

Comparison of three methods of DNA isolation for PCR study on *Babesia* spp., *Rickettsia* spp., *Borrelia* spp.

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During recent years infectious diseases in animals caused by viruses, bacteria and protozoa transmitted by ticks have emerged as an important problem of public health and veterinary practice. To date, only limited data about the presence of ticks and circulation of tick-borne pathogens in Ukraine are available. The area investigated in this study is situated in western Ukraine. It is characterized by fields, pastures and forested areas. During 2018 year ticks were collected form vegetation by dragging method in Khmelnitsky region. Immediately after collection, ticks were immersed in 70% ethanol and finally stored at 4°C. In total, 72 ticks (60 *Dermacentor reticulatus* and 12 *Ixodes ricinus*) were screened by PCR for the presence of genetic material of *Babesia* spp., *Rickettsia* spp., *Borrelia* spp. Three different methods were utilized to extract DNA: (i) shredding the tick with scissors and lysis in ammonium hydroxide (Guy and Stanek, 1991), (ii) shredding with scissors or (iii) homogenization of ticks by SPEX SamplePrep followed by DNA extraction with kit Genomic Mini AX Tissue Spin (A&A BIOTECHNOLOGY, Poland).

As a result, with the first method of DNA isolation prevalence of Rikettsia spp. in the analysed ticks was 12%, *Babesia* spp. – 3%, *Borrelia* spp. – 32%; second method – *Rikettsia* spp. – 54%, *Babesia* spp. – 4%, *Borrelia* spp – 35%; at the third *Rikettsia* spp. – 40%, *Babesia* spp. – 4%, *Borrelia* spp. – 36%. As an outcome of this study, it can be concluded that the machine homogenization of ticks followed by DNA isolation with dedicated kits is effective method, which enhance detectability of pathogen's genetic material in ticks.