# PATHOGENICITY

## VIRULENCE OF *LISTERIA MONOCYTOGENES* IN IMMUNOCOMPROMISED AND NORMAL MICE

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Listeriosis is a significant foodborne pathogen with high rates of mortality principally in immunocompromised populations. Differences in the ability of *L. monocytogenes* strains to cause illness may in part be attributed to differences in virulence factors associated with different strains.

The objective of this study was to determine the virulence based on $LD_{50}$ and infectivity, of ten *L. monocytogenes* strains in immunocompromised mice and three *L. monocytogenes* strains in normal mice. Infectivity was determined by quantitating the colony-forming units (cfu's) of *L. monocytogenes* in the mouse liver and spleen.

For $LD_{50}$ studies, 10 pathogenic isolates (H9666, ScottA, H7550, G3982, 12375, 12443, Vacherin, F6854, H7776, and 101M) were used in immunocompromised mice and three pathogenic isolates (12443, G3982, and H9666) were used in normal mice. Normal and immunocompromised female ICR mice were intraperitoneally (i.p.) injected with test strains in 0.1 ml PBS at $10^0$-$10^9$ cfu. For the determination of $LD_{50}$, five mice were injected with each *Listeria* strain and observed for 5 days. *Listeria* isolates that caused at least one death in 5 days were considered pathogenic. In addition pulsed-field gel electrophoresis (PFGE) patterns of isolates from tissues were compared with the original treatment strains to verify their stability during passage through the mouse. Data were analyzed statistically using analysis of variance in SAS statistical software.

As expected, the dose for the $LD_{50}$ at 3-days post treatment was higher than the dose for the $LD_{50}$ at 5-days for all strains. In immunocompromised mice, the most virulent strain of *L. monocytogenes* as determined by the $LD_{50}$ at both 3-days and 5-days post-exposure was G3982. In normal (not immunocompromised) mice, strain 12443 was the most virulent based on the 5-day $LD_{50}$ determinations. However, there was statistically no significant difference ($P < 0.05$) between 12443, G3982 and H9666 strains for the 3-day $LD_{50}$ dose in normal mice, indicating that they are all about equally virulent. When comparing normal and immunocompromised mice, there was no significant difference ($P < 0.05$) in strain 12443 for the 3- or 5-day $LD_{50}$'s. However, there was a significant difference ($P < 0.05$) for immunocompromised and normal mice for strains G3982 and H9666 for $LD_{50}$'s at both 3-days and 5-days. The 5-day $LD_{50}$ of G3982 for immunocompromised mice was $3.00 \times 10^2$ cfu and $1.45 \times 10^6$ cfu for normal mice. Similarly, for H9666 strain, the $LD_{50}$ at 5-days was $5.37 \times 10^2$ for immunocompromised mice and $2.06 \times 10^6$ cfu for normal mice.

With all three strains of *L. monocytogenes* the viable counts recovered from liver of infected normal mice were about the same as those, obtained from the spleen. Selected colonies from each treated group of mice were tested by PFGE analysis. PFGE patterns were indistinguishable from the treatment strains in all cases.

Conclusions: For ten *L. monocytogenes* strains tested in immunocompromised mice, G3982 was the most virulent based on a 5-day $LD_{50}$ assay. Three strains (G3982, H9666, and 12443) were tested in both immunocompromised and normal mice. When comparing results from normal and immunocompromised mice, there was no significant difference ($P < 0.05$) in 3- day or 5-day $LD_{50}$ determinations, for strain 12443, but there was a significant difference ($P < 0.05$) for strains G3982 and H9666. In this study we identified three strains that appear to be more virulent than the others tested. Further characterization of genetic differences and virulence factors of strains will aid in understanding the factors affecting the variation in virulence among strains.

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## DEVELOPMENT OF A DNA MICROARRAY CHIP FOR IDENTIFICATION OF *LISTERIA* SPECIES, PARTIAL SEROTYPING AND ASSESSMENT OF THE VIRULENCE POTENTIAL OF *LISTERIA MONOCYTOGENES* ISOLATES

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*Listeria monocytogenes* is a leading cause of death attributed to foodborne bacterial pathogens. Lack of sufficient scientific information has led regulatory agencies to consider any strain of *L. monocytogenes* to be potentially pathogenic for humans although several lines of evidence indicate there are differences in virulence potential among *L. monocytogenes* isolates. Our ultimate goal is to develop a DNA microarray chip that can

differentiate and identify *Listeria* species, including *L. monocytogenes,* as well as subtype and assess the virulence potential of *L. monocytogenes* isolates. Our initial approach to developing this chip was to design a set of 128 probes targeting 16S rRNA and *iap* genes of *Listeria* spp. (for differentiation and identification of *Listeria* spp.), and the *iap*, *gltA-gltB*, and *inlB* genes of *L. monocytogenes* (for partially serotyping and assessing the virulence potential of *L. monocytogenes* isolates). The probes (17- to 37-mer) were designed through the following steps: retrieving all available related gene sequences from GenBank; alignment of retrieved sequences using Clustral-X version 1.80; manual editing of alignment by SeaView; identification of regions for probe design; and design of specific probes using probe design software Sarani. For every probe, a control probe containing one central mismatch was included for accurate discrimination between true signal and random hybridization. The probes were synthesized with 5' or 3' amine modification for covalent attachment to substrate slides and several probes were synthesized with additional 12-mer spacers for studying the effect of general steric hindrance. Construction of the microarray chip is underway using an OmniGrid Accent Microarrayer and the functionality of the chip will be validated through serial hybridization with genomic DNAs from a collection of *Listeria* strains. The developed DNA microarray chip will enable rapid and accurate discrimination among six *Listeria* species and partial serotyping, and assess the virulence potential of *L. monocytogenes* isolates, such as those of food origin. With addition of more probes that specifically target virulence-associated genetic markers, the chip should be useful for rapid and more accurate global assessment of the virulence potential of any *L. monocytogenes* isolate. This DNA chip can be used for large-scale population genetic analysis of *L. monocytogenes* isolates and information gained through such studies would be the scientific basis for differentiating highly virulent from less virulent or avirulent strains of *Listeria.* This would provide an assay to enable food processors, regulatory agencies, and public health organizations to differentiate significant public health concerns such as food contaminated with a highly virulent strain of *L. monocytogenes* in contrast to an unimportant avirulent *L. monocytogenes* food contaminant. In addition, with resolution beyond the species level, the chip may be a useful subtyping tool for tracking contamination sources in the food-processing environment.