DE GRUYTER OPEN

DOI: 10.1515/fv-2017-0009

FOLIA VETERINARIA, 61, 1: 60—65, 2017

COLLECTION OF *LISTERIA MONOCYTOGENES* ISOLATES FROM MILK, DAIRY PRODUCTS AND FOOD PROCESSING ENVIRONMENTS IN SLOVAKIA FOR THE PURPOSES OF EUROPEAN MOLECULAR DATABASE

Kubicová, Z., Filipová, M., Jurovčíková, J., Cabanová, L.

State Veterinary and Food Institute, Veterinary and Food Institute in Dolný Kubín
Jánoškova 1611/58, 026 01 Dolný Kubín
Slovakia

miriam.filipova@svpu.sk

ABSTRACT

The molecular typing of *Listeria monocytogenes* isolates is an important tool for monitoring the spread of the strains in food chains, providing evidence for epidemiological investigations and for the detection of outbreaks. The demand of European typing data centralization, collection and sharing stimulated the generation of "EURL *L. monocytogenes* Database (EURL Lm DB)" in 2012 led by the European Union Reference Laboratory (EURL) for *L. monocytogenes* (ANSES Maisons-Alfort Laboratory for Food Safety, France) in close collaboration with Applied Maths. This database includes the typing results and epidemiological information on strains isolated from food, environmental or animal samples and it is in connection with human strains database TESSy (The European Surveillance System) led by the ECDC (European Centre for Disease Prevention and Control). In total 147 *L. monocytogenes* isolates were examined by PFGE (pulsed field gel electrophoresis) in 2014—2015 in VFI Dolny Kubin from different sources. Nearly half (68) of the 147 isolates in the national Slovak database came from milk or dairy products samples and the related manufacturing environment. In this work, 68 isolates associated with milk were selected and divided into 27 clusters (95 % similarity level) after combined comparison analysis (AscI and ApaI) by BioNumerics 6.6 software. Eight clusters included three or more similar PFGE profiles.

Key words: database; *Listeria monocytogenes*; molecular typing; PFGE

INTRODUCTION

The molecular typing of bacterial DNA by different methods (pulsed field gel electrophoresis — PFGE, multi locus variable-number tandem repeat analysis — MLVA, whole genome sequencing — WGS) has become a standard process of pathogenic foodborne bacteria characterization. The PFGE typing of foodborne microorganisms is very important for surveillance purposes, especially monitoring the spread of strains in food chains, ensuring evidence for

epidemiological investigations and revelation of national or international outbreaks. PFGE is regarded as the "gold standard" among typing methods [6].

A surveillance network based on PFGE method including food and clinical isolates (PulseNet) has been used in USA and Canada for many years and it helped to detect numerous outbreaks [1, 2, 3]. PulseNet Europe was created with the same aim in 2003 [8], but it was cancelled in 2006 due to lack of funding [9]. In 2012 the European Centre for Disease Prevention and Control — ECDC developed a pilot Molecular Surveillance System (MSS) as a component of The European Surveillance System — TESSy. The scope of this databasing system is to share epidemiological information and molecular typing data on *L. monocytogenes* strains isolated in cases of human disease [10]. However, there was a constant need to collect and share information on European *L. monocytogenes* isolates sourced from food, animal and environmental samples. This need was satisfied in 2012 by the creation of "EURL *L. monocytogenes* Database (EURL Lm DB)" led by European Union Reference Laboratory (EURL) for *Listeria monocytogenes* (ANSES Maisons-Alfort Laboratory for Food Safety, France).The National Reference Laboratories (NRLs) across Europe can store and also share their molecular and epidemiological data on *L. monocytogenes* strains isolated from food, animal and environmental samples. The principle of data sharing is based on internet communication using BioNumerics software (Applied Maths Saint-Martens-Latem, Belgium) [5].

MATERIALS AND METHODS

In sum, 147 *L. monocytogenes* isolates from different sources were typed by PFGE in NRL for *L. monocytogenes* at the Veterinary and Food Institute (VFI) in Dolný Kubín during 2014—2015. However, this study was focused on 68 isolates from milk, dairy products and related food processing environments. The isolates originated in official control samples or from samples of producer's self-control. All *L. monocytogenes* isolates were analysed according to the EURL protocol [7]. Agarose plugs with bacterial DNA were prepared and restriction enzymes AscI and ApaI (Thermo Scientific, USA) were used for PFGE profiles production. Electrophoresis was performed in 1 % agarose (SeaKem Gold Agarose, Lonza, USA) on a CHEF Mapper® XA (Bio-Rad, USA). *Salmonella* Braenderup H9812 DNA cleaved with XbaI enzyme (Thermo Scientific, USA) was used as a reference system. The molecular profiles obtained by the PFGE procedures were analysed by BioNumerics v6.6 software (Applied Maths, Belgium) using the Dice coefficient and unweighted pair group method (UPGMA) with arithmetic mean analysis, with "optimization" and "tolerance" settings of 1 %. The inclusion of PFGE profiles into one group — cluster — was done according to 95 % similarities among ApaI/AscI compared profiles.

RESULTS

From 2014 to 2015, a total of 147 *L. monocytogenes* from different sources were examined by PFGE in NRL for *L. monocytogenes* at VFI Dolný Kubín. These milk-associated isolates (milk, dairy products and related manufacture environments) formed nearly half (46.26 %) of all investigated isolates. The combined clustering analysis of 68 ApaI/AscI profiles resulted in 27 clusters with 95 % similarity.

Only 8 of the 27 clusters contained 3 or more identical, similar or nearly similar PFGE profiles (Fig. 1, Table 1). All 8 clusters were comprised of 44 isolates in total. The biggest cluster IV was composed of *L. monocytogenes* isolates from one specific source — sheep (sheep milk — 2 samples, products from sheep milk — 8 samples and specific sheep milk processing environments — 2 samples), and from one manufacturer. Conventional serotyping classified all 12 isolates into 1/2a serogroup. The epidemiological data of the other 7 clusters (I.—II., V.—VIII.) were also investigated in detail, but there was no confirmation of either the presence of isolates from the same source and time, or the connection with one manufacturer.

The remaining 19 clusters were formed only by one or two isolates per cluster and were comprised of 24 isolates in total (data not shown).

DISCUSSION

A surveillance of the *L. monocytogenes* spread in food chains is an essential part in preventing disease and monitoring public health issues. The surveillance may include sampling of food or environments for the presence or absence of the organisms which may contribute to the identification of the risky food batches or identify colonization of

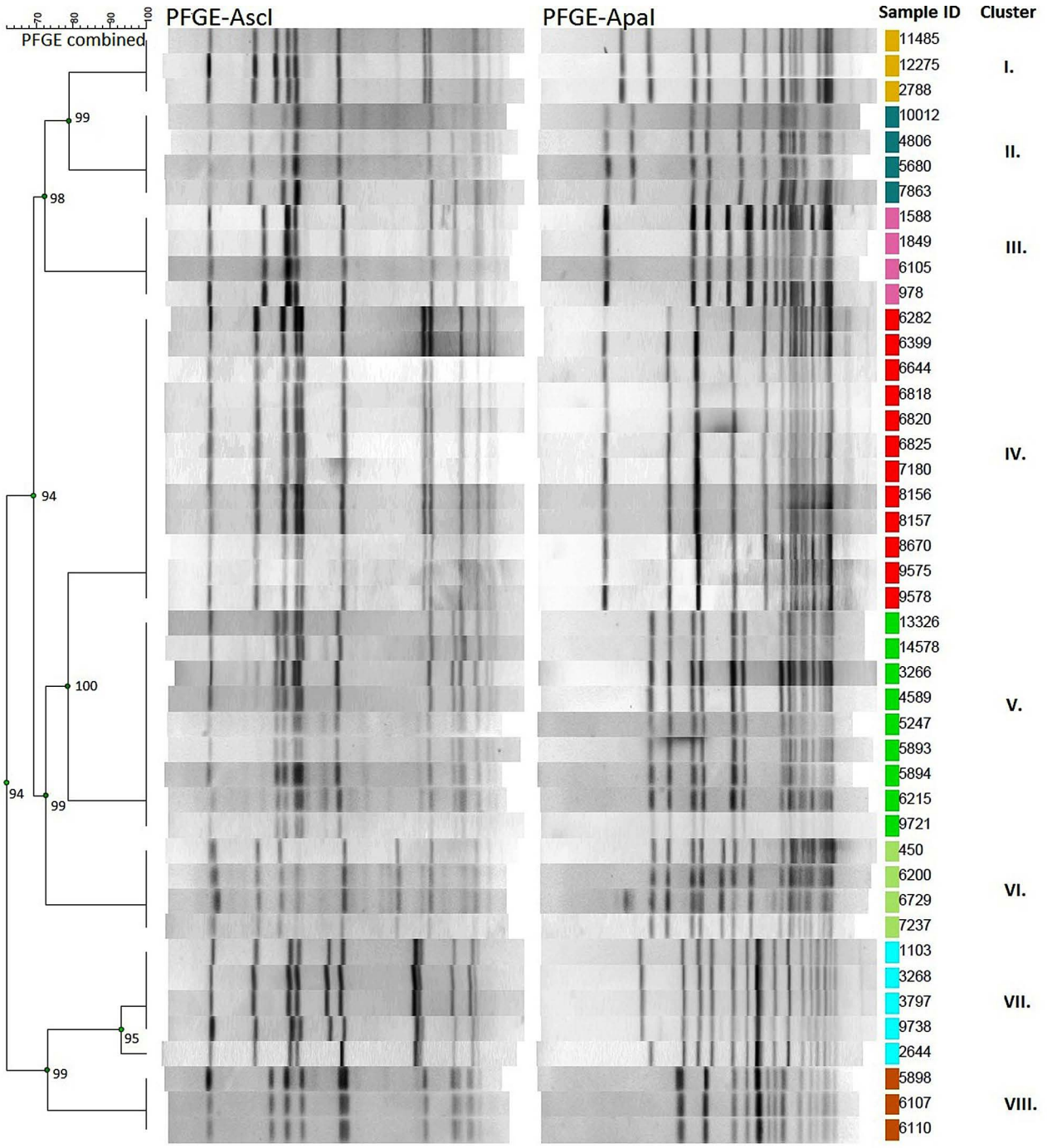

Fig 1. Similarity dendrogram of 8 PFGE clusters identified in the positive samples of milk, dairy products and related food processing environments

Combined analysis of AscI and ApaI profiles of 44 *L. monocytogenes* strains resulted in 8 clusters (I.—VIII.). Cluster IV was composed of 12 *L. monocyogenes* samples isolated from a specific source and one manufacturer. Despite the profiles similarity, all of the other clusters did not show a connection either with the source and time of sampling or with one producer

Table 1. Detailed epidemiological data concerning the samples of 8 PFGE clusters

Cluster	Sample ID	Conventional serotype	Source	Sample category	Sampling date
I.	11485	1/2a	Sheep	Cheese category not specified	2014-09-30
	12275	1/2a	Sheep	Soft cheese	2014-10-17
	2788	1/2a	Sheep	Cheese category not specified	2014-03-28
II.	10012	1/2a	–	Food processing environment	2014-08-26
	4806	1/2a	Sheep	Cheese category not specified	2014-05-13
	5680	1/2a	Sheep	Cheese category not specified	2024-05-26
III.	7863	1/2a	Sheep	Cheese category not specified	2015-07-16
	1588	1/2a	Bovine	Semi soft cheese	2015-03-05
	1849	1/2a	Sheep	Cheese category not specified	2014-03-07
	6105	1/2a	–	Food processing environment	2014-06-03
IV.	978	1/2a	Bovine	Cheese category not specified	2015-02-17
	6282	1/2a	Sheep	Soft cheese	2015-06-16
	6399	1/2a	Sheep	Cheese category not specified	2015-06-17
	6644	1/2a	Sheep	Soft cheese	2015-06-23
	6818	1/2a	–	Food processing environment	2015-06-24
	6820	1/2a	–	Food processing environment	2015-06-24
	6825	1/2a	Sheep	Milk	2015-06-24
	7180	1/2a	Sheep	Semi soft cheese	2015-07-01
	8156	1/2a	Sheep	Semi soft cheese	2015-07-23
	8157	1/2a	Sheep	Soft cheese	2015-07-23
	8670	1/2a	Sheep	Cheese category not specified	2015-07-29
	9575	1/2a	Sheep	Soft cheese	2015-08-21
	9578	1/2a	Sheep	Milk	2015-08-21
V.	13326	1/2a	Sheep	Soft cheese	2014-11-10
	14578	1/2a	Sheep	Soft cheese	2014-12-11
	3266	1/2a	Sheep	Cheese category not specified	2015-04-16
	4589	1/2a	Sheep	Soft cheese	2014-05-07
	5247	1/2a	Sheep	Soft cheese	2014-05-20
	5893	1/2a	Bovine	Semi soft cheese	2011-05-28
	5894	1/2a	Sheep	Milk	2014-05-26
	6215	1/2a	Sheep	Soft cheese	2014-06-05
	9721	1/2a	Bovine	Other dairy product	2014-08-25
VI.	450	1/2a	Bovine	Semi soft cheese	2014-01-28
	6200	1/2a	Bovine	Semi soft cheese	2014-06-05
	6729	1/2a	Bovine	Semi soft cheese	2014-06-17
	7237	1/2a	Bovine	Semi soft cheese	2014-06-25
VII.	1103	4b	Bovine	Semi soft cheese	2014-02-11
	3268	4b	Bovine	Semi soft cheese	2015-04-16
	3797	4b	Bovine	Semi soft cheese	2015-04-28
	9738	4b	Sheep	Cheese category not specified	2015-08-26
	2644	4b	Sheep	Cheese category not specified	2015-03-30
VIII.	5898	1/2b	Bovine	Semi soft cheese	2014-05-28
	6107	1/2b	–	Food processing environment	2014-06-03
	6110	1/2b	–	Food processing environment	2014-06-03

environments (farms) with organism and potential cross-contamination of the food. Using more advanced tools (strains serotyping and molecular typing by PFGE), better epidemiologic understanding can be achieved. 46.26 % occurrence of milk associated isolates in a total 147 investigated *L. monocytogenes* isolates is consistent with the fact that milk and dairy product are one of the most frequent source of listeriosis [4]. The combined analysis of AscI and ApaI patterns of 68 *L. monocytogenes* strains isolated from dairy product and related environments resulted in strain discrimination into 27 clusters (95 % similarity level). After the selection of clusters with 3 or more profiles, 44 samples were grouped into 8 clusters and one of them (cluster IV) showed the close relationship between samples. All 12 samples from cluster IV were identified to be from one source and one producer. In this case, the subtyping data of *L. monocytogenes* isolates facilitated finding the source of *L. monocytogenes* presence in final dairy products and, of course, allowed the producer to optimize the technical and sanitation measures to be taken to ensure hygiene of the food production.

Moreover, all PFGE profiles were sent for curation processing in EURL Lm DB. The majority of them were accepted and involved in the transnational database for sharing with other European countries.

In Slovakia, there have been only a few similar studies focused on solving the secondary contamination problems in food producers using modern typing methods. In one of them, Veghova et al. [11] examined 20 *L. monocytogenes* strains isolated from sheep milk products and related food processing environments. In contrast with our results, the dominated serogroup in the collection was IIa. The authors identified 14 clusters at a similarity level of 100 % without confirmation of epidemiological clone ECI-ECIII. This high strain similarity suggested the external environment as an origin of contamination [11]. Using molecular tools on national and international levels one can better understand the potential route of disease transmission through the food chains. Databasing system based on PFGE in NRL for *Listeria monocytogenes* in Slovakia is connected with European databasing system and enables as to compare profiles from all participating countries, not only in the case of disease outbreak, but also for surveillance purposes and epidemiological investigations.

CONCLUSIONS

L. monocytogenes is a serious foodborne pathogen in human health, and also has important economic influence due to its persistence in food processing environments and potential food product contaminations. In the case of its source and route of contamination detection, suitable analytical method, such as molecular typing, needs to be used. In our surveillance, we referred the potential source and route of milk and dairy products contamination by the identification a cluster (cluster IV) of *L. monocytogenes* isolates from a specific source (sheep milk, products from sheep milk and related food processing environments) and from one producer. However, another 7 clusters mentioned in Fig. 1 did not show a connection in the context of source and season of contamination or coherence with a specific manufacturer. This example points out the importance and advantages of molecular typing and creating a national database of molecular profiles. Using national databasing systems we were able to search and compare molecular profiles collected through the years. Joining the European databasing system allows the comparison of molecular data with all participating countries and brings benefits during disease outbreak detection, epidemiological investigation and it is also helpful in the case of monitoring and surveillance programmes.

REFERENCES

1. **CDC U, 2010:** Outbreak of invasive listeriosis associated with the consumption of hog head cheese — Louisiana, 2010. *MMWR Morbidity and Mortality Weekly Report*, 60, 401—405.
2. **CDC U, 2011:** Multistate outbreak of listeriosis associated with Jensen Farms cantaloupe — United States, August — September 2011. *MMWR Morbidity and Mortality Weekly Report*, 60, 1357—1358.
3. **Choi, M. J., Jackson, K. A., Medu, C., Beal, J., Rigdon, C. E., Cloyd, T. C. et al., 2014:** Notes from t8156he field: multistate outbreak of listeriosis linked to soft-ripened cheese — United States, 20815713. *MMWR Morbidity and Mortality Weekly Report*, 63, 294—295.
4. **EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2015:** The European Union summary report on trends and sources

of zoonoses, zoonotic agents and food-borne outbreaks in 2014. *EFSA Journal*, 13: 4329, 191 pp.

5. **Felix, B., Danan, C., VanWalle, I., Lailler, R., Texier, T., Lombard, B., Brisabois, A., Roussel, S., 2014:** Building a molecular *Listeria monocytogenes* database to centralise and share PFGE typing data from food, environmental and animal throughout Europe. *J. Microbiol. Method*s, 104, 1—8.
6. **Graves, L. M., Swaminathan, B., 2001:** PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. *Int. J. Food Microbiol.*, 65, 55—62.
7. **Marault, M., Roussel, S., 2011:** Molecular subtyping of *Listeria monocytogenes* using pulsed-field gel electrophoresis. M*éthode Anses Maisons-Alfort CEB04, version anglaise*, rév. 06, date 28 Février 2011, https://eurl-listeria.anses.fr/en/minisite/listeria/eurl-lm-house-method-pfge-typing-l-monocytogenes
8. **Martin, P., Jacquet, C., Goulet, V., Vaillant, V., De Valk, H., 2006:** Pulsed field gel electrophoresis of *Listeria monocytogenes* strains: the PulseNet Europe Feasibility Study. *Foodborne Pathogens and Disease*, 3, 303—308.
9. **Swaminathan, B., Gerner-Smidt, P., Ng L. K., Lukinmaa, S., Kam, K. M., Rolando, S. et al., 2006:** Building PulseNet International: an interconnected system of laboratory networks to facilitate timely public health recognition and response to foodborne diseases. F*oodborne Pathogens and Disea*se, 3, 36—50.
10. **Van Walle, I., 2013:** ECDC starts pilot phase for collection of molecular typing data. *Euro Surveill.*, 18, pii: 20357.
11. **Véghová, A., Koreňová, J., Minarovičová, J., Drahovská, H., Siekel, P., Kaclíková, E., 2015:** Isolation and characterization of *Listeria monocytogenes* from the environment of three ewes' milk processing factories in Slovakia. *Journal of Food and Nutrition Research*, 54, 252—259.

Received November 14, 2016
Accepted February 3, 2017