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# GENOTYPING OF *FRANCISELLA TULARENSIS* ISOLATES FROM UKRAINE

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**Summary.** The aim of the work is to provide fingerprinting of 20 tularemia isolates collected at the territory of Ukraine and find their relationships with other typed tularemia isolates. The 20 randomly sampled *Francisella tularensis* thermolysates were collected during 1997–2016 on the territory of Ukraine. The panel included samples from 11 regions of Ukraine. Samples were collected from patients (3 samples), rodents (8 samples) and lagomorphs (1 sample), ticks (6 samples) and environment (2 samples). The genotyping and processing of data was carried out using the MLVA 12+1 method. Data were statistically processed using the method of unweighted pair group method with arithmetic mean-clustering (UPGMA). It was found that all specimens belong to the same subgenus *F. tularensis* subsp. *holarctica* and belong to three genotypes: Russian-Azerbaijan (5 samples), Czechoslovakia (1 sample) and European (14 samples) one. The European genotype is inherent in the 70% of studied samples almost in every studied year and can be named as the leading genotype that distributed on the territory of all Ukraine and is related to European genotypes of *F. tularensis*.

**Keywords:** MLVA, tularemia, Ukraine, UPGMA, *Francisella tularensis* subsp. *holarctica*

**Introduction.** Tularemia agent — *Francisella tularensis* — is a Gram-negative, facultative, bacterial pathogen that occurs naturally in lagomorphs and rodents, especially microtine rodents such as voles, vole rats and muskrats, as well as in beavers. In addition, the infection has been reported in a wide variety of other mammals, birds, amphibians and arthropods. There is a high risk of human infection with *F. tularensis*, as the infective dose is extremely low (beginning from 10 bacteria) and infected animals excrete bacteria with urine and feces. Humans can get infection through the simple contact with animals (WHO, 2007; Oyston, Sjöstedt and Titball, 2004). Thus, *Francisella tularensis* is a bacterium that could be potentially used for biological weapon development (Oyston, Sjöstedt and Titball, 2004). Tularemia is an endemic disease in most European countries. Natural foci of tularemia may exist for centuries, showing themselves as periodic disease outbreaks. That is why tularemia often becomes a public health problem, especially on the territory of former Soviet Union and USA (WHO, 2007; Oyston, Sjöstedt and Titball, 2004; Farlow et al., 2001).

The epidemiological and epizootological situation in Ukraine varied over the years and depends on the level of diagnosis and quality of the implementation of appropriate response measures. The first confirmed cases of tularemia were documented in the 1940's and were associated with occupational exposure among furriers. Then, in 1960's, tularemia was registered in the south of Ukraine where several arthropods and small mammals were recognized as vectors and hosts of the disease. The Ministry of Health of Ukraine had been reporting data on 3,086 positive isolates of *F. tularensis* from 1941–2008 collected at 1,084 locations form all regions of Ukraine. The distribution of *F. tularensis* in Ukraine is primarily connected to croplands followed by forests, grasslands (steppe), and water (Hightower et al., 2014). According to this, outbreaks occur the most often in north and south regions of Ukraine, and nearby river channels. Nowadays, the problem of tularemia infection is particularly important for uncontrolled territories in the east of the country, because of increasing density of rodent's population, how it was in Kosovo (Reintjes et al., 2002).

MLVA (Multi-locus variable number tandem repeat)-analysis of tularemia agent can determine its genotypic characteristics, as well as the frequency and distribution of individual genotypes. It is also called 'fingerprinting', and it may differentiate suspected, fast-evolving bacterial strains from an outbreak even though those strains might look the same using other methods of genotyping. This can be used for tracing back the pathways of pathogen circulation at the territory of one country and between countries and continents (Vogler et al., 2009; Johansson et al., 2004).

**The aim of our work** was to provide fingerprinting of 20 *F. tularensis* isolates collected at the territory of Ukraine and find their relationships with other typed tularemia isolates.

**Materials and methods.** The thermolysates from 20 *F. tularensis* cultures were kindly provided for MLVA-genotyping by the State Institution 'Public Health Center of the Ministry of Health of Ukraine'. All samples were collected during 1997–2016 on the territory of Ukraine. The panel included samples from 11 regions of Ukraine (Sumy, Chernihiv, Rivne, Volyn, Lviv, Poltava, Odesa, Crimea, Mykolaiv, Zaporizhia, Vinnytsia). Samples were collected by Regional Laboratory Centers from patients (3 samples), rodents (8 samples) and

lagomorphs (1 sample), ticks (6 samples) and environment (2 samples) (Table 1).

**Table 1 —** The list of collected samples from the territory of Ukraine

| # | Strain number | Source | Date of sample collection | Region |
|---|---|---|---|---|
| 1 | 222 | *Mus musculus* | 18.05.1999 | Crimea |
| 2 | 86 | *Dermacentor reticulatus* | 26.05.2011 | Chernihiv |
| 3 | 317 | *Apodemus uralensis* | 16.11.2010 | Chernihiv |
| 4 | 138/15 | *Microtus arvalis* nest | 28.04.1998 | Mykolaiv |
| 5 | 201 | *Ixodes ricinus* | 17.07.2006 | Rivne |
| 6 | 201 | *Micromys minutus* | 13.04.2005 | Lviv |
| 7 | 205/15 | Bubo punctate | 29.11.2000 | Sumy |
| 8 | 103 | *Apodemus agrarius* | 31.03.1999 | Poltava |
| 9 | 456 | *Mus spicilegus* | 01.02.2000 | Crimea |
| 10 | 21 | Water from the Uday River | 04.03.2003 | Poltava |
| 11 | 128 | *Lepus europaeus* brain | 05.05.1998 | Odessa |
| 12 | 523 | Hay | 29.12.1999 | Chernihiv |
| 13 | 562/278o | *Sorex araneus* | 05.11.2008 | Sumy |
| 14 | 60 | Unknown ticks | 23.07.1997 | Zaporizhia |
| 15 | 351/278o | Inguinal node punctate | 13.05.2005 | Sumy |
| 16 | 37 | *Hyalomma plumbeum* | 13.05.1999 | Crimea |
| 17 | 359/278o | Inguinal node punctate | 16.09.2005 | Sumy |
| 18 | 493 | *Myodes glareolus* | 12.09.1996 | Vinnytsia |
| 19 | 132 | *Dermacentor reticulatus* | 28.06.2016 | Volyn |
| 20 | 66 | | 10.06.2008 | Rivne |

The genotyping and processing of data was carried out at the Bundeswehr Institute of Microbiology (Munich, Germany) within the Ukrainian-German Biosafety Program using the MLVA 12+1 method (Vogler et al., 2009; Svensson et al., 2009). Genotyping was performed using the Genetic Analyzer 3130 (Applied Biosystems, USA) device. Data were statistically processed in Genemapper (Thermoficher Scientific, USA) and Bionumerics (Applied Maths, USA) programs using the method of unweighted pair group method with arithmetic mean-clustering.

**Results.** It was found that all specimens belong to the same subgenus *Francisella tularensis* subsp. *holarctica*, which coincides with the OIE and WHO data for the distribution of various subtypes of *F. tularensis* throughout the globe. Within this subtype, three distinct genotypes were conventionally named according to the genotypes of other related countries: Russian-Azerbaijan (5 samples), Czechoslovakia (1 sample) and European (14 samples) cluster. These clusters are emphasized with red circles (Fig. 1).

The European cluster included samples from all studied regions: Sumy, Chernihiv, Rivne, Volyn, Lviv, Poltava, Odesa, Crimea, Mykolaiv, Zaporizhia, Vinnytsia (strain numbers 222, 86, 317, 138/15, 201 (2 samples), 205/15, 103, 21, 128, 351/278o, 359/278o, 132, 66). The Russian-Azerbaijan cluster included samples from Crimea, Zaporizhia, Sumy, and Vinnytsia regions (strain numbers 456, 60, 37, 562/278o, 493). The Czechoslovakia cluster was detected in Chernihiv region and included strain number 523 (Fig. 2).

The most commonly encountered genotype related to the European cluster. This genotype was registered during the study in 1996, 1998–2000, 2003, 2005, 2006, 2008, 2010, 2011, and 2016 in all studied regions of Ukraine (Fig. 3).

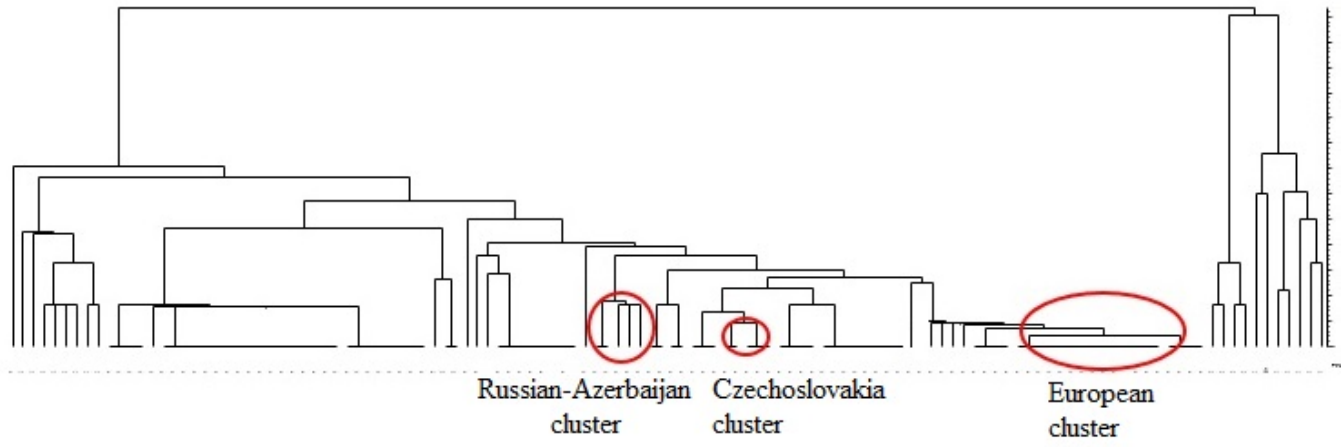


**Figure 1.** Clustering of the studied MLVA samples using the unweighted pair group method with arithmetic mean-clustering

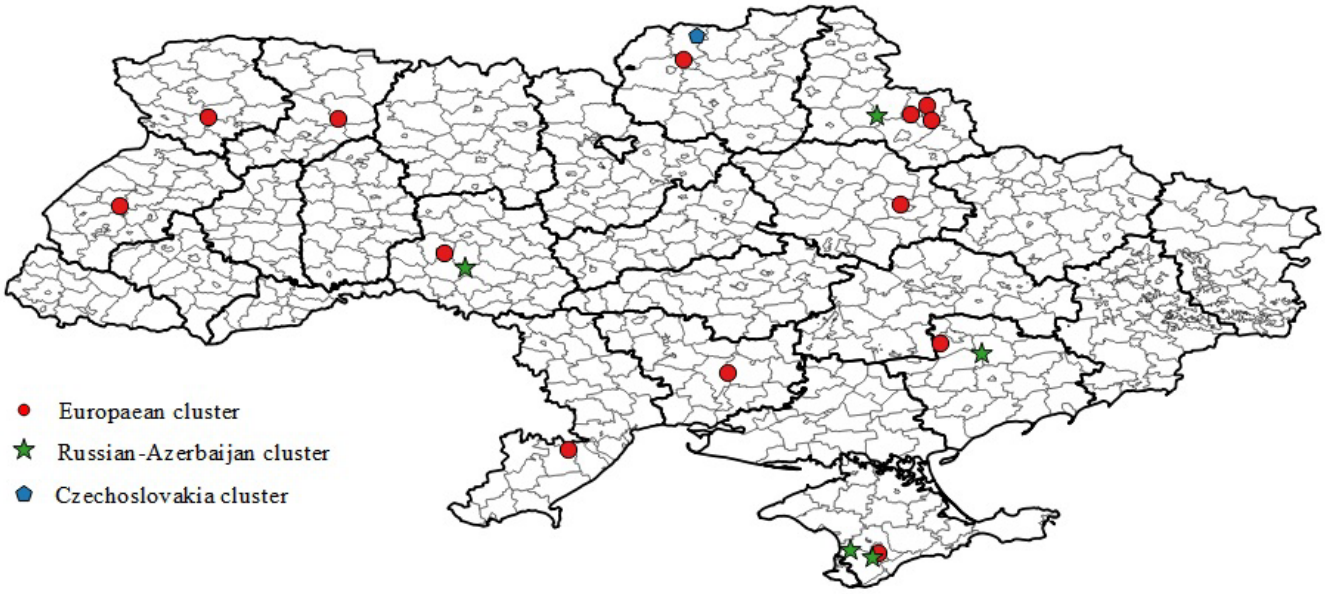


**Figure 2.** The geographical distribution of studied samples

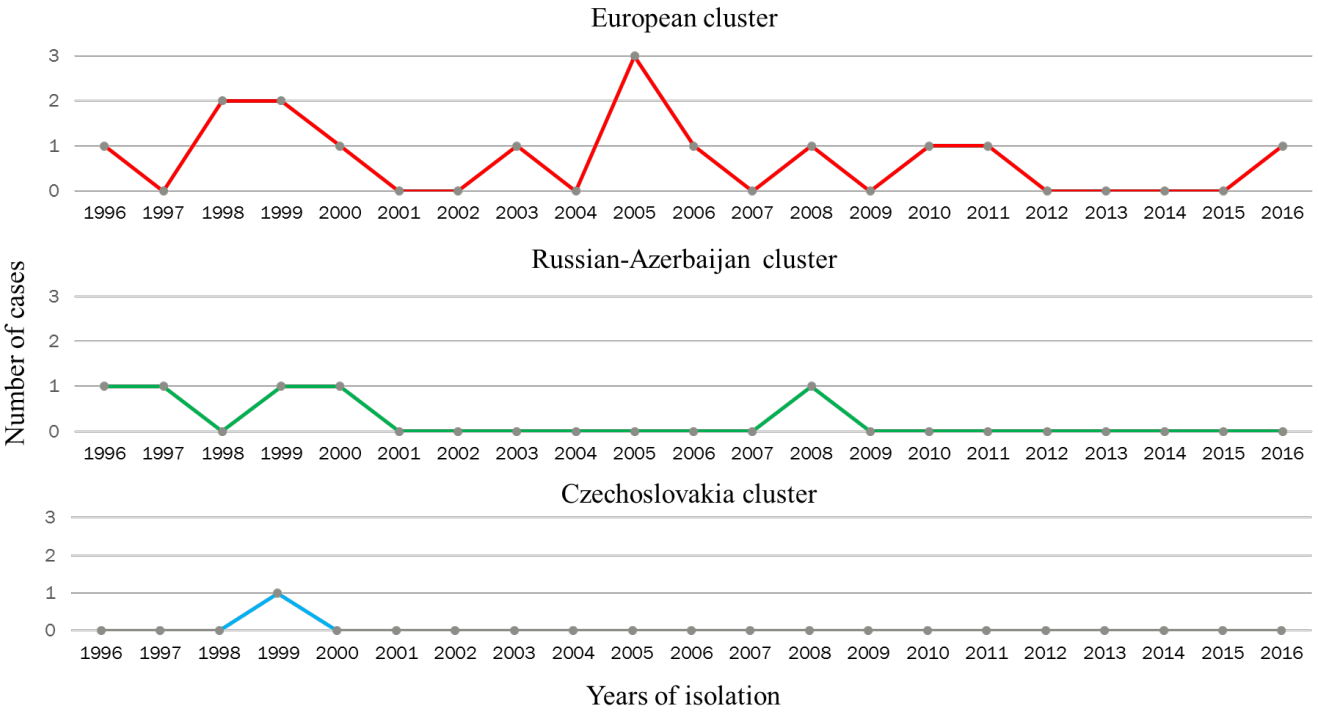


**Figure 3.** The cluster-distribution of studied samples according to years of isolation

It can be assumed that this European genotype is typical for both the territory of Ukraine and many European countries. The sample, which was attributed to the Czechoslovak genotype was isolated from hay in Chernihiv region and may have been accidentally brought to the country.

**Conclusions.** The studied panel included 20 samples that were randomly chosen for MLVA-analysis. This panel is quite small, but it includes samples collected during different years from different regions of Ukraine that makes possible to proceed data in time and space. Considering these output conditions, singling out the European genotype that inherent in the 70% of studied samples almost in every studied year can say that we found the leading genotype that distributed on the territory of all Ukraine and is related to European genotypes of *Francisella tularensis*. Other genotypes need studies in larger panels for more correct traceability and conclusions.

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