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VETERINARSKI FAKULTET

Završni rad specijalističkog studija „Veterinarska medicina i javno zdravstvo“

Smjer: „Zarazne bolesti životinja“

„METODE DIJAGNOSTIKE BOLESTI PLAVOG JEZIKA KOD DOMAĆIH ŽIVOTINJA“

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SAŽETAK

U ovom radu ispitivana je dijagnostika virusa plavog jezika (Bluetongue virus, BTV) kod ovaca sa područja općine Neum primjenom kombinacije seroloških, virusoloških i molekularnih metoda. Istraživanje je obuhvatilo uzorke krvnog seruma klinički sumnjivih životinja, kao i dodatne uzorke dobijene tokom eksperimentalnih procedura u svrhu izolacije i molekularne analize virusa.

Serološka ispitivanja izvedena su primjenom agar-gel imunodifuzionog testa (AGID) i kompetitivnog ELISA testa (cELISA), uz korištenje komercijalnih kitova i standardizovanih laboratorijskih protokola za detekciju specifičnih antitijela protiv BTV. AGID test je izvođen metodom difuzije u agaroznom gelu uz upotrebu referentnih seruma i virusnog antigena, dok je cELISA izvedena u mikrotitar pločama obloženim antigenom virusa plavog jezika uz upotrebu monoklonskih antitijela konjugovanih peroksidazom i spektrofotometrijsko očitavanje rezultata.

Molekularna detekcija virusa izvršena je RT-qPCR metodom nakon ekstrakcije virusne RNK primjenom QIAamp Viral RNA Mini Kita. Analizirani su različiti tipovi uzoraka, uključujući punu krv, homogenat tkiva embriona i supernatant ćelijske kulture nakon pasaže. Amplifikacija je provedena na real-time PCR uređaju Stratagene Mx3005P, ciljajući konzervirani segment 10 genoma BTV, uz upotrebu specifičnih prajmera i fluorescentne detekcije signala.

Virusološka izolacija vršena je na dva biološka sistema: embrioniranim kokošijim jajima i BHK-21 (baby hamster kidney) ćelijskoj kulturi. Inokulacija embrioniranih jaja izvedena je intravenoznim putem u krvne sudove embrija uz prethodnu pripremu inokuluma homogenizacijom i centrifugiranjem uzoraka. Inkubacija je trajala sedam dana uz kontinuirano

lampiranje i praćenje vitalnosti embrija. U ćelijskoj kulturi korišteni su EMEM i DMEM mediji sa dodatkom fetalnog goveđeg seruma, a inokulacija je praćena u više pasaža uz evaluaciju citopatogenog efekta (CPE) i citotoksičnog efekta (CTE).

Priprema uzoraka uključivala je standardne laboratorijske postupke kao što su centrifugiranje, filtracija kroz 0,45 μm filtere, homogenizacija tkiva i ultrazvučna obrada u cilju oslobađanja virusnih čestica. Svi postupci su izvođeni u uslovima biosigurnosnog nivoa II.

Korištene metode omogućile su paralelnu analizu serološkog odgovora, prisustva virusnog genoma i mogućnosti izolacije infektivnog virusa, čime je izvršena sveobuhvatna procjena dijagnostičkih pristupa u detekciji BTV. Poseban naglasak stavljen je na uporednu primjenu seroloških metoda (AGID i cELISA), molekularne metode (RT-qPCR) i virusološke izolacije u embrioniranim jajima i ćelijskoj kulturi.

Ključne riječi: virus plavog jezika (BTV), ovce, AGID, kompetitivni ELISA (cELISA), RT-qPCR, virusološka izolacija, BHK-21 ćelijska kultura, molekularna dijagnostika.

UNIVERSITY OF SARAJEVO
FACULTY OF VETERINARY MEDICINE

Specialist Study Programme in Veterinary Medicine and Public Health
Specialization: Infectious Diseases of Animals

„DIAGNOSTIC METHODS FOR BLUETONGUE DISEASE IN DOMESTIC ANIMALS“

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Sarajevo, 2026

SUMMARY

In this study, the diagnostic approach for Bluetongue virus (BTV) infection in sheep from the Neum municipality area was evaluated using a combined application of serological, virological, and molecular methods. The investigation included serum samples from clinically suspected animals, as well as additional samples generated during experimental procedures for virus isolation and molecular analysis.

Serological testing was performed using the agar gel immunodiffusion test (AGID) and a competitive enzyme-linked immunosorbent assay (cELISA), employing commercial kits and standardized laboratory protocols for the detection of BTV-specific antibodies. The AGID test was conducted by double immunodiffusion in agarose gel using reference positive and negative sera and viral antigen, while the cELISA was performed in microtiter plates coated with BTV antigen. Detection was based on monoclonal antibodies conjugated with horseradish peroxidase and spectrophotometric measurement of optical density.

Molecular detection of BTV was carried out using real-time reverse transcription PCR (RT-qPCR) following viral RNA extraction with the QIAamp Viral RNA Mini Kit. Different sample types were analyzed, including whole blood, embryonic tissue homogenates, and cell culture supernatants after passage. Amplification targeted the conserved segment 10 (Seg-10) of the BTV genome using specific primers and fluorescent probe-based detection on a Stratagene Mx3005P real-time PCR platform.

Virus isolation was attempted in two biological systems: embryonated chicken eggs and BHK-21 (baby hamster kidney) cell culture. Inoculation of embryonated eggs was performed via intravenous administration into the embryo’s vasculature following sample preparation

through homogenization, centrifugation, and sonication. Embryos were incubated for seven days with continuous candling and viability monitoring. In cell culture systems, EMEM and DMEM media supplemented with fetal bovine serum were used, and viral growth was assessed through multiple passages by monitoring cytopathic effect (CPE) and cytotoxic effect (CTE).

Sample preparation included standard laboratory procedures such as centrifugation, filtration through 0.45 μm filters, tissue homogenization, and ultrasonic treatment to release viral particles. All procedures were conducted under biosafety level 2 (BSL-2) conditions.

The applied methodological approach enabled parallel assessment of serological responses, viral genome detection, and the potential for infectious virus isolation, providing a comprehensive evaluation of diagnostic strategies for BTV detection. Particular emphasis was placed on comparative performance of serological assays (AGID and cELISA), molecular detection (RT-qPCR), and virological isolation in embryonated eggs and cell culture systems.

Keywords: Bluetongue virus (BTV), sheep, agar gel immunodiffusion (AGID), competitive enzyme-linked immunosorbent assay (cELISA), RT-qPCR, virus isolation, BHK-21 cell culture, molecular diagnostics.