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Workshop on Q fever

Dear reader,

On May 18–19, 2017, the Institute of Virology, Biomedical Research Center of Slovak Academy of Sciences in Bratislava, Slovakia, was hosting a general meeting of the project 21610493 that was sponsored by the International Visegrad Fund. The meeting was accompanied by a Workshop on Q fever with public attendance. Experts from all four Visegrad countries and Germany were discussing a current status of this re-emerging zoonosis in Europe and trends in the field.

Q fever is a febrile illness caused by *Coxiella burnetii.* It was first documented by Edward Derrick in 1937 as a human disease among abattoir workers in Queensland, Australia. The non-specific symptoms of fever, intense headache, malaise, and anorexia led to a search for infectious causes of typhus, typhoid and paratyphoid fever, and leptospirosis. When all the tests of these infections were negative, the hitherto unknown disease was a designated as a Query (Q) fever. This investigation in Australia concurred with the isolation of an unknown agent from ticks collected in Nine Mile Creek, Montana, USA. The initial report of the American team rejected it as a true bacterium and assigned it viral characteristics since it was unable to grow in cell-free culture.

Further studies of Q fever "virus" indicated rickettsia-like properties. It resulted in the initial designation to the genus *Rickettsia* due to morphological similarity. However, based on the sequence of its 16S rRNA, the *C. burnetii* has been phylogenetically reclassified from the order of *Rickettsiales* to *Legionellales,* the family *Coxiellaceae*. Closest relatives are *Legionella, Francisella,* and *Rickettsiella* species. Thus, the bacterium is a Gram-negative coccobacillus. Typical for this family is the intracellular and intravacuolar lifestyle in cells of invertebrate or vertebrate hosts. Its life cycle contains two morphologically distinct forms. The small cell variant (SCV) is the extracellular form that is metabolically inactive. In contrast, the large cell variant (LCV) is metabolically and divisionally active. It develops inside the monocytes or macrophages after infection of the host's immune cells by SCV. The trigger for this differentiation seems to be the low pH inside the parasitophorous vacuole.

In addition, the bacterium undergoes antigenic phase variation. The cells isolated from natural sources have full-length lipopolysaccharide (LPS) molecules with O-antigen in the membrane that representing the phase I variant. The phase shift may occur in the laboratory when serial in vitro passages in embryonated hen eggs or tissue culture results in truncated LPS molecules. This form is referred to the phase II bacterium. The phase II LPS has the same Lipid A as phase I but lacks the *C. burnetii*-specific carbohydrates virenose and dihydrohydroxystreptose (Skultety *et al*., 1998). This phase variation phenomenon is associated with loss the of virulence in immune-competent animals and human (Toman *et al*., 2013). The cells in phase I are virulent, while the bacterium in phase II does not cause the disease. Moreover, the inactivated form of phase II cells applied as a vaccine does not protect against subsequent infection. This phase shift has a huge impact also to serological response. During the acute infection, an antibody against phase II is first detected, followed by antibodies against phase I antigens. On the other hand in chronic infection, antibody titers to phase I antigens are persistently present.

The research on Q fever began due to human outbreaks more than 80 years ago and two decades later also in Visegrad countries. Since then, some but not all characteristics of this causative bacterium and its interaction to host were revealed. Although Q fever is considered to be an occupational disease, outbreaks have a major public health impact and attract the most attention. The Q fever outbreak in Nederlands which resulted in 4,026 human cases during the years 2007–2010, is an example of how Q fever can re-emerge from an endemic state into an outbreak of abrupt dimension. In this epidemic, the link between dairy goats and human cases was confirmed by genotyping for the first time. The same was also achieved during the last outbreak in southern Hungary in spring and summer of 2013. Seventy human infections were associated with merino sheep flock of 450 ewes, in which 44.6% (25/56) seropositivity was detected. The multispacer sequence typing examination of *C. burnetii* DNA revealed the sequence type 18 in the human and manure samples (Gyuranecz *et al*., 2014).

In Poland, the first outbreak of Q fever was recognized in Owczary (Podkarpackie), formerly Rzeszów province in 1956. The infection was confirmed by serological methods in 63 farm workers. Epidemiological investigation revealed that the source of infection was a sheep flock imported from Romania. After several small epidemics, the largest one occurred in 1982 in Ulhówek, formerly Zamość Province that resulted in 1300 human cases. Thus, the surveillance, and control-Q fever in livestock is of particular importance (Szymańska-Czerwińska *et al*., 2017). The authors investi-

gated the seroprevalence of *C. burnetii* in horses and ruminants including cattle, sheep, and goats in Poland. The role of the vector in the pathogen circulation in natural foci was also evaluated (Sallay *et al*., 2017).

Within the period of 1972–1982 large-scale vaccination of the ruminants and their veterinary control were implemented in former Czechoslovakia, which resulted in significant reduction of Q fever cases in the country. Nevertheless, the largest outbreak of human Q fever occurred in 1993 in the village Jedľové Kostoľany, Nitra District, Slovakia with 113 human cases. Epidemiological investigation revealed that the source of infection was a goat flock imported from Bulgaria (Flores-Ramirez *et al*., 2017). It was later recognized that the complex nature of whole cell antigens may impair the sensitivity and specificity of the used diagnostic kits. Therefore, the discovery of active immunogenic proteins became a subject of interest to researchers for decades (Gerlach *et al*., 2017). These compounds might not only improve *C. brunetii* diagnosis, but they can be beneficial also in subunit vaccine development. Flores-Ramirez *et al.* (2017) have analyzed the proteins present in phase I *C. burnetii* soluble antigen prepared trichloroacetic acid extraction which in the past was used as a chemovaccine against Q fever extraction in men and discovered 12 immunoreactive proteins.

These analyses were based on mass spectrometry. Using the same technique, the partner from the Czech Republic has introduced a novel molecular classification tool. The rapid evaporative ionization mass spectrometry was evaluated not only as an ionization technique coupled with the surgical device but also as a biomarker discovery tool for analyzing bacterial colonies. For a more complex understanding of pathological processes at the cellular and molecular levels, the importance of multimodal approach in imaging applications was also shown in the context of fiducial markers needed for hyperspectral data fusion collected by molecular mass spectrometry imaging and optical microscopy (Luptáková *et al*., 2017). Finally, they also presented a methodological approach for creation color pictures from the grayscale image taken simultaneously from at least three different detectors of the scanning electron microscope. They have shown several color images, including bacterial cells (Kofroňová and Benada, 2017). These two methological papers indicate the emerging analytical portfolio which soon can be applied also in *Coxiella* research.

Although *C. burnetii* seems to have a potential to cause epidemics in man, it is not known yet what factors are essential for its eruption. To prevent Q fever outbreaks, a better understanding of these factors and their interaction is necessary.

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