* Dairy Cows; Polymerase Chain Reaction

1. Introduction

Salmonella is found worldwide in cattle and is considered the most important animal related zoontic diseases today Anderson *et. al* (1999).

Salmonella is endemic in many intensive dairy farms however outbreaks of disease are relatively infrequent and typically reflect a combination of environmental conditions and management House and Smith *et. al* (2004).

Traditional laboratory culture techniques consume long time before getting a positive or negative result. Molecular techniques using genetic probes and polymerase chain reaction give rapid, sensitive and specific detection of pathogens in the environment Jose phson *et. al*. (1991).

Microbiological culture of faecal samples was preferable than rectal swap culturing particularly in the latent infections Bager and Baggesen *et. al.* (1993)

Dairy cows may serve as asymptomatic carriers of Salmonella. The potential herd carrier status increases with herd size and Salmonella shedding may be triggered by stresses placed on the animals. Hume *et. al.*(2004)

Detection of Salmonella serovars in clinical samples from pigs, horses and cattle by polymerase chain reaction is more rapidly than conventional culture techniques, the sensitivity and specificity of this assay were 100% compared with culture techniques. The method could be applied for rapid routine diagnosis Stone *et. al.* (1994).

Salmonella organisms were screened in poultry faecal samples using a20 –h real time PCR, The test supplies the growing demand for validated diagnostic PCR methods for screening of samples in meat production chain to assure safe food Lofstrom *et. al.* (2010). The objectives of this study are isolation and typing of Salmonella organisms using different bacteriological methods and identification of Salmonella using polymerase chain reaction.

2. Material and Methods

1- Samples:

Twenty seven Salmonella strains were isolated and identified serologically from 298 fecal samples collected from dairy cows 173 apparently healthy dairy cows, 68 diarrheic dairy cows, 24 apparently healthy calves and 15 diarrheic calves with age over one month from Suez Canal area. Salmonella strains were subjected for molecular characterization using polymerase chain reaction. Cruickshank *et. al.* (1975), Kauffman, (1974) and Edwards and Ewing (1972).

2- DNA extraction

Salmonella strains were cultivated onto Luria Bertani (LB) agar for 24 hours at 37^{o}c then extraction of DNA was done according to Sambrook *et. al*. (1989).

3-Primers:

Two 24-mers oligonucleotides primers as mentioned in Table (2) were obtained from (MWG Biotech AG,

Germany) were used as pooling primers for Salmonella invA gene.

Oligo Name	Oligo number	Primers sequence (5 – 3)	length	Melting point	GC %
A1	70425X137F 03 23/30	5 ACA GTG CTC GTT TAC GAC CTG AAT- 3	24	64.0	45.8 %
A2	70425X137F 04 24/30	5 AGA CGA CTG GTA CTG ATC GAT AAT- 3	24	62.0	41.7 %

4- Polymerase chain reaction

Amplification program is performed according to Singer *et. al.* (2006) PCR was carried out in 25 ul reaction volumes 12.5 ul 2× PCR master mix 0.47 ul inv A 1 (inv A1 0.47 ul (0.3 uM)), 0.48 ul inv A2 inv A 0.48 ul (0.3uM), and 1.7 ul NA template c1 ug, . The reaction was completed upto 25 ul with distilled water.

The PCR system was programmed to 5 min for denaturation at 94°C, 30 cycles of denaturation at 94°C for 30 sec, Annealing at 56°c for 30 sec and extension at 72°C for 2min. after completion of the final cycle, the reaction was held at 72°c for 7 min then stored at 4°c.

5- Agarose gel electrophoresis

was done according to Sambrook *et. al. (*1989). PCR products were electrophoreses at 2% (wt/ vol) agarose and 0.5 ug of ethidium bromide (Sigma) permlin TBE buffer. The samples were electrophorsed at 85 volt for 1.5 to 1.8 hour. A300 nm uv transillumination was used to detect. The bands which were then photographed with a Polaroid camera.

3. Results and Discussion

Table (1): Number and percentage of *Salmonellae* isolates in dairy cows according to biochemical identification:

Animal	Total no.	Positive faecal samples		Negative faecal samples	
		No.	%	No.	%
Apparently healthy dairy cows	173	12	6.9 %	161	93 %
Diarrheic dairy cows	86	10	11.6%	76	88 %
Total	259	22	8.5%	237	91.5 %

Table (2): Number of *Salmonella* isolates in calves according to biochemical identification:

Animal	Total no.	Positive faecal samples		Negative faecal samples	
		No.	%	No.	%
Apparently healthy calves	24	6	25 %	18	75 %
Diarrheic calves	15	8	53 %	7	47 %
Total	39	14	35.9%	25	64.1 %

Table (3): Serological diagnosis of *Salmonella* in cultural and biochemical positive samples in dairy cows:

*% was calculated upon the no. of positive biochemical isolates

Animal	No. of positive samples	Positive faecal samples		Negative serology samples	
		No.	%	No.	%
Apparently healthy dairy cows	12	3	25 %	9	75 %
Diarrheic dairy cows	10	6	60 %	4	40 %
Total	22	9	40.9 %	13	59 %

Table (4):Serological diagnosis of *Salmonella* in cultural and biochemical positive samples in calves:

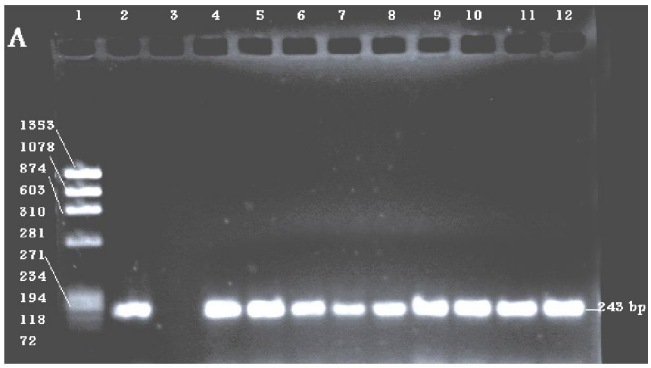
*% was calculated upon the No. of positive biochemical isolates.

Animal status	No. of positive samples	Positive serology samples		Negative serology samples	
		No.	%*	No.	%*
Apparently healthy calves	6	2	33.3%	4	66.6 %
Diarrheic calves	8	7	87.5%	1	12.5 %
Total	14	9	64.3%	5	35.7 %

Table (5): The prevalence of *Salmonella* serovars among dairy cows and calves:

Animal status	*Salmonella* spp.	No.	%
Apparently healthy dairy cows (173)	serovar Dublin serovar Typhimurium	2 1	1.2 % 0.6 %
Diarrheic dairy cows (86)	Subsp. *III arizona* serovar Typhimurium	4 2	4.7 % 2.3 %
Apparently healthy calves (24)	Subsp. *III arizona* serovar Typhimurium	1 1	4.2 % 4.2 %
Diarrheic calves (15)	Subsp. *III arizona* serovar Typhimurium serovar Newport	1 3 3	6.7 % 20 % 20 %
Total (298)	-	18/298	6.2 %

Photo (1): PCR detection of serologically positive *Salmonella* isolates using invA primer.



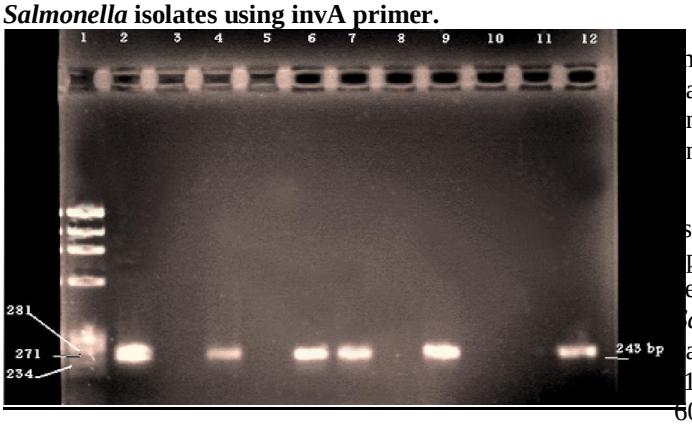
Lane 1: marker DNA.

Lane 2: control +ve. (243bp)

Lane 3: control –ve .

Lane 4 – 12: +ve PCR product

Photo (2): PCR detection of serologically negative *Salmonella* isolates using invA primer.



Lane 1 : DNA marker

Lane 2 : control + ve

Lane 3 : control – ve

Lane 4,6,7,9 and 12 + ve PCR products

Lane 5,8,10 and 11: - ve PCR products

In this study the percentage of *Salmonella* isolated from diarrheic cattle was 11.6% (Table 1). These results nearly agree with Kim-YongHwan *et al*. (2000) that isolated *Salmonella* spp. with percent of (8.7 %) from faecal samples of dairy cows and disagree with Sato *et al.* (2001) who isolated *Salmonella* ssp. at higher rate reach (43. 8 %) from diarrheic dairy cows and Murinda *et al.* (2002) isolated *Salmonella* spp. with percent of (25.3 %).

Concerning the total percentage of *Salmonella* isolated from apparently healthy calves, it was 25% (Table 2). These results disagree with Acha *et al.* (2004) who isolated *Salmonella* spp. with percentage of (2 %).

In diarrheic calves the prevalence of *Salmonella* was 53% (Table 2). These results were nearly agreed with Vena *et al.* (1984) isolated *Salmonella* with percent of (30 %); Kaura (1990) who stated that diarrhea more prevalent in winter than in summer (48.57 %) and Tanios *et al.* (1999) who isolated *Salmonella* spp. with an incidence rate of (34 – 85 %) and disagree with Sato *et al*. (1993) who recorded a relatively small scale outbreaks of *S. dublin* infection occurred repeatedly in these years (1989 to 1991); Hoda (1994) isolated *Salmonella* in lower rate (9.4 %) from faecal swabs of diarrheic claves and Aydin *et al*. (2001) who isolated 1 *Salmonella typhimurium* strain (0.99%) on contrary isolated other pathogenic bacteria and parasite (93 *Escherichia coli* (92.07%), 2 *Campylobacter jejuni* (1.98%),) 6 *Eimeria spp.* (5.94%), 10 *Toxocara vitolorum* (9.90%) and 6 *Cryptosporidium spp.* (5.94%)) of faecal specimens from diarrheic calves.

These variations in prevalence of *Salmonella* n apparently healthy and diarrheic dairy cows and alves may be attributed to the management and the nvironment and the immune status of affected nimals (House *et al.*, 2003).

Concerning the serotyping of *Salmonella* solates from dairy cows and calves (Table 5) it was ppear that *Salmonella enterica* subsp. *enterica* erovar Typhimurium considered the major cause of *almonella* infection among the dairy cows and alves and this result was agree with Farid *et al.* 1987) who recovered 25 *Salmonella* strains out of 600 samples and typed as *Salmonella typhimurium* with presence of other serotypes (*Salmonella dublin, Salmonella bovismorbificans, Salmonella reading, Salmonella derby* and *Salmonella enteritidis*) but those strains were not isolated at this study except *Salmonella dublin* which isolated from apparently dairy cows; Ahmed *et al*. (1989) also serotyped *Salmonella typhimurium* from apparently healthy calves; McLaren and Wray, (1991) serotyped *Salmonella typhimurium* which was the commonest cause of salmonellosis in calves ranging from 4 months to two years in 5 calf farms and *Salmonella* persisted in the farm for years; Riad *et al*. (1998) who isolated *Salmonella typhimurium* in a percentage of (18. 2 %) by bacteriological examination of 66 faecal samples collected from calves suffered from watery diarrhea; Pasmans *et al*. (2000) isolated *Salmonella* serotype Typhimurium. Seven out of 8 phage-typed Typhimurium strains belonged to phage type DT104 and Veling *et al.* (2002) who reported 47 case farms

experienced a clinical outbreak of salmonellosis which was confirmed with a positive bacteriologic culture for serovar Typhimurium in one or more samples. Serovar Typhimurium phage type 401 and 506 (definitive type 104, DT104) were the most frequently isolated phage types (13 isolates). On most farms (66%), clinical signs were seen only among adult cows.

Because multiple serotypes of *Salmonellae* which cause disease, genus specific identification is useful for diagnosis and prevention of salmonellosis. Using developed oligonucleotide primers for the polymerase chain reaction that enable genus specific detection of members of the genus *Salmonella* but not other bacteria (Cohen, *et al.* 1995).

It is recorded that traditional laboratory techniques take long time from days to weeks before getting positive or negative results, molecular techniques as DNA probes and PCR provide rapid , sensitive and specific detection of pathogens (Josephson et al. 1991) .

The ability of *Salmonella* specific primers to detect *Salmonella* species rapidly and accurately is primarily due to the primer sequences that are selected from the gene invA of serovar Typhimurium. The invA gene code for protein is found in inner membrane of bacteria, which is necessary for invasion to epithelial cells (Salehi *et al.*, 2005).

Our finding of using PCR as technique for diagnosis of *Salmonella* it was found that all PCR products of isolates include positive control, screened by PCR, resulted in 243bp amplified fragment. No amplified DNA fragments were obtained from non-*Salmonella* species (Photos 1 and 2).These results agree with (Stone et al.,1994) who stated that detection of Salmonella serovars in clinical samples from cattle by PCR gives more rapidly than with conventional culture techniques. The sensitivity and specificity of PCR results were 100 % and can be applied for routine diagnosis.

Photo 2 reveals that non serologically identified Salmonella isolates can be detected using PCR. This result may be attributed to the presence of rough mutant strains which lack the specific side chains responsible for O specificity and some of them have additional abnormalities of the core structure (Topley and Wilson's, 1990). It was found that the PCR is relatively rapid and highly sensitive (Singer *et al.*, 2006).

4. References

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