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P0779 Development of enzyme-linked immunosorbent assay for detection of specific antibodies to hepatitis E virus in rabbits

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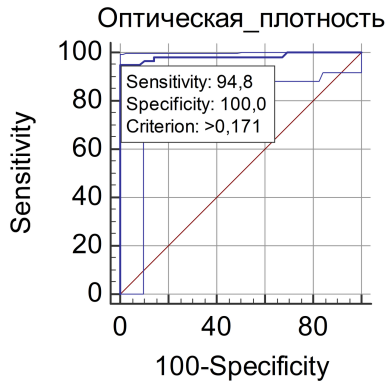
Background: Hepatitis E virus (HEV) is classified within the family *Hepeviridae*, genus *Hepevirus*. HEV genotype 3 (HEV3) infections are endemic in Europe and in America and cause infections in humans and different animals. There are deficiency of available serological assays to detect specific anti-HEV antibodies in animals, including rabbits.

Materials/methods: Recombinant proteins of HEV3 open reading frame (ORF) 2 and 3 were coated onto polystyrene ELISA plates in concentrations 1, 2, 4 and 8 µg/ml. After incubation of rabbit sera samples, bound anti-HEV antibodies were detected with anti-rabbit-IgG conjugate. For further validation of the assay and setting the cutoff value, a batch of 107 rabbit sera samples was used. All rabbit sera samples were tested for HEV RNA and anti-HEV-IgG using the reverse transcript PCR-analysis and adapted serologic assay based on using peroxidase conjugate of protein A. Since there is no gold standard available for HEV antibody testing, further validation and a definite setting of the cutoff of the developed HEV3 assay were performed using a statistical approach.

Results: Optimal concentrations for sorption recombinant ORF2 and ORF3 proteins were 2 µg/ml and 1µg/ml respectively. The cutoff value was equal to 0,171. Diagnostic sensitivity of the developed method was 94,8% and diagnostic specificity – 100% (picture). Coefficients of variation of intra-serial reproducibility and inter-serial reproducibility were 3,5% 12,4% respectively. These data demonstrated obtaining of highly reproducible results and detection of specific antibodies to HEV in all positive rabbit's serum samples. The described diagnostic method can be widely used in practice for the further study of the seroprevalence of anti-HEV-IgG in rabbits.



Evaluation of diagnostic sensitivity and specificity of the ELISA method

Conclusions: The developed antibody assay showed effective detection of HEV-specific antibodies in rabbits. This assay can contribute to an improved detection of HEV antibodies and estimation the prevalence of HEV in rabbits from different regions.