JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

Adzitey et al. 2013 : 3 (1) 87-93

GENOTYPING OF SALMONELLA STRAINS ISOLATED FROM DUCKS AND THEIR ENVIRONMENTS IN PENANG, MALAYSIA USING REPETITIVE EXTRAGENIC PALINDROMIC (REP)

Frederick Adzitey[1], Gulam Rusul Rahmat Ali[*2], Nurul Huda[2] and Rosma Ahmad[2]*

Address(es): *Gulam Rusul Rahmat Ali*[*2],*
[1]University for Development Studies, Faculty of Agriculture, Animal Science Department, Box TL 1882, Tamale, Ghana. Phone number: +60122103046.
[2]Universiti Sains Malaysia, School of Industrial Technology, Food Technology Division, 11800 Pulau Pinang, Malaysia.

*Corresponding author: gulam@usm.my

ARTICLE INFO

Received 17. 1. 2013
Revised 29. 7. 2013
Accepted 29. 7. 2013
Published 1. 8. 2013

Regular article

OPEN ACCESS

ABSTRACT

Salmonella are important foodborne pathogens of worldwide concern. The objective of this study was to determine the genetic diversity of 107 *Salmonella* strains isolated from ducks, their rearing and processing environments in Penang, Malaysia using repetitive extragenic palindromic-polymerase chain reaction (REP-PCR). REP-PCR of the *Salmonella* strains produced DNA bands of different sizes for differentiation purposes. The DNA band sizes ranged from 105-7692 bp for *S.* Typhimurium, 116-7033 bp for *S.* Hadar, 127-7399 bp for *S.* Enteritidis, 140-7497 bp for *S.* Braenderup and 123-5857 bp for *S.* Albany. Cluster analysis at a coefficient of 0.85 grouped the *Salmonella* strains into various clusters and singletons. *S.* Typhimurium were grouped into 4 clusters and 26 singletons at a discriminatory index (*D-value*) of 0.98, *S.* Hadar were grouped into 3 clusters and 13 singletons at a *D*-value of 0.914, *S.* Enteritidis were grouped into 3 clusters and 9 singletons at a *D*-value of 0.971, *S.* Braenderup were grouped into 2 clusters and 11 singletons at a *D*-value of 0.981, and *S.* Albany were grouped into 3 clusters and 7 singletons at a *D*-value of 0.978. With the exception of *S.* Hadar strains which were grouped into two major groups (genotypes) by REP-PCR, the rest were grouped into three major genotypes. REP-PCR successfully typed all the *Salmonella* strains and proved to be a useful typing tool for determining the genetic diversity of the duck *Salmonella* strains. Determining the genetic diversity among *Salmonella* strains, other foodborne pathogens and their sources of isolation is important to trace their primary or potential sources and the sources of human infection.

Keywords: Ducks, genetic diversity, REP-PCR, *Salmonella* strains

INTRODUCTION

Salmonellae are important foodborne pathogens in both developed and developing countries. Salmonellae are involved in a number of foodborne disease outbreaks which have resulted into human illnesses, hospitalizations or deaths**. CDC (2010)** reported a total of 190 illnesses in the United State of America due to the outbreak of *Salmonella* Heidelberg which was linked to the consumption of contaminated kosher broiled chicken livers. Among all foodborne bacterial infections in the United State of America, salmonellae have been estimated as the second largest cause of human illnesses and first in hospitalization and human deaths **(Scallan *et al.*, 2011)**. In the United Kingdom, 9,685 cases of human salmonella infections were reported in 2010 **(Defra, 2010)**. In most developing countries, data on salmonella infections or outbreaks is unavailable due the lack of effective monitoring and reporting systems of foodborne infections.
Effective monitoring and reporting systems of foodborne pathogens will depend largely on effective surveillance studies, effective methods of isolating foodborne pathogens and/or effective characterization or typing of foodborne pathogens **(Adzitey and Corry, 2011**; **Adzitey and Nurul, 2011; Frederick and Huda, 2011; Adzitey *et al.*, 2012a).** Typing of foodborne pathogens have be achieved using molecular methods like pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST), random amplified polymorphic deoxyribonucleic acid (RAPD), enterobacterial repetitive intergenic consensus (ERIC), repetitive extragenic palindromic (REP), ribotying and many more **(Versalovic *et al.*, 1991; Jersek *et al.*, 1999; Bennasar *et al.*, 2000; Adzitey *et al.*, 2012a; Adzitey *et al.*, 2013a, b).** These typing methods are used to analyze foodborne pathogens isolated from various sources and by comparing the DNA bands of these pathogens, the genetic relatedness or diversity can be established. Comparing the DNA bands of foodborne pathogens can also lead to establishing the source of human infection and/or major source of contamination.
Duck production is an important agricultural business in Malaysia. FAO statistics in 2009 indicated that Malaysia is the third world producer of duck meats **(FAO, 2009)**. Current agricultural policies in Malaysia also continue to encourage duck farmers to increase their production for local consumption and export purposes (**Adzitey *et al.*, 2012b**). Important foodborne pathogens like *Campylobacter* species, *Salmonella* species, *Listeria* species and *Escherichia coli* have been isolated from ducks in Malaysia **(Adzitey *et al.*, 2011a, b; Adzitey *et al.*, 2012b, c, d, e; Adzitey *et al.*, 2013a).** Contact with ducklings or the consumption of duck eggs, meats or products contaminated with salmonellae have been associated with salmonellosis, hospitalization and/or death of affected persons **(Merritt and Herlihy, 2003; Noble *et al.*, 2012)**. Therefore, determining the genetic relatedness or diversity among duck foodborne pathogens and their environments is essential to give an idea about the distribution of these foodborne pathogens in various samples and their possible roles in human infections.
Thus this study was carried out to determine the genetic relatedness of *Salmonella* strains isolated from ducks and their environmental sources in Penang, Malaysia.

MATERIAL AND METHODS

Salmonella strains

A total of 107 *Salmonella* strains isolated from ducks, their rearing and processing environments were used in this study (**Adzitey *et al.*, 2012b**). The *Salmonella* strains were made up of 37, 26, 15, 15 and 14 of *S.* Typhimirium, *S.* Hadar, *S.* Enteritidis, *S.* Braenderup, and *S.* Albany, respectively. They were isolated from duck faeces (n=36), intestines (n=25), cloaca swabs (n=14), soils (n=11), wash water (n=8), pond water (n=3), carcass rinses (n=3), drinking water (n=2), floor swabs (n=2), transport crate swabs (n=2), feed (n=1) and table swab (n=1).

Extraction of deoxyribonucleic acid (DNA)

A single colony of pure *Salmonella* was inoculated into 10 ml Trypticase Soy Broth and incubated at a temperature of 37 ^{o}C overnight. One ml of the overnight culture was centrifuged for 2 min at 14,000 x g. Pelleted bacterial cells were subjected to DNA extraction using Wizard® Genomic DNA Purification Kit by

following the manufacturer's instructions (**Anonymous, 2011**). Briefly, bacterial cells were lysed in 600 µl nuclei lysis solution (for 5 min at 80 °C) and in 3 µl RNase solution (for 30 min at 37 °C). The protein was precipitated in 200 µl of protein precipitate solution, incubated on ice for 5 min and centrifuged for 5 min at 14,000 x g. DNA precipitation was achieved by transferring the supernatants into 600 µl isopropanol (centrifuged for 3 min at 14,000 x g) and into 600 µl 70% ethanol (centrifuged for 3 min at 14,000 x g). The ethanol was aspirated and the pellet air-dried for 10-15 min at room temperature. Finally, DNA pellets were rehydrated in 100 µl rehydration solution for 1 h at 65 °C and the concentration adjusted to 100 ng/µl for further use.

REP analysis of *Salmonella* strains

Extracted DNA was subjected to a modified REP-PCR described by **Versalovic *et al*. (1991) and Jersek *et al*. (1999)**. The (18-mer) primer REP1R-I (5'-IIIICGICGICATCIGGC-3') and REP2-I (5'-ICGICTTATCIGGCCTAC-3) was used for the REP-PCR. REP-PCR was carried out in a 25 µl volume containing 12.5 µl GoTaq mastermix (M5132, Promega, USA), 7 µl nuclease free water, 2 µl 25 mM $MgCl_2$, 2.5 µl template DNA and 0.5 µl of each primer (2 µM concentration). Amplification was done with the following temperature cycle: 1 cycle at 95 °C for 2 min; followed by 35 cycles at 90 °C for 30 s, 52 °C for 1 min and 65 °C for 8 min; and 1 cycle at 65 °C for 16 min. The amplification was performed using Biometra® TProfesssional thermocycler, Germany. Amplicons (10 µl) were stained with EZ-Vision® One DNA Dye (2 µl), loaded on a 1.5% agarose gel and electrophoresed at 90 V for 1h 30 min. VC 1 kb and 100 bp DNA ladders (Vivantis, USA) were used as the molecular weight marker and the amplicons were visualized under UV transilluminator gel imaging system (Bio-Rad Gel Imaging System, USA).

Cluster analysis and calculation of discriminatory index

Cluster analysis and calculation of discriminatory index were done individually for the various *Salmonella* strains. DNA band sizes were determined using the detect band button of the NTSYSpc Version 2.2 programme. Band sizes were then scored as presence of DNA band (a score '1') and absence of DNA band (a score '0'). These scores were entered in NTedit to obtain a data matrix and then inserted in NTSYSpc Version 2.2 computer software for the construction of dendogram based on simple matching coefficient and UPGMA (Unweighted Pair-Group Arithmetic Average Clustering) cluster analysis to determine the genetic relatedness of the *Salmonella* strains. Clustering was defined at a coefficient of 0.85 and *Salmonella* strains not belonging to any particular cluster were referred to as singletons (single isolates). The discriminatory index (*D-value*) was calculated according to **Hunter and Gaston (1988)**.

RESULTS AND DISCUSSION

Repetitive extragenic palindromic-polymerase chain reaction (REP-PCR) analysis of the 107 *Salmonella* strains isolated from ducks, their rearing and processing environments in Penang, Malaysia between 2009 to 2010 produced DNA bands/fingerprints of different sizes for differentiation among the strains of *Salmonella* and their sources of isolation. The DNA band sizes of *S.* Typhimurium ranged from 105-7692 bp, *S.* Hadar ranged from 116-7033bp, *S.* Enteritidis ranged from 127-7399 bp, *S.* Braenderup ranged from 140-7497 bp and that of *S.* Albany ranged from 123-5857 bp. Dendograms were constructed separately for the various *Salmonella* serovars thus *S.* Typhimurium (Figure 1a), *S.* Hadar (Figure 1b), *S.* Enteritidis (Figure 1c), *S.* Braenderup (Figure 1d), and *S.* Albany (Figure 1e). Clustering at a coefficient of 0.85 and the calculation of discriminatory index based on the number of clusters and singletons categorized the 37 *S.* Typhimirium into 4 clusters and 26 singletons at a *D-value* of 0.980, the 26 *S.* Hadar into 3 clusters and 13 singletons at a *D-value* of 0.914, the 15 *S.* Enteritidis into 3 clusters and 9 singletons at a *D-value* of 0.971, the 15 *S.* Braenderup into 2 clusters and 11 singletons at a *D- value* of 0.981, and the 14 *S.* Albany into 3 clusters and 7 singletons at a *D-value* of 0.978. **Hunter and Gaston (1988)** reported that if the *D-value* of a typing result is greater than 0.900, the typing result is desirable and can be interpreted with confidence. This study showed that the discriminatory indexes of the various *Salmonella* serovars at a coefficient of 0.85 were all greater than 0.900.

Clusters consisted of 2 or more *Salmonella* strains, for example, *S.* Typhimurium cluster 1 (TRep1), *S.* Hadar cluster 1 (HRep1), *S.* Enteritidis cluster 1 (ERep1), *S.* Braenderup cluster 1 (BRep1), *S.* Albany cluster 1 (ARep1) and so on (Figures 1a to 1e). *Salmonella* strains in the same cluster are clonal and genetically closely related. *Salmonella* serovars in the same cluster but isolated from different sources suggest possible cross contamination. Examples of such clusters are *S.* Typhimurium cluster 2 (TRep2) which consists of one isolate each isolated from duck intestines and wash water sample (Figure 1a), *S.* Hadar cluster 3 (HRep3) which consists of two isolates each isolated from duck cloaca, duck faces and soil sample, and one pond water sample (Figure 1b), and *S.* Braenderup cluster 2 (BRep2) which consists of one isolate each isolated from duck intestines and wash water sample (Figure 1d). *Salmonella* strains isolated from cloaca, wash water, soil and pond water might have taken their origin from duck intestines and faeces. This is because duck intestines and faeces are known as primary sources of *Salmonella* species rather than cloaca, wash water, pond water and wash water samples (**Defra, 2010; Adzitey *et al*., 2012b; Adams and Moss, 2008; EFSA, 2012**). Singletons (single isolates) include *S.* Typhimurium singleton i (TRepi), *S.* Hadar singleton i (HRepi), *S.* Enteritidis singleton i (ERepi), *S.* Braenderup singleton i (BRepi), *S.* Albany singleton i (ARepi) and so on (Figures 1a to 1e). Singleton strains are genetically heterogeneous to other *Salmonella* strains.

From Figures 1a to 1e, the various *Salmonella* serovars could also be grouped generally into three major groups (genotypes) except for *S.* Hadar strains which were grouped into 2 major groups. This is expected since the *Salmonella* strains were isolated from the same animal species, similar environment and geographical area. Furthermore, each of this major group consists of *Salmonella* strains isolated from different sources and places (Table 1-3). For example, *S.* Typhimurium group I consists of *Salmonella* strains isolated from soil (farm 1), wash water (processor 1, processor 3), intestines (processor 1, processor 2) and faeces (farm 1) (Table 1), *S.* Hadar group I consist of *Salmonella* strains isolated from drinking water (farm 4), pond water (farm 4), cloacal (farm 3, farm 4) and faeces (farm 3, farm 4) (Table 2), *S.* Braenderup group 1 consist of *Salmonella* strains isolated from cloacal (farm 1), intestines (processor 1), wash water (processor 1) and faeces (farm 1) (Table 3), and other major groups (Tables 1 to 3). The afore-mentioned examples strongly suggest that *Salmonella* strains of similar genotypes were distributed within ducks and their environmental samples in Penang, Malaysia between 2009 to 2010.

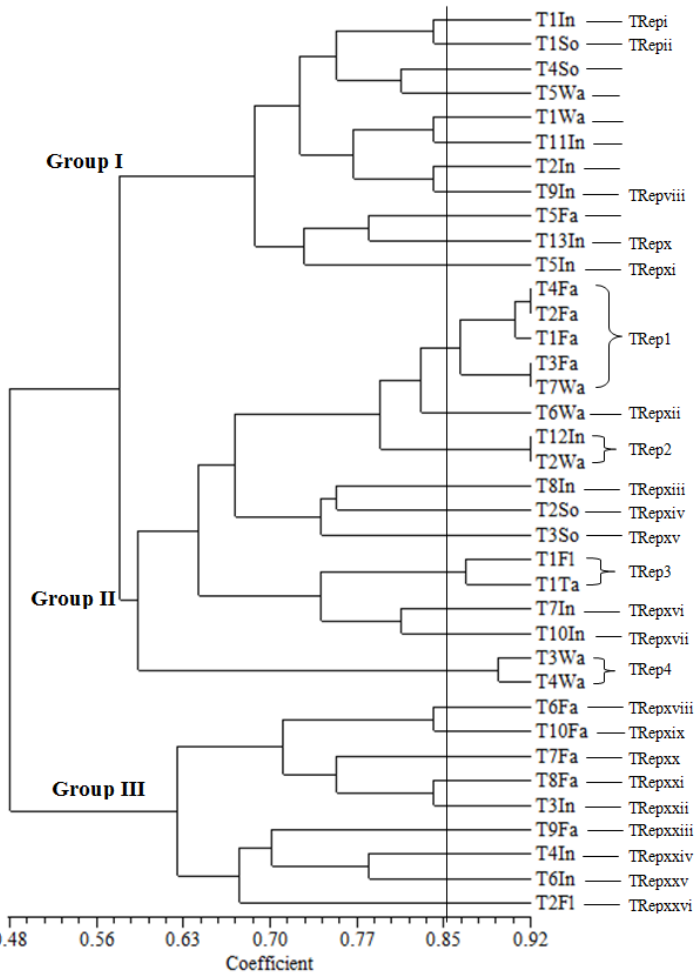

Figure 1a Dendogram showing the genetic relatedness of *S.* Typhimirium isolated from ducks and their environmental sources performed by REP-PCR. TRep1-TRep4 = *S.* Typhimirium cluster 1-4 by REP; TRepi-TRepxxvi = *S.* Typhimirium singleton i-xxvi by REP; T = *S.* Typhimirium; 1-13 = strain number; In = intestines; Fa = faeces; Wa = wash water; So = soil; Fl = floor swab; and Ta = table swab.

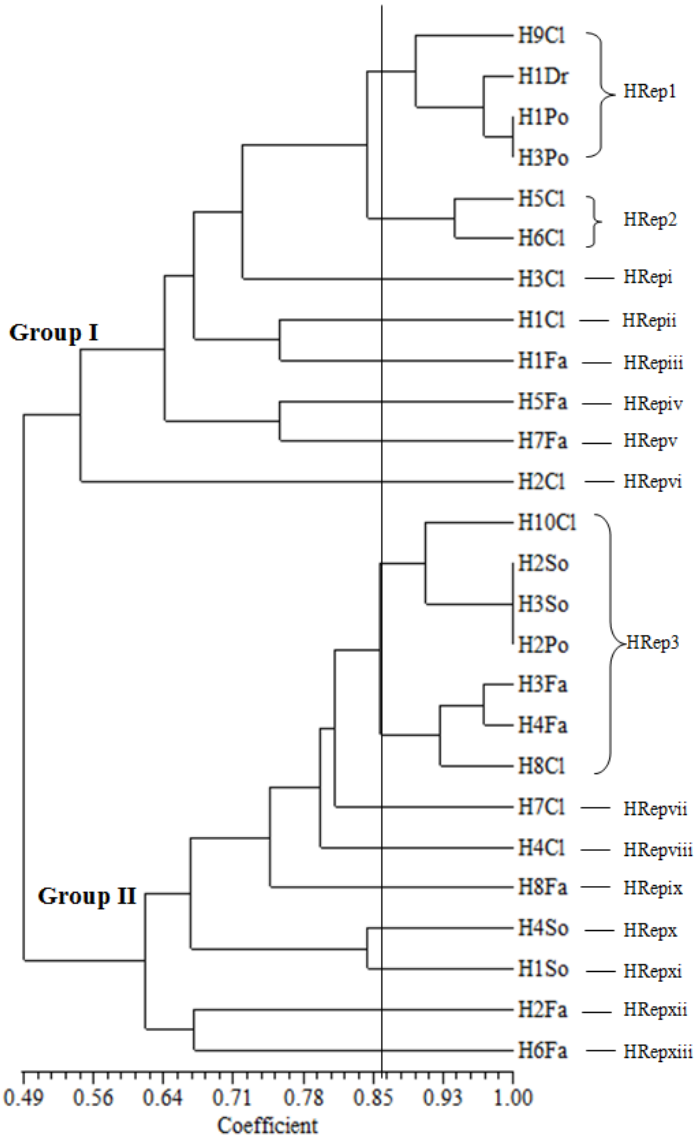

Figure 2b Dendogram showing the genetic relatedness of *S.* Hadar isolated from ducks and their environmental sources performed by REP-PCR. HRep1-HRep3 = *S.* Hadar cluster 1-3 by REP; HRepi-HRepxiii = *S.* Hadar singleton i-xiii by REP; H = *S.* Hadar; 1-10 = strain number; Cl = cloaca swab; Po = pond water; So = soil; Fa = faeces; and Dr = drinking water.

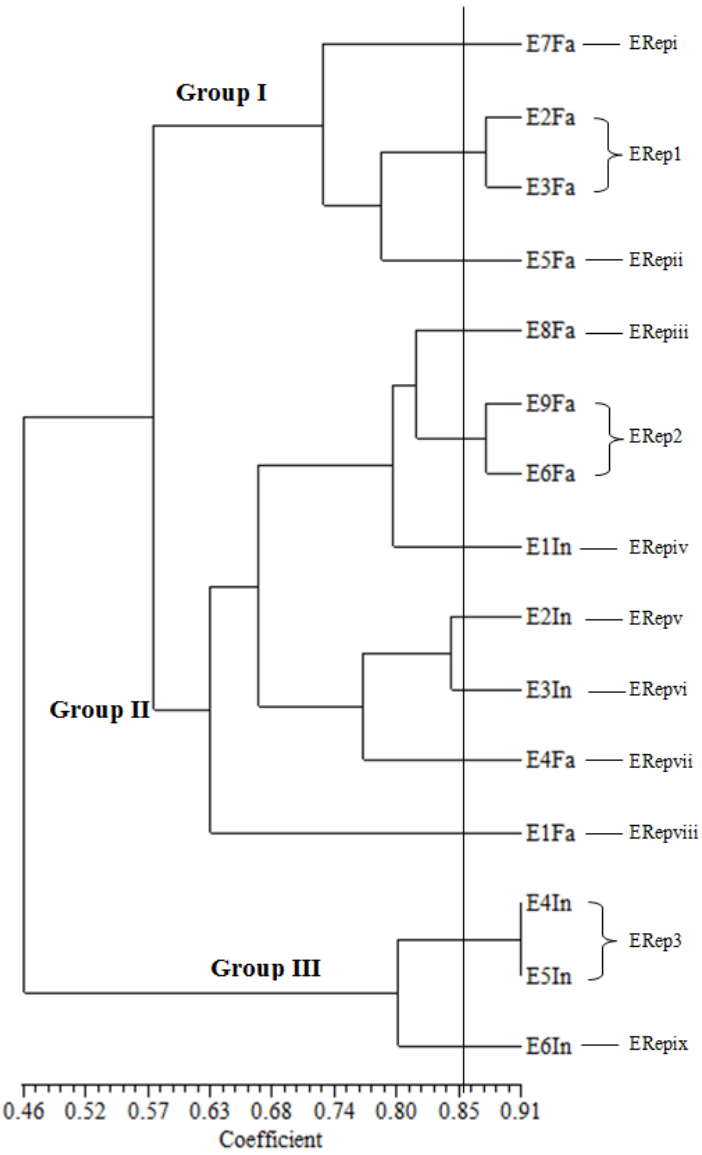

Figure 3c Dendogram showing the genetic relatedness of *S.* Enteritidis isolated from ducks and their environmental sources performed by REP-PCR. ERep1-ERep3 = *S.* Enteritidis cluster 1-3 by REP; ERepi-ERepix = *S.* Enteritidis singleton i-ix by REP; E = *S.* Enteritidis; 1-9 = strain number; Fa = faeces; and In = intestines.

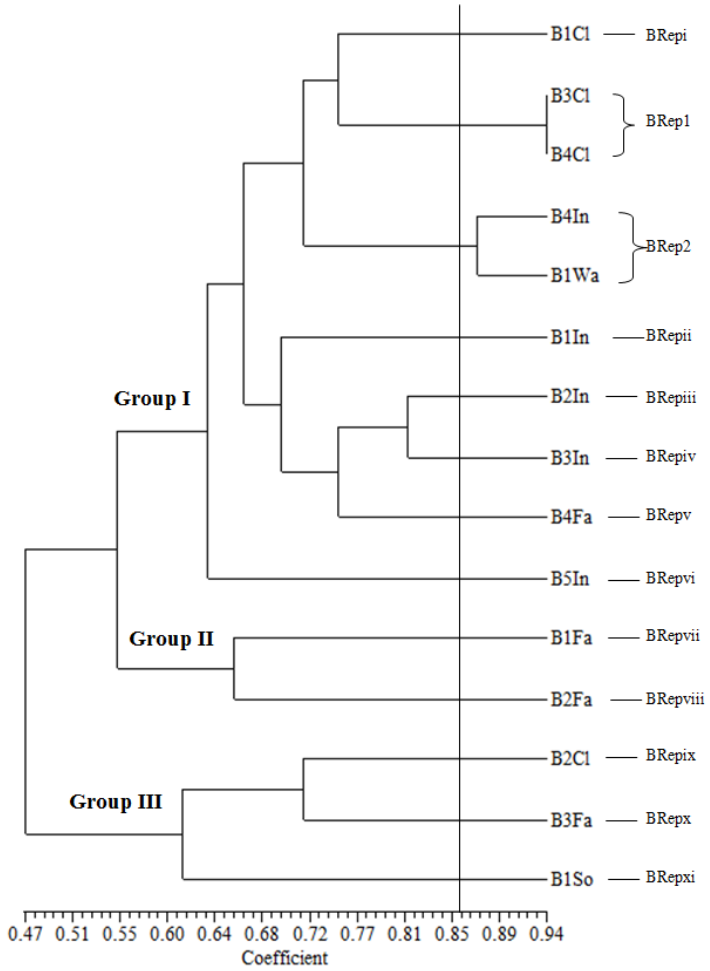

Figure 4d Dendogram showing the genetic relatedness of *S.* Braenderup isolated from ducks and their environmental sources performed by REP-PCR. BRep1-BRep2 = *S.* Braenderup cluster 1-2 by REP; BRepi-BRepxi = *S.* Braenderup singleton i-xi by REP; B = *S.* Braenderup; 1-5 = strain number; Cl = cloaca swab; So = soil; In = intestines; Fa = faeces; and Wa = wash water

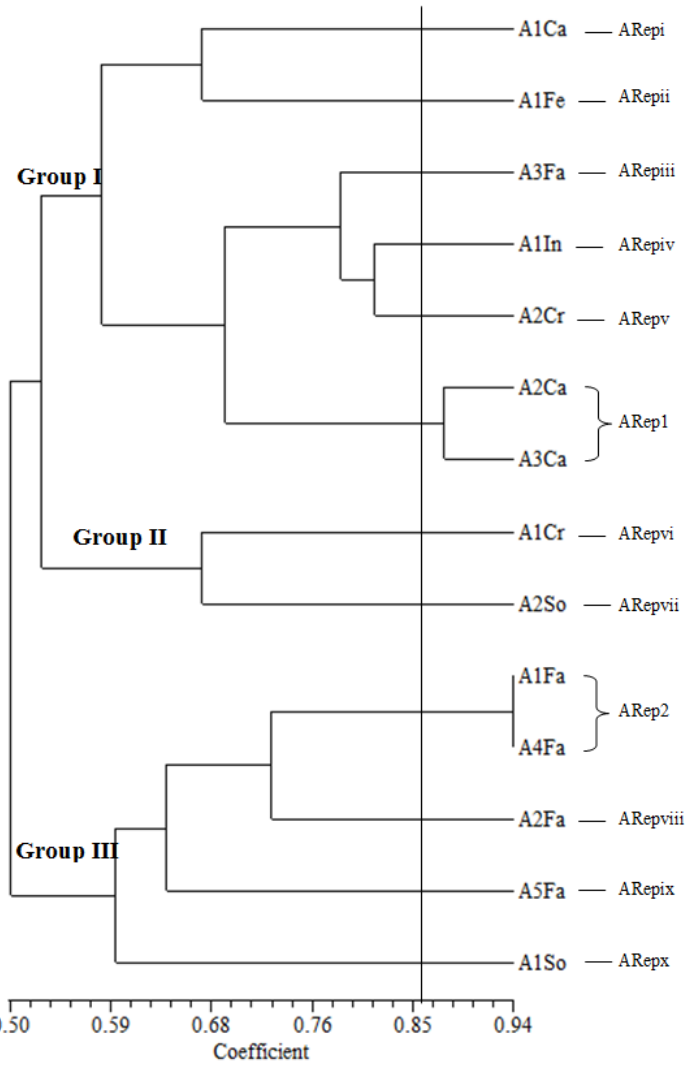

Figure 5e Dendogram showing the genetic relatedness of *S.* Albany isolated from ducks and their environmental sources performed by REP-PCR. ARep1-ARep2 = *S.* Albany cluster 1-2 by REP; ARepi-ARepx = *S.* Albany singleton i-x by REP; A = *S.* Albany; 1-5 = strain number; Ca = carcass rinse; So = soil; Cr=crate swab; Cl = cloaca swab; Fe = feed; In = intestines; and Fa = faeces.

REP depends on repetitive DNA elements present in salmonellae which are amplified during PCR process and when electrophoresed produce DNA bands of different sizes and numbers. The differences in band sizes and numbers are used/analyzed to determine the genetic relatedness or diversity among different salmonellae or foodborne pathogens. **Kerouanton *et al.* (1996)** used REP-PCR to differentiate 32 *S.* Dublin strains isolated from cattle and reported that, REP-PCR grouped the 32 *S.* Dublin strains into one type. **Bennasar *et al.* (2000)** also used REP to determine the genetic diversity of *S.* Enteritidis, *S.* Typhimurium and *S.* Virchow strains. They reported that these three serovars gave reproducible and distinguishable profiles using REP, ERIC or ITS, and the conserved patterns in each serovar allowed for easy differentiation from other serovars of *Salmonella*. **Albufera *et al.* (2009)** showed that REP-PCR analysis of *Salmonella* isolates from human and food sources generated different profiles for isolates of the same serogroup for differentiation purposes. This present study also revealed that REP-PCR could discriminate between *Salmonella* strains of the same serogroup.

Table 1 Major groups of *S.* Typhimurium according to REP-PCR analysis

No.	Strain	Source of isolation	Strain code	Place of isolation	
1	*S. Typhimurium*	Wash water	T7Wa	Processor 1	Group II
2	*S. Typhimurium*	Faeces	T1Fa	Farm 1	Group II
3	*S. Typhimurium*	Faeces	T2Fa	Farm 1	Group II
4	*S. Typhimurium*	Faeces	T3Fa	Farm 1	Group II
5	*S. Typhimurium*	Faeces	T4Fa	Farm 1	Group II
6	*S. Typhimurium*	Intestines	T12In	Processor 2	Group II

7	*S. Typhimurium*	Wash water	T2Wa	Processor 3	Group II
8	*S. Typhimurium*	Table swab	T1Ta	Processor 4	Group II
9	*S. Typhimurium*	Floor swab	T1Fl	Processor 4	Group II
10	*S. Typhimurium*	Wash water	T3Wa	Processor 1	Group II
11	*S. Typhimurium*	Wash water	T4Wa	Processor 1	Group II
12	*S. Typhimurium*	Intestines	T1In	Processor 1	Group I
13	*S. Typhimurium*	Soil	T1So	Farm 1	Group I
14	*S. Typhimurium*	Soil	T4So	Farm 1	Group I
15	*S. Typhimurium*	Wash water	T5Wa	Processor 1	Group I
16	*S. Typhimurium*	Wash water	T1Wa	Processor 3	Group I
17	*S. Typhimurium*	Intestines	T11In	Processor 1	Group I
18	*S. Typhimurium*	Intestines	T2In	Processor 1	Group I
19	*S. Typhimurium*	Intestines	T9In	Processor 1	Group I
20	*S. Typhimurium*	Faeces	T5Fa	Farm 1	Group I
21	*S. Typhimurium*	Intestines	T13In	Processor 2	Group I
22	*S. Typhimurium*	Intestines	T5In	Processor 1	Group I
23	*S. Typhimurium*	Wash water	T6Wa	Processor 1	Group II
24	*S. Typhimurium*	Intestines	T8In	Processor 1	Group II
25	*S. Typhimurium*	Soil	T2So	Farm 1	Group II
26	*S. Typhimurium*	Soil	T3So	Farm 1	Group II
27	*S. Typhimurium*	Intestines	T7In	Processor 1	Group II
28	*S. Typhimurium*	Intestines	T10In	Processor 1	Group II
29	*S. Typhimurium*	Faeces	T6Fa	Farm 1	Group III
30	*S. Typhimurium*	Faeces	T10Fa	Farm 1	Group III
31	*S. Typhimurium*	Faeces	T7Fa	Farm 1	Group III
32	*S. Typhimurium*	Faeces	T8Fa	Farm 1	Group III
33	*S. Typhimurium*	Intestines	T3In	Processor 1	Group III
34	*S. Typhimurium*	Faeces	T9Fa	Farm 1	Group III
35	*S. Typhimurium*	Intestines	T4In	Processor 1	Group III
36	*S. Typhimurium*	Intestines	T6In	Processor 1	Group III
37	*S. Typhimurium*	Floor swab	T2Fl	Processor 4	Group III

Table 2 Major groups of *S*. Hadar and *S*. Albany according to REP-PCR analysis

No.	Strain	Source of isolation	Strain code	Place of isolation	
1	*S. Hadar*	Drinking water	H1Dr	Farm 4	Group I
2	*S. Hadar*	Pond water	H1Po	Farm 4	Group I
3	*S. Hadar*	Pond water	H3Po	Farm 4	Group I
4	*S. Hadar*	Cloacal swab	H9Cl	Farm 4	Group I
5	*S. Hadar*	Cloacal swab	H5Cl	Farm 4	Group I
6	*S. Hadar*	Cloacal swab	H6Cl	Farm 4	Group I
7	*S. Hadar*	Faeces	H3Fa	Farm 4	Group II
8	*S. Hadar*	Faeces	H4Fa	Farm 4	Group II
9	*S. Hadar*	Soil	H2So	Farm 4	Group II
10	*S. Hadar*	Soil	H3So	Farm 4	Group II
11	*S. Hadar*	Pond water	H2Po	Farm 4	Group II
12	*S. Hadar*	Cloacal swab	H8Cl	Farm 4	Group II
13	*S. Hadar*	Cloacal swab	H10Cl	Farm 4	Group II

14	*S. Hadar*	Cloacal swab	H3Cl	Farm 4	Group I
15	*S. Hadar*	Cloacal swab	H1Cl	Farm 3	Group I
16	*S. Hadar*	Faeces	H1Fa	Farm 3	Group I
17	*S. Hadar*	Faeces	H5Fa	Farm 4	Group I
18	*S. Hadar*	Faeces	H7Fa	Farm 4	Group I
19	*S. Hadar*	Cloacal swab	H2Cl	Farm 3	Group I
20	*S. Hadar*	Cloacal swab	H7Cl	Farm 4	Group II
21	*S. Hadar*	Cloacal swab	H4Cl	Farm 4	Group II
22	*S. Hadar*	Faeces	H8Fa	Farm 4	Group II
23	*S. Hadar*	Soil	H4So	Farm 4	Group II
24	*S. Hadar*	Soil	H1So	Farm 4	Group II
25	*S. Hadar*	Faeces	H2Fa	Farm 3	Group II
26	*S. Hadar*	Faeces	H6Fa	Farm 4	Group II
1	*S. Albany*	Carcass rinse	A2Ca	Processor 4	Group 1
2	*S. Albany*	Carcass rinse	A3Ca	Processor 4	Group 1
3	*S. Albany*	Faeces	A1Fa	Farm 2	Group III
4	*S. Albany*	Faeces	A4Fa	Farm 2	Group III
5	*S. Albany*	Carcass rinse	A1Ca	Processor 4	Group 1
6	*S. Albany*	Feed	A1Fe	Farm 4	Group 1
7	*S. Albany*	Faeces	A3Fa	Farm 2	Group 1
8	*S. Albany*	Intestines	A1In	Processor 4	Group 1
9	*S. Albany*	Crate swab	A2Cr	Processor 4	Group 1
10	*S. Albany*	Crate swab	A1Cr	Processor 4	Group II
11	*S. Albany*	Soil	A2So	Farm 2	Group II
12	*S. Albany*	Faeces	A2Fa	Farm 2	Group III
13	*S. Albany*	Faeces	A5Fa	Farm 3	Group III
14	*S. Albany*	Soil	A1So	Farm 2	Group III

Table 3 Major groups of *S.* Braenderup and *S.* Enteritidis according to REP-PCR analysis

No.	Strain	Source of isolation	Strain code	Place of isolation	
1	*S. Braenderup*	Cloacal swab	B3Cl	Farm 1	Group I
2	*S. Braenderup*	Cloacal swab	B4Cl	Farm 1	Group I
3	*S. Braenderup*	Intestines	B4In	Processor 1	Group I
4	*S. Braenderup*	Wash water	B1Wa	Processor 1	Group I
5	*S. Braenderup*	Cloacal swab	B1Cl	Farm 1	Group I
6	*S. Braenderup*	Intestines	B1In	Processor 1	Group I
7	*S. Braenderup*	Intestines	B2In	Processor 1	Group I
8	*S. Braenderup*	Intestines	B3In	Processor 1	Group I
9	*S. Braenderup*	Faeces	B4Fa	Farm 1	Group I
10	*S. Braenderup*	Intestines	B5In	Processor 1	Group I
11	*S. Braenderup*	Faeces	B1Fa	Farm 1	Group II
12	*S. Braenderup*	Faeces	B2Fa	Farm 1	Group II
13	*S. Braenderup*	Cloacal swab	B2Cl	Farm 1	Group III
14	*S. Braenderup*	Faeces	B3Fa	Farm 1	Group III
15	*S. Braenderup*	Soil	B1So	Farm 1	Group III

1	*S. Enteritidis*	Faeces	E2Fa	Farm 1	Group I
2	*S. Enteritidis*	Faeces	E3Fa	Farm 1	Group I
3	*S. Enteritidis*	Faeces	E6Fa	Farm 1	Group II
4	*S. Enteritidis*	Faeces	E9Fa	Farm 1	Group II
5	*S. Enteritidis*	Intestines	E4In	Processor 2	Group III
6	*S. Enteritidis*	Intestines	E5In	Processor 3	Group III
7	*S. Enteritidis*	Faeces	E7Fa	Farm 1	Group I
8	*S. Enteritidis*	Faeces	E5Fa	Farm 1	Group I
9	*S. Enteritidis*	Faeces	E8Fa	Farm 1	Group II
10	*S. Enteritidis*	Intestines	E1In	Processor 1	Group II
11	*S. Enteritidis*	Intestines	E2In	Processor 1	Group II
12	*S. Enteritidis*	Intestines	E3In	Processor 2	Group II
13	*S. Enteritidis*	Faeces	E4Fa	Farm 1	Group II
14	*S. Enteritidis*	Faeces	E1Fa	Farm 1	Group II
15	*S. Enteritidis*	Intestines	E6In	Processor 1	Group III

CONCLUSION

We report for the first time on the use of REP-PCR to determine the genetic relatedness or diversity of salmonellae isolated from ducks and their environmental samples in Malaysia. REP-PCR analysis of the 107 salmonellae resulted in differences and similarities among the strains of salmonellae isolated from the same or different sources. REP-PCR successfully type all the *Salmonella* strains and was a useful tool for determining the genetic relatedness or diversity of the *Salmonella* strains isolated from ducks, their rearing and processing environments.

Acknowledgments: This research was supported by Postgraduate Research Grant Scheme (1001/PTEK1ND/843007) of the Universiti Sains Malaysia.

REFERENCES

ADAMS, R., MOSS, M. O. 2008. Food microbiology. RSC Publishing, ISBN 978-0-85404-284-5.

ADZITEY, F., ALI, G. R. R., HUDA, N., COGAN, T., CORRY, J. 2013a. Prevalence, antibiotic resistance and genetic diversity of *Listeria monocytogenes* isolated from ducks, duck rearing and processing environments in Penang, Malaysia. *Food Control,* 32, 607-614.

Adzitey, F., Ali, G.R.R., Huda N., Rosma, A. 2013a. Genotyping of *Salmonella* strains isolated from ducks, their rearing and processing environments in Penang, Malaysia, using RAPD. *3 Biotech*, DOI: 10.1007/s13205-013-0115-7.

ADZITEY, F., HUDA, N., ALI, G. R. R. 2012a. Molecular techniques for detecting and typing of bacteria, advantages and application to foodborne pathogens isolated from ducks. *3 Biotech* 2012a, DOI 10.1007/s13205-012-0074-4.

ADZITEY, F., RUSUL, G., HUDA, N. 2012b. Prevalence and antibiotic resistance of *Salmonella* serovars in ducks, duck rearing and processing environments in Penang, Malaysia. *Food Research International,* 45, 947-952.

ADZITEY, F., HUDA, N., GULAM, R. 2012c. Prevalence and antibiotic resistance of *Campylobacter*, *Salmonella* and *L. monocytogenes* in ducks: A review. *Foodborne Pathogens and Diseases,* 6, 498-505.

ADZITEY, F., ALI, G. R. R., HUDA, N., COGAN, T., CORRY, J. 2012d. Prevalence, antibiotic resistance and RAPD typing of *Campylobacter* species isolated from ducks, duck rearing and processing environments in Penang, Malaysia. *International Journal of Food Microbiology,* 154, 197-205.

ADZITEY, F., LIEW, C. Y., ARONAL, A. P., HUDA, N. 2012e: Isolation of *Escherichia coli* from ducks and duck related samples. *Asian Journal of Animal and Veterinary Advances,* 7, 351-355.

ADZITEY, F., Corry, J. 2011. A comparison between hippurate hydrolysis and multiplex PCR for differentiating *C. coli* and *C. jejuni*. *Tropical Life Sciences Research*, 22:57–64.

ADZITEY, F., NURUL, H. 2011. *Campylobacter* in poultry: incidences and possible control measures. *Research Journal of Microbiology,* 6, 182-192.

ADZITEY, F., HUDA, N., GULAM, R. 2011a. Comparison of media for the isolation of *Salmonella* (XLD and Rambach) and *Listeria* species (ALOA and Palcam) in naturally contaminated duck samples. *Internet Journal of Food Safety*, 13, 20-25.

ADZITEY, F., HUDA, N., GULAM, R. 2011b. Isolation of *Campylobacter* species from the large intestines of domestic Pekin ducks obtained from a Wet Market in Penang, Malaysia. *Internet Journal of Food Safety*, 13, 170-174.

ALBUFERA, U., BHUGALOO-VIAL, P., ISSACK, M. I., JAUFEERALLY-FAKIM, Y. 2009. Molecular characterization of *Salmonella* isolates by REP-PCR and RAPD analysis. *Infection Genetics and Evolution*, 3, 322-327.

Anonymous, 2011. Available at: http://www.promega.com/resources/protocols/technical-manuals/0/wizard-genomic-dna-purification-kit-protocol/ accessed on 18/02/2012.

BENNASAR, A., DE LUNA, G., CABRER, B., LALUCAT, J. 2000. Rapid identification of *S.* Typhimurium, *S.* Enteritidis and *S.* Virchow isolates by polymerase chain reaction based fingerprinting methods. *International Microbiology,* 3, 31-38.

CDC. 2011. Multistate outbreak of human *Salmonella* Heidelberg infections linked to "Kosher broiled chicken livers" from Schreiber Processing Corporation. Available at: http://www.cdc.gov/salmonella/heidelberg-chickenlivers/index.html, accessed on 13/09/2012.

DEFRA. 2010. Zoonoses report UK. Available at: http://www.defra.gov.uk/animal-diseases/ zoonotic/, accessed on 23/08/2011.

EFSA. 2012. Scientific report of EFSA and ECDC the European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in 2010. *EFSA Journal,* 10, 2597.

FAO. 2009. FAOSTAT on main producer country of duck meat in 2007. Available at: http://faostat.fao.org/site/569/DesktopDefault.aspx?PageID=569#ancor, accessed on 18/02/2011.

FREDERICK, A., HUDA, N. 2011. *Salmonellas*, poultry house environments and feeds: a review. *Journal of Animal and Veterinary Advances,* 10, 679-685.

HUNTER, P.R., GASTON, M. A. 1988. Numerical index for the discriminatory ability of typing systems. An application of Simpson's index of diversity. *Journal of Clinical Microbiology,* 26, 2465-2466.

JERSEK, B., GILOT, P., GUBINA, M., KLUN, N., MEHLE, J., TCHERNEVA, E., RIJPENS, N., HERMAN, L. 1999. Typing of *Listeria monocytogenes* strains by repetitive element sequence-based PCR. *Journal of Clinical Microbiology,* 37, 103-109.

KEROUANTON, A., BRISABOIS, A., GROUT, J., PICARD, B. 1996. Molecular epidemiological tools for *Salmonella* Dublin typing. *FEMS Immunology and Medical Microbiology,* 14, 25-29.

MERRITT, T.D., HERLIHY, C. 2003. *Salmonella* outbreak associated with chicks and ducklings at childcare centres. *Medical Journal of Australia,* 179, 63-64.

NOBLE, D.J., LANE, C., LITTLE, C. L., DAVIES, R., DE PINNA, E., LARKIN, L., MORGAN, D. 2012. Revival of an old problem: an increase in *Salmonella enterica* serovar Typhimurium definitive phage type 8 infections in 2010 in England and Northern Ireland linked to duck eggs. *Epidemiology and Infection,* 140, 146-149.

SCALLAN, E., HOEKSTRA, R. M., ANGULO, F.J., TAUXE, R. V., WIDDOWSON, M. A., ROY, S. L., JONES, J. L., GRIFFIN, P.M. 2011. Foodborne illness acquired in the United States-major pathogens. *Emerging and Infectious Diseases,* 17, 7–15.

VERSALOVIC, J., KOEUTH, T., LUPSKI, J.R. 1991. Distribution of repetitive DNA sequences in Eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Research*, 19, 6823-6831.