



"PARAZITAR KASALLIKLAR DIAGNOSTIKASIDA MOLEKULAR GENETIK USULLARNI QO'LLASH, DAVOLASH VA PROFILAKTIKASINING ZAMONAVIY JIHLATLARI"

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EVALUATION OF A CHELEX 100-BASED DNA EXTRACTION PROCEDURE FOR MORPHOLOGICALLY IDENTIFIED ADULT CYATHOSTOMINS

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UDC: 619:616.995.1:577.2**EVALUATION OF A CHELEX 100-BASED DNA EXTRACTION
PROCEDURE FOR MORPHOLOGICALLY IDENTIFIED ADULT
CYATHOSTOMINS****Demkina O.V.¹, Kotelnikov D.D.²**¹ *Far Eastern State Agrarian University**86 Polytechnicheskaya St., Blagoveshchensk, Amur Region, Russia*² *Institute for Agrarian Studies, HSE University**11 Pokrovsky Boulevard, Moscow, 109028, Russia.*

Cyathostomins (Nematoda: Strongylida: Cyathostominae) are common gastrointestinal nematodes of horses and comprise a taxonomically diverse group. Morphological identification of adult specimens remains important for species determination, whereas molecular approaches provide additional possibilities for taxonomic verification and analysis of genetic variation, including polymorphisms associated with anthelmintic resistance [1, 2]. Molecular assays for small strongyles require DNA of sufficient quality for amplification; therefore, a simple procedure that permits individual morphologically identified specimens to be processed separately is useful for combined morphological and molecular studies. Coles et al. [1] also emphasized that the integrity of DNA used in molecular assays may be assessed by amplification of independent gene sequences.

The aim of the study was to evaluate an adapted Chelex 100-based procedure for DNA extraction from individual morphologically identified adult cyathostomins and to assess the yield and spectrophotometric characteristics of the obtained DNA. PCR amplification was used only as an additional functional test of DNA suitability for subsequent molecular analysis.

Eleven adult cyathostomins were morphologically identified and processed individually. Each specimen was transferred into 50 µL of a 5% Chelex 100 m/w, and 2 µL of proteinase K (20 mg/mL) was added. Samples were incubated at 56°C for 1 h and subsequently heated at 99°C for 8 min. After centrifugation at 15,000 × g for 3 min, the supernatant containing DNA was collected without disturbing the Chelex resin. Before PCR, DNA extracts were diluted 1:10 with nuclease-free water.

DNA concentration and absorbance ratios A₂₆₀/A₂₈₀ and A₂₆₀/A₂₃₀ were determined by microvolume spectrophotometry. Measurable nucleic acid was obtained from all 11 specimens. DNA concentrations ranged from 1.9 to 106.6 ng/µL, with a median of 12.5 ng/µL and a mean of 29.9 ng/µL. The A₂₆₀/A₂₈₀ ratio ranged from 1.80 to 6.33; the median value was 1.97, and six of the eleven extracts had values between 1.80 and 2.00. The highest A₂₆₀/A₂₈₀ ratio (6.33) occurred in the extract with the lowest DNA concentration (1.9 ng/µL), indicating that absorbance ratios should be interpreted cautiously when absolute absorbance values are low. A₂₆₀/A₂₃₀ values were highly variable and were therefore not used as the principal criterion of extract suitability.

The extracts were additionally tested by PCR. Amplification was obtained from the prepared DNA samples, confirming the presence of amplifiable template.

In the two PCR runs, the amplification curves crossed the preset fluorescence threshold at approximately cycles 14–30 and 20–30, respectively. These values were used only as a functional characteristic of the extracts and were not interpreted quantitatively. Detailed evaluation of amplification performance and taxon-specific PCR was outside the scope of the present study.

The Chelex 100/proteinase K procedure provided DNA from individually processed, morphologically identified adult cyathostomins without column purification or organic solvents and with a limited number of sample-transfer steps. This is relevant when individual parasite specimens must remain analytically independent for subsequent molecular characterization. PCR amplifiability provided a more direct indication of downstream suitability than spectrophotometric ratios alone, particularly for low-concentration extracts.

In conclusion, the adapted Chelex 100-based procedure allowed DNA to be recovered from all examined adult cyathostomins and produced templates suitable for subsequent PCR amplification. The method can be used as a simple preparatory approach in studies combining morphological identification of individual cyathostomins with molecular genetic analysis.

References

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