Improvement of diagnostic tools for molecular epidemiology of listeriosis: Virulence genes and their implication in the pathogenesis of rhombencephalitis in ruminants.

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Key words
Listeria monocytogenes, real-time PCR, encephalitis, rhombencephalitis, virulence-associated genes, MLVA

Aim of the study
To evaluate the use of a multiplex real-time PCR for detection and confirmation of L. monocytogenes in clinical isolates and in brain tissue samples from animal listerial encephalitis.
To investigate the genetic relatedness of L. monocytogenes strains from cases of encephalitis and in particular rhombencephalitis by multi locus variable number tandem repeat analysis (MLVA).

Material and methods
The collection contained 109 strains from brains of ruminants with listeric rhombencephalitis, 4 strains from bovine placenta, 20 strains from human infections, 50 strains from non-outbreak-related food from Switzerland and Greece, and 14 strains of unknown origin. Moreover, 156 brainstem tissue samples from ruminants were analyzed by real-time PCR.

Results and significance
The Listeria real-time PCR was able to confirm the presence of L. monocytogenes in 87% of the samples from cases of encephalitic listeriosis, whereas the bacterial isolation method detected only 64%. Additionally, no cross-reactivity was observed when brain tissue samples with histopathological lesions caused by other aetiological agents were tested by real-time PCR, showing a high specificity of the direct real-time PCR method. The real-time PCR enabled fast, sensitive and specific detection of L. monocytogenes in brainstem tissue samples of infected ruminants. The MLVA allelic profile-based comparisons clustered L. monocytogenes strains into 3 clonal complexes. Clonal complexes A and C emerged to be the main ones. The clonal complex A consisted of 95.5% (64/67) of strains isolated from brain samples independently of the species. Moreover, strains isolated from bovine brain samples, mostly from rhomb-encephalitis, were restricted to the clonal complex A. In contrast, the majority of food and environmental strains were clustered in the clonal complex C, and none of food and environmental strains were located in clonal complex A. The association between clonal complexes and the origin of the strain appeared to be statistically highly significant. Subsequently, the strains were analyzed by PCR for the presence of 12 virulence-associated genes (prfA, actA, inlA, inlB, inlC, inlE, inlF, inlG, inlJ, inlC2, auto, and vip), which revealed significant differences in the actA and inlJ genes between clinical and non-clinical strains. The presence of particular alleles of actA and inlJ suggests that actA and inlJ are very important in the pathogenesis of L. monocytogenes infections.

Publications, posters and presentations
Balandyte, L. (2010) MLVA typing of Listeria monocytogenes strains isolated from brain samples of sheep, goat and cattle, and comparison with strains of human, food and environmental origin. Inaugural-Dissertation, Vetsuisse-Fakultät, Universität Bern.
Fauser, A. (2009) Evaluation of a multiplex real-time PCR for detection of Listeria monocytogenes and Listeria innocua in clinical brain tissue samples. Inaugural-Dissertation, Vetsuisse-Fakultät, Universität Bern.

Project *1.08.11*

Project duration *February 2008 – February 2010*