Investigation of listeriosis outbreaks in small ruminants using pulsed-field gel electrophoresis and whole-genome sequencing

Bojan Papić*, Mateja Pate, Darja Kušar

*bojan.papic@vf.uni-lj.si

University of Ljubljana, Veterinary Faculty, Institute of Microbiology and Parasitology, Slovenia

INTRODUCTION

- *Listeria monocytogenes* is an important zoonotic pathogen with a high (>30%) mortality rate.
- In animals, listeriosis mostly affects cattle and small ruminants.
- Animal listeriosis outbreaks are often neglected and the potential of animal *L. monocytogenes* strains to cause disease in humans remains understudied.

METHODS

- Based on the epidemiological and pulsed-field gel electrophoresis (PFGE) typing data, seven small listeriosis outbreaks in small ruminants were retrospectively identified in the period 2008–2018 in the database of the Slovenian Reference Laboratory for *L. monocytogenes*.
- Presumable outbreak-associated isolates underwent whole-genome sequencing (WGS) and were typed with BioNumerics v7.6.3 software by three different approaches: **wgSNP**, **cgMLST** and **wgMLST**.
- wgSNP phylogenetic trees were constructed using RAxML v8.1.22, options -m GTRGAMMA -# 1000.
- In 6/7 outbreaks, clinical isolates were available, whereas in one case, environmental isolates were also obtained, enabling source investigation.

RESULTS

- All isolates within a single outbreak had indistinguishable *Asc*I-*Apa*I PFGE profiles (data not shown).
- All seven presumable outbreaks were confirmed by WGS, suggesting a common source of infection with a single outbreak strain: the outbreak-associated isolates differed in 0–12 wgMLST alleles, 0–7 cgMLST alleles and 0–12 SNPs (**Figs. 1–3**).
- The outbreak strains belonged to four different sequence types (STs): ST1 (*n*=3), ST18 (*n*=1), ST21 (*n*=2) and ST184 (*n*=1).
- In the case of ST1 and ST21 isolates, the epidemiologically unlinked isolates showed a high (≤10 allelic differences) genetic relatedness to the outbreak strains (**Figs. 1–3**), calling for caution when using a fixed similarity threshold to delineate the outbreak clusters.
- In the case of **ST184 outbreak**, the most probable source(s) of infection were silage and/or surface water (**Fig. 2A)**.
- While ST1 and ST21 are common among human clinical isolates, ST184 is a rare clone with a single human isolate currently available in the NCBI Pathogen Detection and BigsDB-*Lm* database.

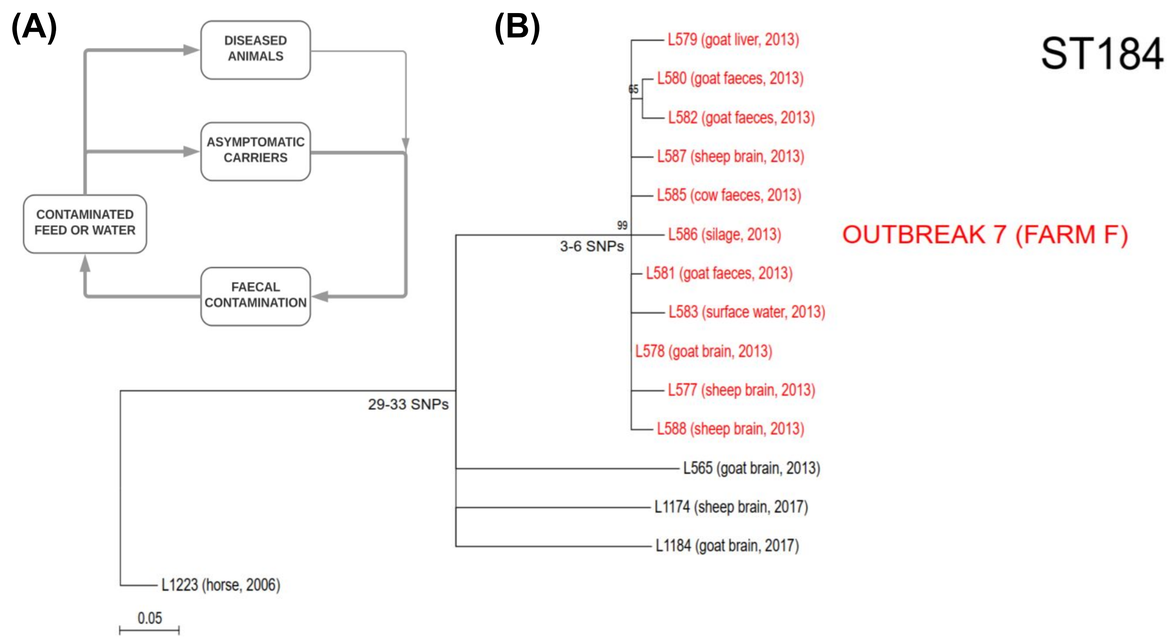

Fig. 2: ST184 outbreak cluster. (A) Most probable on-farm transmission routes. Asymptomatic carriers contributed to the faecal contamination of the farm environment. Contaminated silage and/or water were the most probable source(s) of infection of the diseased animals. **(B) Maximum-likelihood phylogenetic tree based on wgSNP analysis.** Outbreak cluster is indicated in red. The numbers at the nodes indicate bootstrap values. Bar, number of nucleotide substitutions per site.

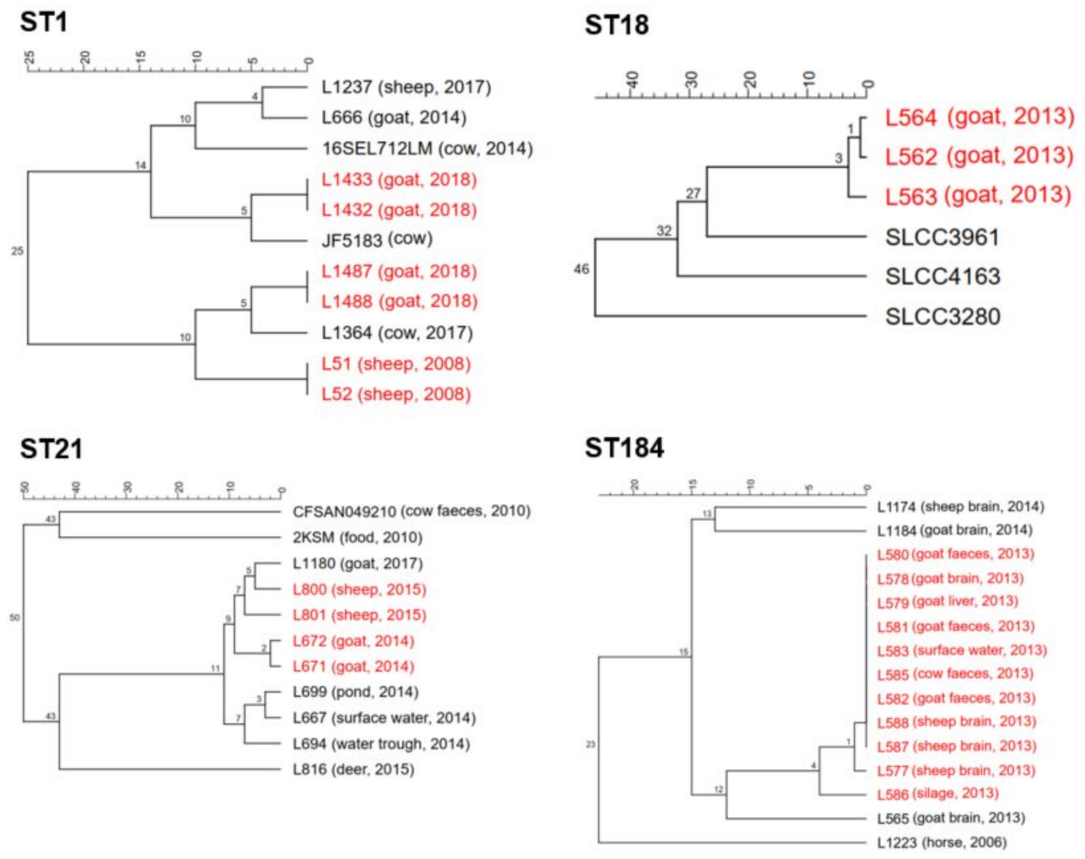

Fig. 3: Dendrograms based on cgMLST analysis. Outbreak clusters are indicated in red. Bar, number of allelic differences in cgMLST scheme.

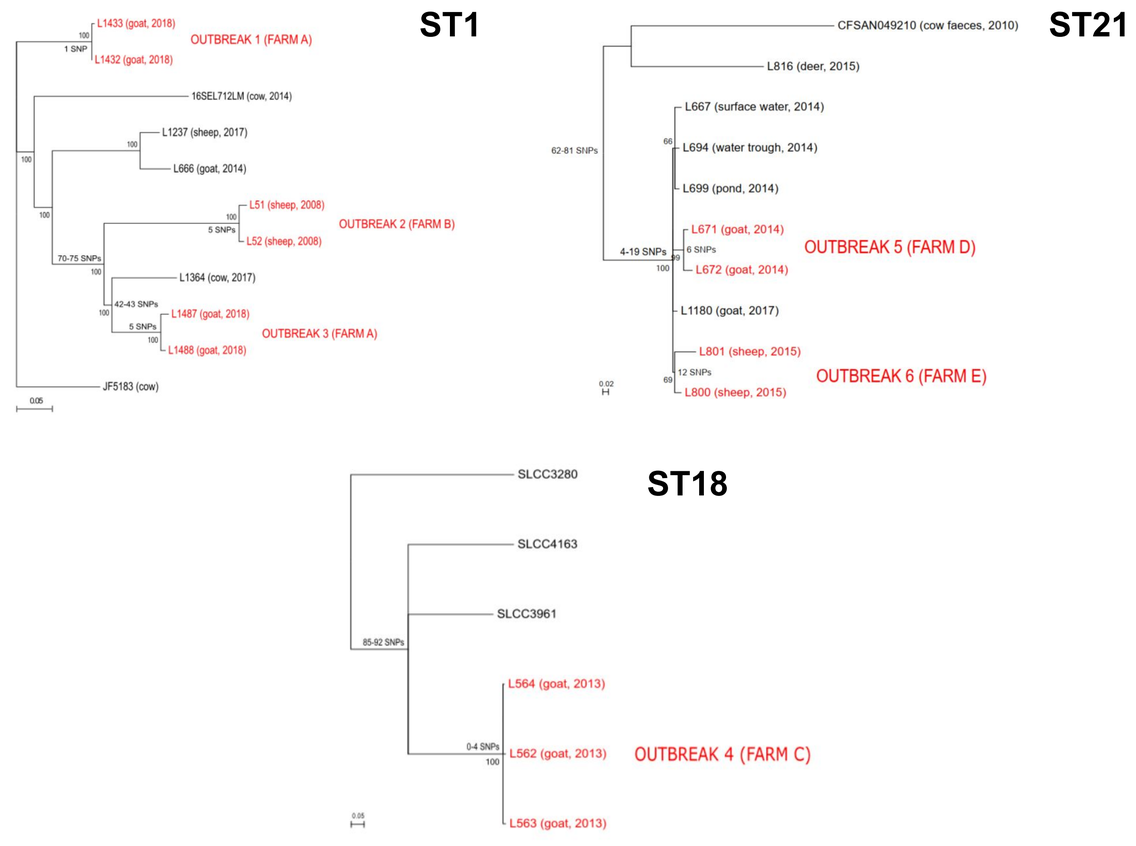

Fig. 1: Maximum-likelihood wgSNP trees of ST1, ST21 and ST18 outbreak clusters. Outbreak clusters are indicated in red. The numbers at the nodes indicate bootstrap values. Bar, number of nucleotide substitutions per site.

CONCLUSIONS

- We propose that in outbreak investigation, allele-based typing is confirmed by wgSNP typing; a phylogenetic tree with bootstrap values should be constructed.
- Choosing a closely related reference is of great importance for reliable wgSNP typing.
- The addition of closely related, but epidemiologically unrelated isolates to the analysis may help to define the outbreak cluster.
- WGS data should always be supported by epidemiological data to confirm an outbreak.
- Further comparative genomics and phenotypic studies of animal clinical strains are needed to assess their potential to cause disease in humans.

#FoodSafetyWGS2019

ACKNOWLEDGEMENTS

The authors thank Maja Kavalič for performing PFGE typing. Dr. Irena Zdovc and Majda Golob are acknowledged for providing and maintaining the isolates. Bojan Papić acknowledges the support of the Slovenian Research Agency (grant for young researchers).