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Veterinarni Medicina

Quantification of the effectiveness of laboratory diagnostics of rabies using classical and molecular-genetic methods

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[fulltext]

In comparative experiments the diagnostic effectiveness of four methods of laboratory diagnostics of rabies – the mouse intracerebral inoculation test (MICIT, MIT), the rabies tissue culture infection test (RTCIT), the rapid rabies enzyme immune diagnosis test (RREID) and a molecular-genetic method, the nested reverse transcription polymerase chain reaction (nRT-PCR) – was quantified by the titration of serial dilutions of brain viral suspensions. The threshold value of the tests, i.e., the highest dilution of a specimen, which the method used is able to detect as a positive one, was determined. Further

tested methods were compared as well. Experimental optimization of procedures for RNA extraction was carried out and the optimum primer for RNA transcription to cDNA was selected. The RREID method was carried out in two variants: detection of the rabies antigen in a clarified (centrifugated) as well as in a non-clarified (noncentrifugated) brain suspension. In the experiments three autochthonous street isolates of rabies virus (in the form of primary isolates) were used; they had been isolated from naturally infected red foxes (*Vulpes vulpes*) and a lynx (*Lynx lynx*). The results of comparative experiments revealed a relative correlation of the diagnostic effectiveness of standard methods (MICIT and RTCIT), with standard MICIT being the more sensitive one, RTCIT however having several other advantages (among others the speed of performance) and thus being preferred. For quantitative comparison of diagnostic effectiveness two other methods (RREID and nRT-PCR) were examined in that street isolates of rabies virus, which revealed the highest titer after titration by MICIT and RTCIT. The sensitivity of the

RREID method proved to be rather low. If used with noncentrifugated brain suspensions this method may yield nonspecific reactions. If compared particularly with RREID the nRT-PCR is characterized by a considerably higher diagnostic effectiveness. The sensitivity of nRT-PCR is not affected by preliminary clarification of the brain suspension. The reverse primer N12 seems to be more suitable for transcription of the extracted RNA to cDNA than random hexamers.

Keywords:

rabies; rabies virus (RABV); MICIT – mouse intracerebral inoculation test; MIT – mouse inoculation test; RTCIT – rabies tissue culture infection test; RREID – rapid rabies enzyme immune diagnostic test; nRT-PCR – nested reverse transcription polymerase chain reaction

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