

Prevalence and Geographic Distribution of *Ehrlichia* spp. among the Dog Population in Georgia

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The main goal of this research was to study the geographical distribution of ehrlichiosis (causative agent: *E. canis*, *E. chaffeensis*, *E. Ewingii*, and other concurrent species) among the dog population in Georgia. Canine blood samples (n=480) were collected from the 16 private veterinary clinics and 5 animal shelters located in different regions of the country. Samples were tested for *E. canis*, *E. chaffeensis* and *E. ewingii* specific antibodies using “Canine Rapid Ehrlichia Test Kit” and PCR techniques. Overall, *Ehrlichia* spp. prevalence was 23.9% and disease was detected in every region of the country. The highest prevalence was found in a region encompassing Kvemo Kartli, Shida kartli, Mtskheta-Tianeti (Central Georgia) 35.4%, 35.1%, and 32.2% respectively. The results of this study provide the first large scale regional documentation of exposure to *Ehrlichia* spp. in canine population, highlighting regional differences in prevalence and providing the basis for heightened awareness of these emerging vector-borne pathogens by veterinarians and public health agencies. © 2022 Bull. Georg. Natl. Acad. Sci.

Ehrlichia spp., canine ehrlichiosis, ehrlichiosis, Georgia

The genus *Ehrlichia* consists of tick-transmitted gram-negative obligate intracellular bacteria from the order Rickettsia and family Anaplasmataceae that primarily infect leukocytes [1]. The three most relevant species found in dogs at this time are *E. canis*, *E. chaffeensis*, and *E. ewingii*, with *E. chaffeensis* having significance as a human pathogen [2].

The vector for *Ehrlichia* spp. are the ticks from the family of Ixodidae (*Rhipicephalus sanguineus*, *Amblyomma americanum*, etc.) which has worldwide distribution throughout tropical and temperate climates [3].

Clinical signs of *E. canis* infection range from non-specific (depression, lethargy, anorexia, weight loss), to red/purple subcutaneous bleeding, nose bleeds, ocular signs (retinal he-

morrhage/Inflammation), and/or neuromuscular signs (e.g. seizures, balance issues, or pain). Diagnosis of canine ehrlichiosis can be made by the observation of infected morulae in macrophages in blood smears or monocytes in tissue aspirates or impression smears [4, 5].

With *E. chaffeensis* infection, clinical signs are similar to, but often less apparent, than those of dogs infected with *E. canis*. Thrombocytopenia is common, but other observations, including identification of morulae, are not routinely observed. With *E. ewingii*, the infection can be mild or unapparent, however symptomatic, infected dogs display signs of fever, lethargy, anorexia, polyarthritis, vomiting, diarrhea, and/or neurologic signs [3].

After infection with Ehrlichia species organisms; an acute, sub-acute, or chronic infection can occur. The acute phase can last from 1 to 4 weeks. Most dogs that are treated appropriately with antibiotics during the acute phase will recover. Dogs that are either untreated or inappropriately treated may clinically recover, but then enter the subclinical phase for months to years. Dogs that are persistently infected may spontaneously recover or develop severe chronic disease. The severity of chronic ehrlichiosis can vary in severity from mild to life-threatening [6].

The problem of ehrlichiosis has not been studied sufficiently in Georgia. Before recently serious attention has not been drawn to the problem of revealing, treating and preventing the disease, since ehrlichiosis was considered as rare disease for the country and only few cases could be connected to the import from subtropical and tropical countries. That's evident for today, climate and relief features of Georgia highly promote to the prevalence of the disease among the country.

The only survey of canines in different regions of Georgia was conducted by Dr. L. Tsitskishvili in cooperation with veterinarian 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 the zones. Almost all clinics from 10 regions of the country participated in the study and provided the requested information on the study subjects. The study contributed to the understanding of the disease distribution in the country and now ehrlichiosis is a well-recognized endemic disease in this geographical area.

As for the incidence of ehrlichiosis in the people population of Georgia, it should be underlined that ehrlichiosis is not subject to mandatory registration and there are no reliable epidemiological data and there is no confirmed case of human ehrlichiosis.

Technical Approach and Methodology

Study population and design. The research was carried out in 16 veterinary clinics and 5 animal shelters that are currently operating in Georgia. The number of animals from each clinic included in the study were determined by its representative status at regional level.

A cross-sectional study involving one-stage design was used in the sampling plan. A sampling frame (with no bias in ordering) containing a list of animals to be examined each day, with individual animal identification codes attached to it, was obtained.

The selection of the animal populations to be involved in the study was based on stratified random sampling (strata was defined based on animal age, sex, brief preliminary health assessment, and geographic origin).

For each individual animal examined by veterinarian, the following information relevant to epidemiological investigation was recorded on a data sheet: 1) Individual animal identity; 2) Age; 3) Sex; 4) breed; 5) lifestyle; 6) husbandry.

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 serum separating tube (2.5 ml), and later processed for molecular and serological analysis.

Serological test. The commercial “VetScan Canine Ehrlichia Rapid” and “SNAP 4Dx Plus” tests were employed for detection of circulating antigen in serum [7, 8].

Molecular testing. The aim of molecular studies was to identify *Ehrlichia* species circulating in Georgia. Genus-specific qPCR assays have been validated and applied to detect the 16S rRNA gene of *Ehrlichia* species [9, 10].

Data analysis. The prevalence was estimated by computing the rate (%) of positive cases in examined animals. The Kruskal-Wallis one-way ANOVA was used for comparing the disease prevalence across the regions. All statistical analyses were conducted in SAS 9.4.

Results. Canine blood samples ($n = 480$) were collected from the 16 private veterinary clinics and 5 animal shelters located in different regions of the country. Samples were tested for *E. canis*, *E. chaffeensis* and *E. ewingii* specific antibodies using Canine Rapid Ehrlichia Test Kit and PCR techniques.

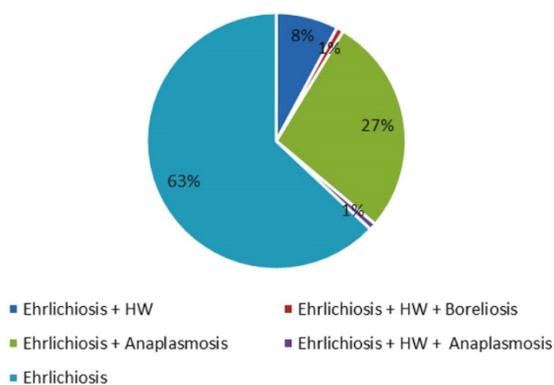


Fig. 1. The percentage correlation of cases complicated by Co-infections.

The study was conducted between 27/02/2019 and 27/12/2021. During this period, 480 animals were investigated throughout Georgia by immunoenzymatic method. 115 (23.95%) animals showed a positive reaction (the Kruskal-Wallis test of variation showed that p -value is .00172. The

result is significant at $p < .01$.), all positive cases were confirmed by PCR test. Among them 73 (63.48%) cases were not complicated by any co-infection, 32 (27.83%) cases were complicated by anaplasmosis, 9 (7.48%) cases by dirofilariasis, 1 (0.87%) cases by dirofilariasis and borreliosis, and 1 (0.87%) with dirofilariasis and anaplasmosis (see Fig. 1).

Regarding the prevalence of canine ehrlichiosis according to the regions of Georgia, the cases of the disease were detected in all regions of the country and its prevalence varies from 8.8% to 35.48%. The highest rate was detected in Kvemo Kartli region 22 (35.48%), and the most reliable region is Adjara-Guria regions, where a total of 3 (8.82%) positive cases were detected (see Table). See Fig. 2 for visualization of the prevalence and geographic distribution of *Ehrlichia* spp. in dogs in Georgia.

Table. Regional Distribution of Investigated Animals and Positive Cases of Canine Ehrlichiosis

Region	# of Investigated animals	# of Positive cases	Ehrlichiosis totale (%)
Achara+Guria*	34	3	8.8235294
Imereti+Racha*	60	9	15
Kakheti	49	10	20.408163
Kvemo Kartli	62	22	35.483871
Mtskheta-Tianeti	31	10	32.258065
Javakheti	26	3	11.538462
Samegrelo	26	5	19.230769
Shida kartli	37	13	35.135135
Tbilisi	155	40	25.806452
Total	480	115	23.958333

* Adjara-Guria and Imereti-Racha regions are considered jointly.

As we can see on the presented map, the central regions of Georgia Kvemo Kartli, Shida Kartli, and Mtskheta-Tianeti are extremely high-risk regions for the spreading of ehrlichiosis. The prevalence of canine ehrlichiosis in these regions is 35.48%, 35.13%, and 32.25% respectively.

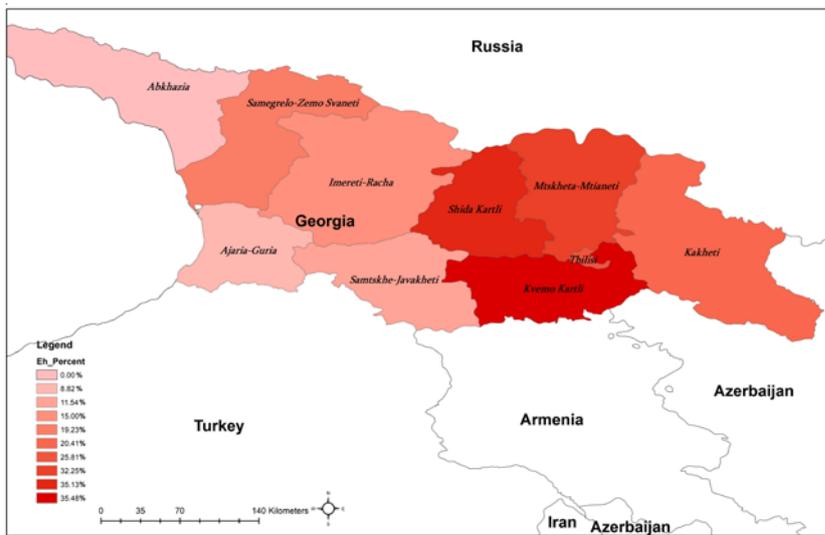


Fig. 2. The prevalence and geographic distribution of *Ehrlichia* spp. in dogs in Georgia.

Conclusion

The study showed that Georgia is an unreliable country concerning the distribution of ehrlichiosis and that there is a necessity for further research, both throughout Georgia and in the South Caucasus region as a whole.

Considering the zoonotic nature of ehrlichiosis, it undoubtedly poses a risk for the population of the South Caucasus. For further research, we consider it necessary to perform a phylogenetic analysis [11]

of *Ehrlichia* spp. circulating in Georgia, which allows us to analyze the genetic relationship between the strains isolated in Georgia and the strains isolated in other countries and assess the public risks burden of this disease for the south Caucasus region.

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ადამიანისა და ცხოველთა ფიზიოლოგია

Ehrlichia spp.-ს პრევალენტობა და გეოგრაფიული გავრცელება ძაღლის პოპულაციაში საქართველოში

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(წარმოდგენილია აკადემიის წევრის გ. ალექსიძის მიერ)

წარმოდგენილი კვლევის მთავარ მიზანს წარმოადგენდა საქართველოში, ძაღლების პოპულაციაში ერლიხიოზის რეგიონალური გავრცელების შესწავლა (გამომწვევი აგენტი: *E. canis*, *E. chaffeensis*, *E. Ewingii* და სხვა თანმხლები სახეობები). ძაღლის სისხლის სინჯები (n=480) შეგროვდა ქვეყნის სხვადასხვა რეგიონში მდებარე 16 კერძო ვეტერინარული კლინიკიდან და ცხოველთა 5 თავშესაფრიდან. ნიმუშები შემოწმდა *E. canis*, *E. chaffeensis* და *E. ewingii* სპეციფიკურ ანტისხეულებზე "Canine Rapid Ehrlichia" ტესტის ნაკრებისა და "PCR" ტექნიკის გამოყენებით. საერთო ჯამში, *Ehrlichia* spp. პრევალენტობამ შეადგინა 23,9%, (P<0,01) დაავადება გამოვლინდა ქვეყნის ყველა რეგიონში. ყველაზე მაღალი გავრცელება დაფიქსირდა ქვეყნის ცენტრალურ რეგიონებში, ქვემო ქართლი (35,4%), შიდა ქართლი (35,1%), მცხეთა-თიანეთი (32,2%). წარმოდგენილი კვლევის შედეგები იძლევა *Ehrlichia* spp.-ის ექსპოზიციის პირველ ფართომასშტაბიან რეგიონალურ შეფასებას ძაღლების პოპულაციაში, ხაზს უსვამს გავრცელების გეოგრაფიულ თავისებურებებს და ქმნის საფუძველს ვეტერინარული და საზოგადოებრივი ჯანდაცვის სექტორისთვის, გააძლიერონ ქმედითი ღონისძიებები ამ განვითარებადი „ვექტორების“ ჩართულობით მიმდინარე გადამდები პათოგენების გავრცელების პრევენციის მიმართულებით.

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