Invited lecture/Scientific contribution

Canine Dirofilariasis in the South Caucasus and its Pathomorphology

Tsitskishvili Levan[1], Makaradze Levan[2], Kurashvili Tengiz[2], Milashvili Nino[2,3] , Makaradze Zurab[2], Sanaia Ekaterina[4,*]

1. Scientific-Research Center of Agriculture (SRCA), Tbilisi, Georgia
2. Samtskhe-Javakheti State University of Georgia (SJSU), Akhaltsikhe, Georgia
3. European University (EU), Tbilisi, Georgia
4. Georgian Technical University (GTU), Tbilisi, Georgia

* Correspondence: Ekaterine Sanaia; esanaia@gmail.com

Abstract:
The main goal of this paper is to provide the results of a study on the regional distribution of dirofilariasis (causative agent: *Dirofilaria immitis*, *Dirofilaria repens*, and other concurrent filarial species in animal reservoirs) among the canine population in Georgia and Armenia and estimate the disease burden in these countries.
In total, 1065 animals were investigated in the different regions of Armenia and Georgia, and 26 positive cases were detected. The prevalence of dirofilariasis is 2.4%. However, it varies across regions from 0% to 25%. The Kruskal-Wallis test showed that variation is statistically significant (P=0.01). The majority of positive cases (11) were from the Kvemo Kartli region (Georgia). The highest number (7) of cases have been found in the Armavir region (Armenia). The other cases were found in Tbilisi / Capital City of Georgia (two cases), Yerevan / Capital City of Armenia (two cases), Shirak Region (two cases), and only one case in regions Achara and Ararat.

Keywords: *Dirofilaria* spp., Canine Dirofilariasis, Georgia, Armenia, Souse Caucasus, Vector-Borne disease (VBD)

Citation: Tsitskishvili L, Makaradze L, Kurashvili T, Milashvili N, Makaradze Z, Sanaia E. Canine Dirofilariasis in the South Caucasus and its Patho-morphology. Proceedings of Socratic Lectures. **2023**, 8; 46-51. https://doi.org/10.55295/PSL.2023.II8

1. Introduction

Dirofilaria are long, thin parasitic roundworms that infect a variety of mammals. Infection is transmitted by mosquito bites. There are many species of Dirofilaria, but human infection is caused most commonly by three species; *D. immitis, D. repens,* and *D. tenuis.* The main natural hosts for these three species are dogs and wild canids, such as foxes and wolves (*D. immitis* and *D. repens*) and raccoons (*D. tenuis*). *D. immitis* is also known as "heartworm."

1.1. Historical overview for south Caucasus region.

The problem of dirofilariasis has not been studied sufficiently in Georgia and Armenia. Until recently, no proper attention were given to prevention, diagnosis, and treatment of *Dirofilaria* spp. in animal reservoirs in the South Caucasus. Dirofilariasis was considered an occasional problem for the region and few identified human cases were thought to be imported from other subtropical and tropical countries.

To our best knowledge, the first study on canines in different regions of Georgia was performed by the first author in cooperation with veterinary clinics during the period 2005-2008. The main goal of the survey was to reveal high risk zones for the disease and determine the ecological and other characteristics of zones. Almost all clinics from 10 regions and 30 cities/towns of the country participated in the study and provided the requested information on the study subjects. 3887 dogs were examined during the study period and 70 cases were identified with confirmed dirofilariasis diagnosis. The study contributed to the understanding of the disease distribution in the country and now dirofilariasis is a well-recognized endemic disease in this geographical area.

Figure 1 shows association between the prevalence of dirofilariasis and climatic conditions of the territory. The maximum prevalence of the disease is revealed in Samegrelo (Kolkheti lowland, above sea level 0-200 m) and Kvemo Kartli (Gardabani vake, above sea level 260-450 m) regions.

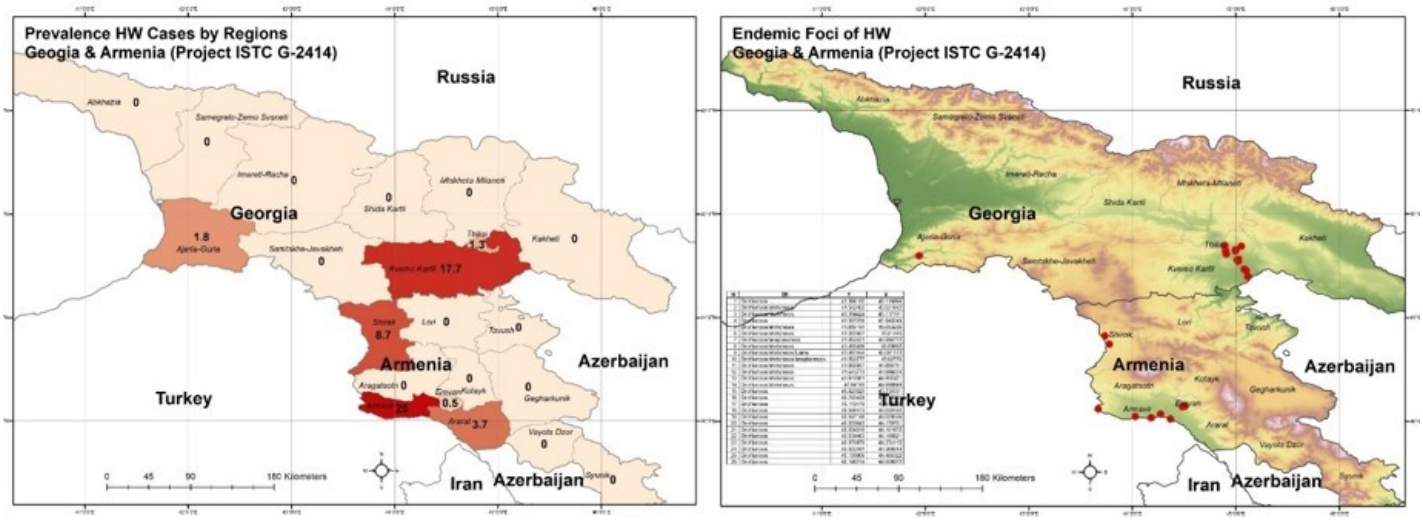

Figure 1. Left: The map of prevalence for all HW positive animals by regions. Right: The origin for all HW-positive animals on the village level.

2. Methods

2.1. Study population and design

The project was carried out in all main veterinary clinics that are currently operating in Georgia and Armenia. The numbers of animals from each clinic included in the study has were determined by the representative status at the regional level. A cross-sectional study involving a one-stage design has been used in the sampling plan. A sampling frame (with no bias in ordering) contained a list of animals that were examined each day, with individual animal identification codes attached. The selection of the animal populations to be involved in the study was based on stratified random sampling (strata were

defined based on animal age, gender, brief preliminary health assessment, and geographic origin). On-site examination lab sampling and data collection procedures of the 10% study population were implemented. For each animal examined by the veterinarian, the following information relevant to the epidemiological investigation was recorded on a data sheet: 1) Individual animal identity; 2) Age; 3) Gender; 4) Breed; 5) Lifestyle; 6) Husbandry.

2.2. Methods of Laboratory Research

Blood was collected from the cephalic vein (5 ml) and stored in tubes with anticoagulant (e.g., EDT; 2.5 ml) or in a serum-separating tube (2.5 ml) and later processed for molecular analyses and for parasitological and serological analysis.

Parasitological and Serological Testing. The modified Knott's technique (KN) and direct smear methods were used for microscopic detection and identification of microfilariae in blood smears. The commercial kit WITNESS®Dirofilaria (WT) (Synbiotics, San Diego, CA, USA) & SNAP 4Dx Plus (IDEXX Laboratories, Inc.) was employed for the detection of *D. immitis* circulating antigen in serum.

Molecular Testing. The aim of molecular studies was to identify *Dirofilaria species* circulating in Georgia and Armenia and to perform sequence analysis of detected species.

A variety of PCR-based methodologies were applied. (Watts et al., 1999; Mar et al., 2002; Rishniw et al., 2006; Latrofa et al.,2012) to diagnose zoonotic filariae in dogs and a combination of PCR and restriction fragment length polymorphism (RFLP) analysis (Nuchprayoon et al., 2003; Nuchprayoon et al., 2005) are employed to differentiate a wide spectrum of the filarial species. In this project, we used a species-specific semi-nested PCR assay, which was based on the amplification of internal transcribed spacer regions ITS1/ITS2 and enables the simultaneous detection and differentiation of filarial species in clinical specimens (Ferreira et al., 2017). Particularly, in a first-round, PCR the entire ITS region was expanded; in second-round PCR, the amplification of ITS1 and ITS2 regions allowed to identify *D. immitis, D. repens,* as well as other filariids - *A. reconditum* and *A. dracunculoides* depending on the size of amplification products.

The PCR products were purified and sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Inc.) in an automated sequencer (ABI-PRISM 377; Applied Biosystems Inc.). All sequences generated has compared to sequences available in GenBank using the Basic Local Alignment Search Tool (BLASTn). Based on the sequencing results, a phylogenetic investigation between species detected in Georgia and Armenia and those reported worldwide has also carried out.

2.3. Data analysis.

Canine dirofilariasis prevalence for each region of Georgia and Armenia was estimated by calculating descriptive statistics and their 95% CI. Logistic regression analysis at 95 % confidence was performed, and the odds ratios (OR) were calculated in order to estimate the epidemiological measure of the association between the variables included in the study (age, gender, breed, lifestyle, husbandry) and the prevalence of canine dirofilariasis. Then this analysis was repeated for each distinct form of canine dirofilariasis separately. The goodness-of-fit for all statistical models has assessed by C-statistics and Kruskal-Wallis one-way ANOVA test. All statistical analysis is done in SAS 9.2.

GIS is used to map the spatial distribution of canine dirofilariasis in the territory of Georgia and Armenia. Maps were created using regions as geographical units in order to display the latter with the number of animals examined and the prevalence (%) of disease for each region of Georgia and Armenia.

3. Results

The cross-sectional study design was employed to collect animal information and blood samples from the canine population. The project has carried out in veterinary clinics and animal shelters in the different regions of Georgia and Armenia. In total, 1065 animals were investigated and there were 26 positive cases. The prevalence of dirofilariasis is 2.4%. (**Figure 1** left, **Table 1**). However, it varies across regions from 0% to 25%. The Kruskal-Wallis test showed that variation is statistically significant (P=0.01). The majority of positive cases

(11) were from the Kvemo Kartli region (Georgia). The following highest number (7) of cases were found in the Armavir region (Armenia). The other cases were found in Tbilisi / Capital City of Georgia (two cases), Yerevan / Capital City of Armenia (two cases), Shirak Region (two cases), and only one case in regions Achara and Ararat.
By using GIS and Google Earth Pro systems, it was possible to study in detail the areas where cases of dirofilariasis had been detected. (**Figure 1,** right)

Table 1. Regional distribution of all investigated animals and positive cases

Regions (Georgia & Armenia)	# of Investigated animals	HW Positive	HW Total %
Achara+Guria	57	1	1.8
Imereti+Racha	61	0	0.0
Kakheti	49	0	0.0
Kvemo Kartli	62	11	17.7
Mtskheta-Tianeti	46	0	0.0
Javakheti	43	0	0.0
Samegrelo	52	0	0.0
Shida kartli	47	0	0.0
Tbilisi	155	2	1.3
Shirak	23	2	8.7
Armavir	28	7	25.0
Ararat	27	1	3.7
Syuniq	12	0	0.0
Yerevan	403	2	0.5
Total	**1065**	**26**	**2.4**

The result is significant at $p < 0.01$ (By Kruskal-Wallis)

Pathomorphology of dirofilariasis

The post-mortem examination of the fallen dogs with dirofilariosis was carried out in the veterinary clinic by the method of complete evisceration (**Figure 2A**). **Figures 2B-F** show representative outcome of an autopsy. The studies were carried out by both macroscopic and microscopic methods. During the study, it is important to note changes that have developed inside and outside the heart. Macromorphological studies have shown that dirofilaries are localized in both the right and left atria of the heart, but in terms of the severity of the location of parasites in the right atrium, their number is significantly higher in the right than in the left half (**Figure 2B**). Studies have also found that with intensive invasion, a certain amount of dirofilariae are localized in the lumen of the vena cava, at the junction with the atrium (**Figure 2C**). Diffuse enlargement of the heart occurs in the cardiac form of dirofilariasis. Microscopically, the enlargement of the heart is myogenic in nature, the muscle fibers are stretched in a transverse direction. The systolic state of the heart is sharply weakened due to myogenic heart failure, which is also confirmed by the mass of clotted blood in the cavities of the heart (**Figure 2D**). The coronary vessels were dilated and filled with blood. Due to a sharp violation of heart function as a complication in various organs, significant pathomorphological changes developed, in most cases incompatible with fever, namely: visible mucous membranes were cyanotic, venous congestion and edema were observed in the lungs, which was accompanied by hypostatic pneumonia, and with chronic course - compaction of the lungs. In the liver, as in other internal organs, cyanosis and dystrophic processes were noted, which could be accompanied by the development of micronecrotic foci and the development of connective tissue (in conditions of a prolonged form of the disease) with subsequent cirrhotic change. The gallbladder was filled, the walls were thickened. Kidneys were involved in the process, where changes characteristic of glomerular nephritis were observed in both acute (at the beginning) and chronic (protracted) form. The bladder was paralyzed and filled with urine, which further complicated the course and outcome of the disease (**Figure 2E**), especially in the cases subjected to complications with uremia. The spleen was enlarged in volume.

In the empty intestine, (especially in the small intestine), a pattern of flatulence was observed (**Figure 2F**). In the acute form, with intensive invasion of the disease, effusion was always recorded in the abdominal cavity and in the pericardium.

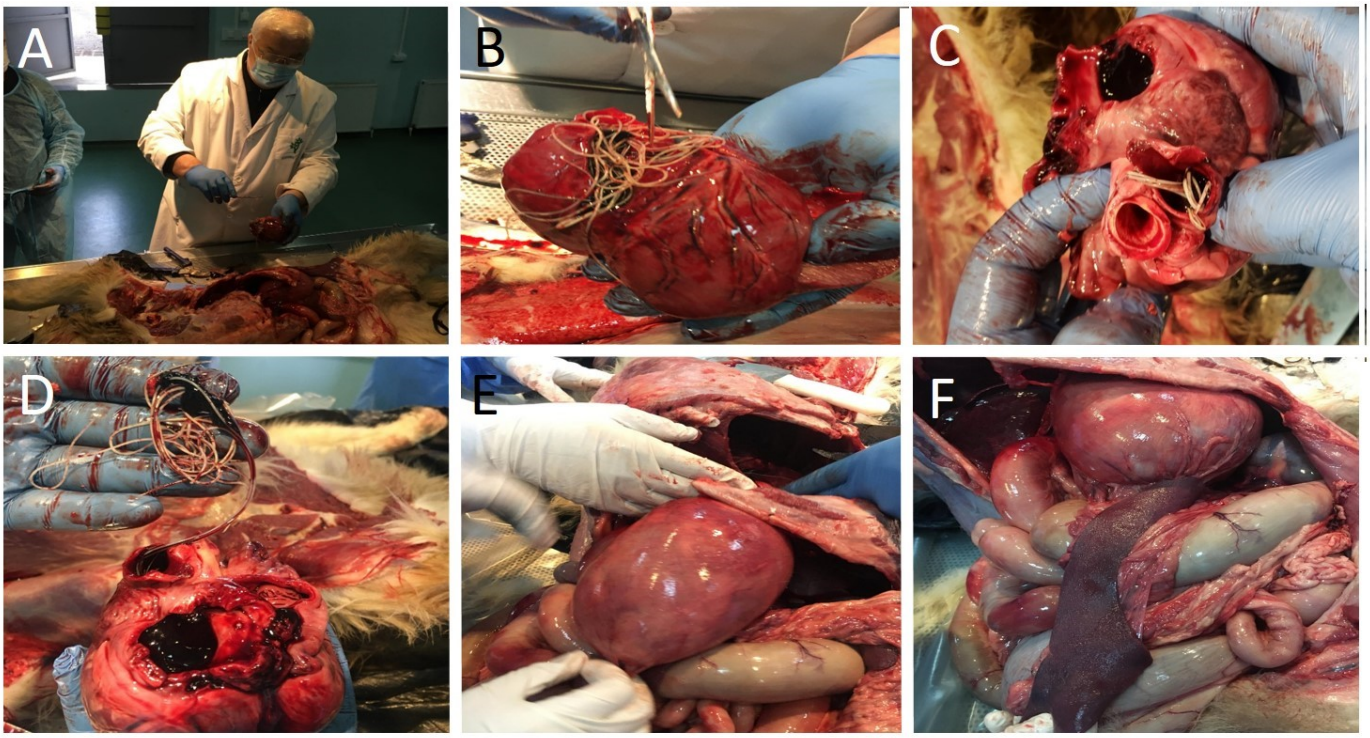

Figure 2. A: Autopsy of a fallen dog with dirofilariosis. B: Dirofilariae in the right atrium. C: Dirofilariae in the vena cava. D: Mass of clotted blood in the heart cavity. E: Paralysis of the bladder, urine overflowing. F: Spleen hyperplasia and flatulence.

4. Discussion

It should be underlined that 11 of the positive cases in Georgia were detected in the Kvemo Kartli region (Gardabani municipality), 2 cases in Tbilisi (Metropolitan city of Georgia), and 1 case in Achara. In Armenia, infected animals were detected in the regions bordering Turkey (Shirak, Armavir, Ararat). The study showed that the South Caucasus region is unreliable concerning the distribution of zoonotic vector-borne pathogens (VBPs) and there is a necessity for further research, in both Georgia and Armenia as well as in the South Caucasus region as a whole. Based on the results of the project, we could identify vector-borne diseases in high-risk regions in Georgia and Armenia. No doubt, it is necessary to continue the study and this study should focus on the environmental factors that contribute to the existence of vectors.

Considering the zoonotic nature of VBPs, it undoubtedly poses a risk for the population of the South Caucasus. For further research, we consider it necessary to perform a phylogenetic analysis of VBPs circulating in the South Caucasus, which allows us to analyse the genetic relationship between the strains isolated in Georgia and Armenia and the strains isolated in other countries and assess the public risk burden of this disease for the South Caucasus region.

In many countries including the Post-Soviet, VBDs are not a nationally notifiable condition and according to local laws, reporting cases to the local health department is not obligatory. Accordingly reliable epidemiological data are not available.

Funding: The study was supported by International Science and Technology Center (ISTC), Project G-2414.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ferreira C, Afonso A, Calado M, et al. Molecular characterization of *Dirofilaria* spp. circulating in Portugal. Parasit Vectors. 2017; 10: 250. DOI: 10.1186/s13071-017-2180-y
2. Latrofa MS, Weigl S, Dantas-Torres F, et al. A multiplex PCR for the simultaneous detection of species of filarioids infesting dogs. Acta Trop. 2012; 122:150-154. DOI: 10.1016/j.actatropica.2012.01.006
3. Mar PH, Yang IC, Chang GN, Fei AC. Specific polymerase chain reaction for differential diagnosis of Dirofilaria immitis and Dipetalonema reconditum using primers derived from internal transcribed spacer region 2 (ITS2). Vet Parasitol. 2002; 106: 243-252. DOI: 10.1016/s0304-4017(02)00032-8
4. Nuchprayoon S, Sangprakarn S, Junpee A, et al. Differentiation of Brugia malayi and Brugia pahangi by PCR-RFLP of ITS1 and ITS2. Southeast Asian J Trop Med Public Health. 2003; 2: 67–73.
5. Nuchprayoon S, Junpee A, Poovorawan Y, et al. Detection and differentiation of filarial parasites by universal primers and polymerase chain reaction-restriction fragment length polymorphism analysis. Am J Trop Med Hyg. 2005; 73: 895–900.
6. Rishniw M, Barr SC, Simpson KW, et al. Discrimination between six species of canine microfilariae by a single polymerase chain reaction. Vet Parasitol. 2006; 135: 303-314. DOI: 10.1016/j.vetpar.2005.10.013
7. Watts KJ, Courteny CH, Reddy GR. Development of a PCR- and probe-based test for the sensitive and specific detection of the dog heartworm, Dirofilaria immitis, in its mosquito intermediate host. Mol Cell Probes. 1999; 13: 425-30. DOI: 10.1006/mcpr.1999.0270