

The Balkans Scientific Center  
of the Russian Academy of Natural Sciences

**4**<sup>th</sup>  
International  
Symposium

**MODERN  
TRENDS IN AGRICULTURAL  
PRODUCTION,  
RURAL DEVELOPMENT  
AGRO-ECONOMY  
COOPERATIVES  
AND ENVIRONMENTAL  
PROTECTION**

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29 - 30 June 2022  
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# **The Balkans Scientific Center of the Russian Academy of Natural Sciences**



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**Modern Trends in Agricultural Production, Rural Development,  
Agro-economy, Cooperatives and Environmental Protection**

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## **SURVIVAL OF YERSINIA PSEUDOTUBERCULOSIS IN SOIL**

**Stanojković-Sebić A.<sup>1</sup>, Trifunović B.<sup>2</sup>, Stojanova M.<sup>3</sup>, Đukić D.<sup>4</sup>,  
Mandić L.<sup>4</sup>, Vlajić S.<sup>5</sup>.**

<sup>1</sup>Institute of Soil Science, T. Drajzera 7, Belgrade, Serbia

<sup>2</sup>City of Čačak, City Administration for Urbanism, Ž. Stracimira 2, Čačak,  
Serbia

<sup>3</sup>Ss. Cyril and Methodius University, Faculty of Agriculture and Food,  
Boulevard of Alexander the Great, Skopje, Northern Macedonia

<sup>4</sup>University of Kragujevac, Faculty of Agriculture in Čačak, Cara Dušana 34,  
Čačak, Serbia

<sup>5</sup>Institute of Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, Serbia

Corresponding author: astanojkovic@yahoo.com

### **ABSTRACT**

*The dynamics of the pseudotuberculous microbes population number in the soil was monitored with the use of bacteriological method. The number of this microbe increased during the first week to  $10^6$ - $5 \times 10^6$  CFU/ml, after which it stabilized until the third week at level  $10^6$ , after which there is a continuous decline in the number of *Yersinia pseudotuberculosis* until the end of the second month, when their growth stops.*

**Key words:** *microbe, survival, soil*

### **INTRODUCTION**

The pseudotuberculous microbe belongs to the group of sapronose agents that are characterized by the ability to live in the external environment outside of any connection with the organism of warm-blooded animals and humans, because they are random parasites of these organisms (Đukić et al., 2011). The specific forms and ways of bacterial populations survival in soil or water have not been sufficiently studied, although Willcocks et al. (2018) stated that *Yersinia pseudotuberculosis* is well adapted to survival in the soil. Accordingly, Santos-Montañez et al. (2015) reported the ability of *Yersinia pseudotuberculosis*

persistence in soil and water and in association with fresh produce, but the mechanism by which it persists is unknown. They also quoted that it has been shown that *Yersinia pseudotuberculosis* co-occurs with protozoans in these environments. However, it is known that some of them can be maintained in the external environment in special forms that do not grow on the usual nutrient media.

Under the influence of a large number of factors, these pseudotuberculous microbes regain the ability to actively grow on nutrient substrates. Such forms, called “uncultivated ” (Đukić et al., 2007; 2015; 2020), are known today in legionella, vibrio cholera, salmonella and a number of other microorganisms (Đukić et al., 2011; Vesković and Đukić, 2017 ). The possibility of bacteria becoming uncultivated significantly complicates the study of their ecology in the saprophytic phase, especially the assessment of population dynamics using traditional microbiological methods.

## MATERIALS AND METHODS

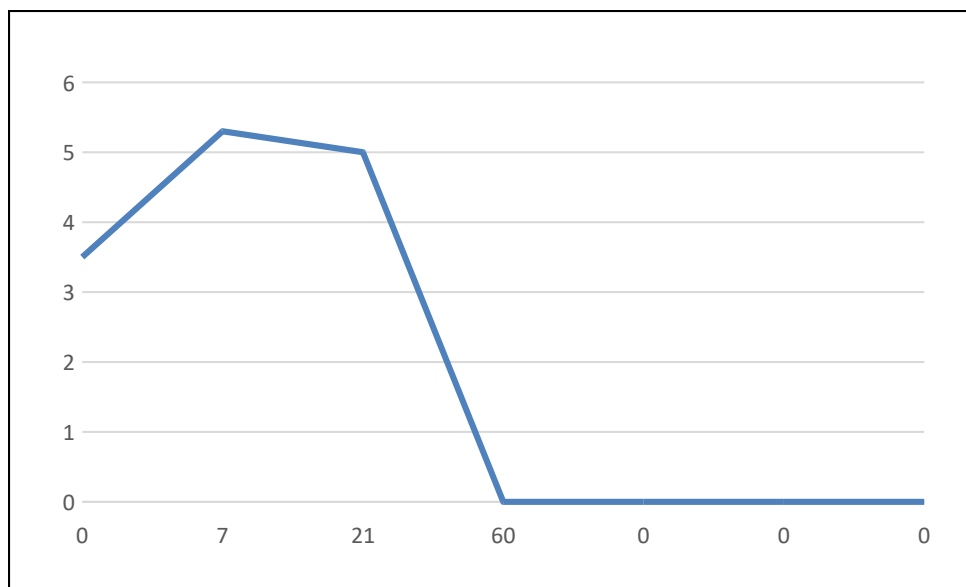
Pseudotuberculosis microbe (*Yersinia pseudotuberculosis*) from the collection of the Laboratory for Microbiology, Institute of Public Health in Čačak, was used in this paper. *Yersinia* colonies were grown on endo-medium. LB-broth and sterile aqueous soil extract (1.2 atm. for 40 minutes) were used as liquid culture media. Cultivation of *Yersinia* in soil extract (initial *Yersinia* concentration- $10^4$ /ml) was performed at room temperature (18-20°C). The number of *Yersinia* during long-term (2 months) presence in the soil was estimated on the basis of CFU.

## RESULTS AND DISCUSSION

During two-month research, it was determined that the number of *Yersinia* increased during the first week to  $10^6 - 5 \times 10^6$  CFU/ml, after which it stabilized at the level of  $10^6$  CFU/ml by the third week. After that period, the number of *Yersinia*, that give colonies on agar, decreased until the end of the second month, when the growth of *Yersinia* stopped (Graph 1). However, it is known that during a longer stay in the external environment there is an increase in the number of uncultivated forms of *Yersinia* at the expense of the cultivated part of the population (Đukić et al., 2009; Mandić et al., 2010). It is not excluded that because of that, *Yersinia* are rarely detected in a low percentage by bacteriological method in soil and other substrates of the external environment. In order to get a



true idea of the presence of yersinia in the environment, it is necessary to determine the quantitative assessment of uncultivated forms of yersinia, using PCR and other methods.



Graph 1. Dynamics of *Yersinia pseudotuberculosis* abundance in sterile soil extract, lg CFU/ml

Further research should clarify not only this, but also many other issues, which are related to the new form of adaptive variability of microorganisms in the environment, with the mechanisms of their transition to uncultivated state, as well as environmental and molecular genetic factors affecting that process.

## CONCLUSION

Based on the obtained results, it can be concluded that the number of *Yersinia pseudotuberculosis* increases until the end of the first week, then stabilizes by the end of the third week, and then follows a continuous decline until the end of the third month. In order to get a more realistic idea of the yersinia presence in the soil, it is necessary, with the use of PCR method (or some other), to assess the qualitative presence of uncultivating forms of these microorganisms.

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