

P1-04

**Molecular typing of *Francisella tularensis* strains isolated in Georgia**

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**Aim:** The National Center for Disease Control and Public Health of Georgia (NCDC) carries out surveillance on especially dangerous pathogens throughout the country. Seasonal outbreaks of tularemia in Georgia represent a public health concern. From 2006 to 2008, two outbreaks occurred in addition to nine sporadic cases. The aim of this study was

- 1) to subtype both new isolates of *Francisella tularensis* and archived strains from NCDC collection,
- 2) to create a reference genotype database for Georgia, and
- 3) to identify epidemiological relationships between geographically diverse environmental strains and clinical samples.

**Methods:** Pulse Field Gel Electrophoresis (PFGE) methodology was performed on archived strains of *F. tularensis* and LVS. A 25 marker Multiple Locus Variable Number Tandem Repeat Analysis (MLVA) system was used to identify genetic relationships among 17 from 87 Georgian *F. tularensis* isolates. Based on observed data a 3-marker MLVA multiplex assay was developed to provide a convenient and rapid subtyping system using markers which showed diversity on a country-wide strain panel. These 3 markers were used for preliminary subtyping of the remaining 70 *F. tularensis* strains, bubo aspirates and tick emulsions.

**Results:** PFGE profiles for all strains were the same. All Georgian isolates of *F. tularensis* typed as *holarctica* (TypeB) using C1-C4 marker. Two MLVA markers (M3-M4) revealed a total of six unique genotypes across all Georgian strains. The genotypes from water samples isolated during the outbreak were identical to those of clinical samples taken in the same region. The multiplex assay containing the two diverse markers and TypeB control (C1-C4) showed robust amplification of all three markers in a single reaction mix.

**Conclusions:**

- a) *F. tularensis* type B is the prevalent subspecies in Georgia and shows little genetic diversity as previously observed in MLVA analyses across globally diverse isolates;
- b) MLVA is useful for subtyping archival isolates, for direct detection and subtyping of clinical diagnostic material, and in subtyping samples directly from vector species preparations;
- c) This multiplex technique can be used in the future for fast and precise typing of clinical and environmental samples as well as newly isolated strains. This assay thus assists in epidemiological analyses and in making predictions regarding the relationships among outbreaks that occurred at different times and different locations.