XXI Middle European Buiatrics Congress

19-22.05.2022

Stare Jabłonki, Poland

Congress Proceedings

COMPARISON OF TWO ANAEROBIC METHODS OF FECAL SAMPLE STORAGE FOR *IN VITRO* DIAGNOSTICS OF ANTHELMINTIC RESISTANCE

M. Mickiewicz[1*], A.V. Potarniche[2], M. Czopowicz[1], Z. Nowek[1], A. Moroz[1], K. Biernacka[1], O. Szaluś-Jordanow[3], I. Markowska-Daniel[1], J. Kaba[1]

[1]Division of Veterinary Epidemiology and Economics, Institute of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 159c, 02-776 Warsaw, Poland

[2]Department of Infectious Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Mănăștur Str. 3-5, 400372, Cluj, Romania

[3]Division of Small Animal Infectious Diseases, Department of Small Animal Diseases with Clinic, Warsaw University of Life Sciences-SGGW, Nowoursynowska 159c, 02-776 Warsaw, Poland

*Correspondence: marcin_mickiewicz@sggw.edu.pl

Background: Parasitic infections, in particular those caused by gastrointestinal nematodes, are responsible for substantial economic losses in goat production worldwide. Their control relies mainly on using anthelmintics of various classes with benzimidazoles being most popular. Unfortunately, extensive and reckless deworming has led to the emergence and spread of anthelmintic resistance in gastrointestinal nematode populations. Anthelmintic resistance to benzimidazoles can be detected using an *in vitro* egg hatch test (EHT), in which nematode eggs are incubated for 48 hours in increasing concentrations of thiabendazole (TBZ) and percentage of hatching eggs is recorded. The shortcoming of EHT is the need to use of fresh fecal samples (less than 3 hours after collection) in which embryonation of nematodes in eggs has not started yet. As embryonation is an aerobic process storage of fecal samples in anaerobic conditions may delay its onset. In this study we compared the effectiveness of two anaerobic methods of fecal sample storage in inhibiting egg hatching and preserving the sample for EHT.

Methods: The eggs of gastrointestinal nematodes were isolated from pooled fecal sample collected from 46 naturally infected goats. Feces were homogenized and divided into two parts. One part was placed in 14 separate 50 ml bottles tightly filled up with tap water. The second part of pooled fecal sample was portioned into 14 bags from which air was removed air by vacuum packing. The EHT was performed daily according to standard procedure on 14 consecutive days with use of samples stored in both anaerobic methods. Eggs were washed and suspended in deionized or tap water at a concentration of 100 eggs per ml and eggs were inspected microscopically to determine if embryonation had already begun. Each sample was tested in duplicate and at least two negative control samples were used. Results of EHT were determined as the percentage of hatched eggs at the discriminating concentration for TBZ (0.1 μg/ml). The number of hatched eggs was corrected for

natural mortality from control wells. The concentration of TBZ inhibiting hatching of 50% (median effective dose, ED_{50}) of eggs was estimated using the four-parameter logistic curve.

Results: The percentage of hatching larvae remained over 90% for 5 days in tap water storage and 6 days in vacuum storage. Then, it dropped abruptly to only 20% on day 10 in tap water storage, while it was still 70% in vacuum storage. On day 14 the percentage of hatching larvae in vacuum storage was still 30%. The ED_{50} value and percentage of hatching larvae at inhibitory TBZ concentration remained stable for 9 days in tap water storage and for the entire 14-day study period in vacuum storage.

Conclusions: Vacuum storage method seems to maintain suitability of a fecal sample for EHT considerably longer than tap water storage. This observation warrants confirmation in a longitudinal study with the representative number of replications.

Keywords: anthelmintics resistance, egg hatch test, goats, gastrointestinal nematodes

The study was financed by the grant from the National Science Center (UMO-2020/37/B/NZ6/00457)