8. Summary

Commonly applied anthelminthics, often in doses which are adjusted not accordingly to invasions occurred, create a risk of appearing drug resistant nematodes worms. This applies in particular to parasites whose elimination requires longer pharmacotherapy. As for carnivores these are hookworms, whipworms and capillarias. Recent reports on benzimidazoles efficiency related to dogs' nematodes are limited. Also observed drugs resistance mechanisms are not fully recognized. It is assumed that they may be similar to *Haemonchus contortus*, the one described in connection with nematodes hookworms. The described mutations of this nematode are related to changes occurring in codons 167, 198 and 200 of the tubulin gene. However, till now, despite conducted research with multiple hookworms types, only individual *Ancylostoma caninum* mutation cases as well as human *Necator americanus* mutations have been reported. In addition, there is no data available on *Uncinaria stenocephala* which is the most commonly widespread hookworm type in Poland.

Another important factor of prevention in case of nematodes invasions is preventing infestation of environment with parasites eggs by decontamination. However, here as well, available efficiency data is limited and refers mainly to *Toxocara spp.*

The purpose of the study was to analyze current invasion status related to dogs' endoparasites, determine benzimidazolic anthelmintics efficiency, as well as to attempt to find drug resistant hookworms in the Lublin region. Additionally, the impact of disinfecting agents on eggs development and dogs' hookworms larvas' survival was analyzed.

500 feaces samples of dogs from urban, rural and dog shelter from Lublin region were examined. The study was conducted from 2008 till 2014 using flotation, decantation and molecular operations, PCR and pyrosequentionning. Then, using flotation-decantation, eggs were separated and tested over larvas' hutching (EHT) in order to determine their benzimidazoles resistance or responsiveness. Isolated eggs were examined in relation to disinfecting agents' efficiency, as well.

The results of conducted coproscopic tests show the prevalence of infection of 56,4% of the dogs from Lublin region. The analysis of the results in relation to a situation in the previous decades presents an increased prevalence of such types as *U. stenocephala* (26,8%) and *Trichuris vulpis* (21,4%). Interestingly, the invasion of

respiratory system nematodes, *Capillaria aerophila* (7,8%) has not been described in Poland. The highest dogs' parasitosis prevalence occurred in May and August. Origin ratios were the highest for rural areas (67,3% of dogs were infested), then 59,9% for dog shelter and 43,7%, respectively, in the urban areas. With regards to age, 96,6% of dogs up to 6 months were infected. The prevalence was negatively correlated with the animals' age. Sex had no impact.

The results of *U. stenocephala* egg hatch test (EHT) show low eggs elimination efficiency of fenbendazole compared to tiabendazole. In case of the first anthelminthic, 33,3% of the parasites continued their development, respectively 51,3% for the second one which allows to conclude that this population is fenbendazole resistant and tiabendazole responsive. Tiabendazole showed particular egg hatch properties, stopping the invasion before larva development stage, while fenbendazole may only stop larvas' hutching process. Such data show decreasing efficiency of fenbendazole – commonly used in dogs' infections cases in comparison with another medicine of the same group which is not applied in case of animals. As for the doses (0,05 μg /ml; 0,1 μg /ml; 0,185 μg /ml), no significant impact of the used dose for parasites elimination efficiency was observed. Molecular studies have shown that dominant species of hookworms in dogs from Poland is *Uncinaria stenocephala.*

Molecular studies have shown that dominant species of hookworms in dogs from Poland is *Uncinaria stenocephala.*

In order to detect polymorphism in codons 167, 198 and 200 of *U. stenocephala* β-tubulin gene using molecular methods, pyrosequencing was applied as this technique allows us to determine the ratio of resistant and susceptible genes in a respective parasites' population. The results of the study do not show any presence of muted homozygotes in analyzed codons, however heterozygotes with resistant alleles were found in two cases. Potential dominating alleles, detected in isolates tested using EHT confirmed the two heterozygotes diagnosed with molecular methods. Those are samples which, in case of fenbendazole, showed poor egg elimination effect (0-5%). Detection of important number of benzimidazoles resistant worms in EHT test allows to conclude that mutations related to drugs resistance may occur also in other areas of *U. stenocephala* genome.

Only two out of eight agents tested stopped *U. stenocephala* eggs' development. Rapicid iodophoric agent and Bioval chloroxylenole stopped development of 50,6% and 22,9% of the eggs respectively. During the stage of an egg with developed larva, the

highest parasites' population was eliminated by the highest concentration of formalin and Virkon potassium monosupersulphate, as well as KOH and NaOH. In case of hutched larvas, 20,2% and 2,7% individuals, respectively, survived the use of formalin and chlorhexidine. The highest number of surviving larvas was observed in case of low concentration of Virkon (93,7%). Other means eliminated all hookworms larvas which left egg membranes. As the majority of disinfectant agents eliminate parasites during the stage of an egg with developed larva, an appropriate decontamination strategy, considering zygote development cycle, must be applied.