WVJ
World's
Veterinary Journal

World's Vet. J. 3(1): 01-04, 2013

Original Article

Tracking the Possible Source of *Listeria monocytogenes* Contamination Using Random Amplified Polymorphic Deoxyribonucleic Acid (RAPD)

Frederick Adzitey

Animal Science Department, Faculty of Agriculture, University for Development studies, P.O. Box TL 1882, Tamale, Ghana, West Africa

*Corresponding author's email: adzitey@yahoo.co.uk

ABSTRACT

Listeria monocytogenes is a Gram positive and ubiquitous organism that has been implicated in a number of foodborne outbreaks. In this study, 15 *Listeria monocytogenes* isolated from duck farms and a wet market were typed using random amplified polymorphic deoxyribonucleic acid (RAPD) to track the source of contamination. Two arbitrary RAPD primers were used in the polymerase chain reaction to determine the genetic relatedness among the *Listeria monocytogenes* isolated from duck farms and a wet market to enable the tracking of their possible source or origin. RAPD primers DAF-4 and OPM-01 characterized the 15 *Listeria monocytogenes* into 4 and 3 RAPD types, respectively. *Listeria monocytogenes* isolated from duck intestines and carcass rinse clustered together in the same RAPD type suggests possible cross contamination. Additionally, OPM-01 grouped *Listeria monocytogenes* isolated from feces and soil into the same RAPD type, while DAF-4 showed that faeces and soil isolates where closely related compared to intestinal and carcass rinse isolates. Tracking the source of foodborne pathogens and routes of transmission is important to help implement effective preventive, control and treatment measures.

KEY WORDS: Cross contamination, Duck farm, *Listeria monocytogenes*, RAPD, Wet market.

INTRODUCTION

Listeria monocytogenes is an important foodborne bacterial pathogen. *Listeria monocytogenes* is referred to as an opportunistic pathogen affecting mainly immuned challenged individuals such as neonates, infants, pregnant women, the elderly, AIDS patients, cancer patients and people with other forms of weak immune systems (Khelef et al., 2006; Bhunia, 2008). *Listeria monocytogenes* causes listeriosis, characterized by fever, watery diarrhoea, nausea, headache, stiff neck, confusion, loss of balance, convulsion, and pains in joints and muscles (Khelef et al., 2006; Bhunia, 2008). Listeriosis is rare foodborne infection but with very high fatality rate (Mead et al., 1999; Adzitey et al., 2010). Understanding and tracking the source and routes of *Listeria monocytogenes* contamination is important in the prevention of the spread of *Listeria monocytogenes* in foods and other products. This will also pave the way for the development of efficient preventive measures, thus reducing the number of listeriosis cases.

In the United Kingdom 176 cases of listeriosis were reported by Defra (2010). In the United States of America an estimated listeriosis cases of 2,518 were reported by Mead et al. (1999). Mead et al. (1999) also reported that, the percentage foodborne transmission, hospitalization rate and case fatality rate is 99, 0.922 and 0.2000, respectively. The incidence of listeriosis in most developing and under developed countries remains unknown. Several foods or their products have also been associated with the transmission, outbreaks and sporadic cases of listeriosis, including meat, milk, vegetable, fish and their products (Mead et al., 1999; Bhunia, 2008; Defra, 2010). Isolation of *Listeria monocytogenes* from ducks and duck related samples have been reported (Chipilev et al., 2010; Adzitey et al., 2011; Adzitey et al., 2012a, Adzitey et al., 2013). Duck meat, eggs or products are consumed by many Asia countries and are important source of nutrients comparable to that of chickens (Adzitey, 2011; Adzitey and Adzitey, 2011; Adzitey et al., 2012b). The consumption of duck meat, eggs or products contaminated with *Listeria monocytogenes* can lead to listeriosis.

Large quantities of ducks are produced in Malaysia. This is evidenced by the fact that Malaysia is the third world producer of duck meats according to the food and agriculture organization report on world duck meat production in 2007 (FAO, 2009). Current agricultural policies also continue to motivate duck farmers and producers to increase their production for local consumption and export purposes (Adzitey et al., 2011; Adzitey et al., 2012b; Adzitey et al., 2012c). The implication is that duck production in Malaysia will continue to increase and problems associated with the

To cite this paper: Adzitey F. 2013. Tracking the Possible Source of *Listeria monocytogenes* Contamination Using Random Amplified Polymorphic Deoxyribonucleic Acid (RAPD). *World's Vet. J.* 3(1): 01-04.
Journal homepage: http://wvj.science-line.com/

increasing diversity and spread of foodborne pathogens is more likely (Adzitey et al., 2013). Knowing the source of contamination of foodborne pathogens will help in the prevention of cross contaminations. Thus this study aims to track the source of contamination of *Listeria monocytogenes* in duck farms and a wet market using RAPD.

MATERIALS AND METHODS

Sources of *Listeria monocytogenes*

Fifteen *Listeria monocytogenes* isolated from duck farms and a wet market in Penang, Malaysia were used. They were isolated from seven duck intestines, five duck faeces, two wash water samples (water used for washing carcasses after dressing) and one soil sample. Intestinal and wash water *Listeria monocytogenes* isolates were isolated from the wet market while faecal and soil isolates were isolated from the farm.

Template DNA preparation

DNA templates were prepared using freshly grown *Listeria monocytogenes* on Trypticase Soy Agar containing 0.6% yeast extract, by adding a loopful to 500 µl sterile distilled water and boiled in a heater block at 100 ^{o}C for 10 min (Adzitey et al., 2012c). Template DNA was stored at -20 ^{o}C until used for PCR reactions.

Typing of *Listeria monocytogenes* using RAPD

Two primers, DAF-4 (5′-CGGCAGCGCC-3′) (Wieldmann-Al-Almed et al., 1994) and OPM-01 (5′-GTTGGTGGCT-3′) (Lawrence et al., 1995) were used. Amplification reaction mixtures (25 µl) consisted of 12.5 µl GoTaq mastermix (Promega, Madison, USA), 2.5 µl 25mM $MgCl_2$, 0.5 µl primers, 7 µl nuclease free water and 2.5 µl DNA template. The reaction mixtures were subjected to the following temperature cycles; denaturation at 95 °C for 5 min, followed by 60 cycles of annealing at 95 °C for 30 s, extension at 45 °C for 30 s, and a final extension at 72 °C for 1 min. For RAPD product analysis, 15 µl of the amplification product was loaded on 1.5% agarose gel (Sigma, Missouri, USA) containing 1 µg ml^{-1} ethidium bromide (Sigma, Missouri, USA) run at 90 V for 1h 30 min. Hyperladder I (Bioline, London, UK) was used as the molecular weight marker and the gels were examined using an ultra violet transilluminator (UVP BioDoc-It^{TM} Imaging System, Cambridge, UK). RAPD profiles were analysed according to Sait et al. (2003).

RESULTS AND DISCUSSION

Listeria monocytogenes isolates were typed in order to determine their genetic diversity and source of possible cross contamination using RAPD; a relatively low cost, rapid, easy to perform technique with good discriminating power and reproducibility, especially if the number of isolates involved is less than 50, more than one primer is used, and the dNTP concentrations are chosen carefully (Zhou and Jiao, 2004; Shi et al., 2010; Adzitey et al., 2012d). RAPD was performed using two primers that is DAF-4 and OPM-01 primers selected on the basis of their performance (Lawrence et al., 1995; Wieldmann-Al-Almed et al., 1994) and after varying other conditions to obtain suitable and better bands. The reproducibility of the RAPD technique was evaluated by running the same experiment twice, and both results were consistent.

The results of typing *Listeria monocytogenes* using DAF-4 and OPM-01 primers are shown in Table 1 and Table 2, respectively. RAPD analysis of the 15 *Listeria monocytogenes* isolates using DAF-4 primer grouped the isolates into four RAPD types, while OPM-01 primer grouped the isolates into three RAPD types. In Table 1, *Listeria monocytogenes* isolated from duck faeces (L1F, L3F, L4F, L8F and L9F) were closely related to each other compared to those isolated from soil (L1S), duck intestines (L3, L5, L8, L9, L11, L12 and L14) and wash water (L2R and L3R). Similarly, *Listeria monocytogenes* isolated from the soil were well separated from those isolated from intestines and wash water but more related to those isolated from duck faeces. The result using DAF-4 primer is not surprising since both soil and faecal samples were obtained from the farm, while intestines and wash water samples were obtained from the same wet market. Intestinal (L3, L5, L8, L9, L11, L12 and L14) and wash water (L2R and L3R) isolates were grouped into the same RAPD type. Thus they are closely related to each other and show possible cross contamination. *Listeria monocytogenes* in wash water samples might have been contaminated with those from the intestines during carcass washing or processing. This will occur when the intestines rupture and release the pathogen into the water used to wash duck carcases (wash water).

From Table 2 using OPM-01 primer, *Listeria monocytogenes* (L3R) isolated from wash water was closely related to those isolated from the intestines (L3, L8, L9, L12, and L14), and wash water might have been cross contaminated by intestinal isolates. In addition, *Listeria monocytogenes* (L1S, L9F, L4F, L1F, L2R, L11, and L5) isolated from soil, faeces wash water and intestines were grouped together and therefore related to each other. Soil samples may have been contaminated by faecal samples while wash water samples may have taken their origin from intestinal samples. The finding of intestinal, faecal and soil samples in the same group (same RAPD type) also suggest that duck intestines are contaminated with *Listeria monocytogenes* which duck can share in their faeces during defaecation, and contaminated faeces can cross contaminate the soil.

The RAPD results using the two arbitrary primers highlight the need to improve hygienic conditions and to take careful precautions to prevent faecal contamination of the duck carcasses and the environment during duck production, processing and marketing. Other workers have also confirmed that RAPD can be a powerful tool for characterizing *L. monocytogenes* isolates and for tracing contamination patterns (Lawrence et al., 1995; Wieldmann-Al-Almed et al., 1994; Zhou and Jiao, 2004; Ozbey et al., 2006).

To cite this paper: Adzitey F. 2013. Tracking the Possible Source of *Listeria monocytogenes* Contamination Using Random Amplified Polymorphic Deoxyribonucleic Acid (RAPD). *World's Vet. J.* 3(1): 01-04.
Journal homepage: http://wvj.science-line.com/

Table 1. RAPD analysis of *Listeria monocytogenes* isolated from duck farms and a wet market obtained using DAF-4 primer

No.	Source	Code	Place	RAPD Type
1	Wash water	L3R	Wet market	One
2	Wash water	L2R	Wet market	One
3	Intestines	L14	Wet market	One
4	Intestines	L12	Wet market	One
5	Intestines	L11	Wet market	One
6	Intestines	L9	Wet market	One
7	Intestines	L5	Wet market	One
8	Intestines	L8	Wet market	One
9	Intestines	L3	Wet market	One
10	Soil	L1S	Farm	Two
11	Faeces	L3F	Farm	Three
12	Faeces	L9F	Farm	Four
13	Faeces	L4F	Farm	Four
14	Faeces	L1F	Farm	Four
15	Faeces	L8F	Farm	Four

Table 2. RAPD analysis of *Listeria monocytogenes* isolated from duck farms and a wet market obtained using OPM-1 primer

No.	Source	Code	Place	RAPD Type
1	Faeces	L8F	Farm	One
2	Wash water	L3R	Wet market	One
3	Intestines	L14	Wet market	One
4	Intestines	L12	Wet market	One
5	Intestines	L9	Wet market	One
6	Intestines	L8	Wet market	One
7	Intestines	L3	Wet market	One
8	Faeces	L3F	Farm	Two
9	Soil	L1S	Farm	Three
10	Faeces	L9F	Farm	Three
11	Faeces	L4F	Farm	Three
12	Faeces	L1F	Farm	Three
13	Wash water	L2R	Wet market	Three
14	Intestines	L11	Wet market	Three
15	Intestines	L5	Wet market	Three

CONCLUSION

RAPD using DAF-4 and OPM-1 successfully typed all 15 *Listeria monocytogenes* into 4 and 3 RAPD types, respectively. Typing of the *Listeria monocytogenes* isolates by RAPD also provided useful information to successfully trace the possible source of *Listeria monocytogenes* contamination in duck farms and a wet market in Penang. This information can be used to prevent the spread of *Listeria monocytogenes* by adhering to effective biosecurity at duck farms and processing ducks under a more hygienic and careful conditions.

Acknowledgment

The author is grateful to IPS-USM, Prof. G.R.R. Ali, Assoc. Prof. Nurul Huda, Dr. J. Corry, Dr. T. Cogan and Dr. L. Sait for their financial support, advice and any form of contribution towards running this project.

REFERENCES

Adzitey F, Ali GRR, Huda N, Cogan T and Corry J, 2913. Prevalence, antibiotic resistance and RAPD typing of *Listeria monocytogenes* isolated from ducks, duck rearing and processing environments in Penang, Malaysia. *Food Control*, 32:607-614.

Adzitey F, Huda N and Ali GRR, 2012a. Prevalence and antibiotic resistance of *Campylobacter*, *Salmonella*, and *L. monocytogenes* in ducks-A review. *Foodborne Pathog. Dis*., 9:498-505.

Adzitey F, Rusul G and Huda N, 2012b. Prevalence and antibiotic resistance of *Salmonella* serovars in ducks, duck rearing and processing environments in Penang, Malaysia. *Food Res. Int.*, 45:947-952.

To cite this paper: Adzitey F. 2013. Tracking the Possible Source of *Listeria monocytogenes* Contamination Using Random Amplified Polymorphic Deoxyribonucleic Acid (RAPD). *World's Vet. J.* 3(1): 01-04.
Journal homepage: http://wvj.science-line.com/

Adzitey F, Rusul G, Huda N, Cogan T and Corry J, 2012c. Prevalence, antibiotic resistance and RAPD typing of *Campylobacter* species isolated from ducks, duck rearing and processing environments in Penang, Malaysia. *Int. J. Food Microbiol*., 154:197-205.

Adzitey F, Huda N and Ali GRR, 2012d. Molecular techniques for detecting and typing of bacteria, advantages and application to foodborne pathogens isolated from ducks. *3 Biotech* DOI 10.1007/s13205-012-0074-4.

Adzitey F, 2011. Production potentials and the physicochemical composition of selected duck strains: a mini review. *Online J. Animal Feed Res.*, 2(1): 89-94.

Adzitey F and Adzitey SP, 2011. Duck production: has a potential to reduce poverty among rural households in Asian communities-a mini review. *The J. World's Poult. Res.*, 1(1):7-10.

Adzitey F, Huda N and Gulam R, 2011. Comparison of media for the isolation of *Salmonella* (XLD and Rambach) and *Listeria* species (ALOA and Palcam) in naturally contaminated duck samples. *Internet J. Food Safety*, 13:20-25.

Adzitey F and Huda N, 2010. *Listeria monocytogenes* in foods: incidences and possible control measures. *African J. Microbiol. Res*., 4:2848-2855.

Adzitey F, Huda N and Gulam R, 2011. Isolation of *Campylobacter* species from the large intestines of domestic Pekin ducks obtained from a Wet Market in Penang, Malaysia. *Internet J. Food Safety*, 13:170-174.

Bhunia AK, 2008. Food-borne microbial pathogens mechanisms and pathogenesis. Chapter 9. pp. 165-182. ISBN: 9780387745367.

Defra, 2010. Zoonoses report UK. http://www.defra.gov.uk/animal-diseases/zoonotic/.

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

Lawrence LM and Gilmour A, 1995. Characterization of *Listeria monocytogenes* isolated from poultry products and from the poultry-processing environment by random amplification of polymorphic DNA and multilocus enzyme electrophoresis. *Appl. Environ. Microbiol*., 61:2139-2144.

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM and Tauxe RV, 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis*., 5:607-625.

Ozbey G, Ertas HB and Kok F, 2006. Prevalence of *Listeria* species in camel sausages from retail markets in Aydin province in Turkey and RAPD analysis of *Listeria monocytogenes* isolates. *Irish Vet. J*., 59:342-344.

Sait L, Galic M, Strugnell RA and Janssen PH, 2003. Secretory antibodies do not affect the composition of the bacterial microbiota in the terminal ileum of 10-week-old mice. *Appl. Environ. Microbiol*., 69:2100-2109.

Shi XM, Long F and Suo B, 2010. Molecular methods for the detection and characterization of foodborne pathogens. *Pure Appl. Chem*., 82:69-79.

Wiedmann-Al-Ahmad M, Tichy HV and Schön G, 1994. Characterization of *Acinetobacter* type strains and isolates obtained from wastewater treatment plants by PCR fingerprinting. *Appl. Environ. Microbiol*., 60:4066-4071.

Zhou X and Jiao X, 2004. Investigation of *Listeria monocytogenes* contamination pattern in local Chinese food market and the tracing of two clinical isolates by RAPD analysis. *Food Microbiol*., 21:695-702.

To cite this paper: Adzitey F. 2013. Tracking the Possible Source of *Listeria monocytogenes* Contamination Using Random Amplified Polymorphic Deoxyribonucleic Acid (RAPD). *World's Vet. J.* 3(1): 01-04.
Journal homepage: http://wvj.science-line.com/