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Biomedicine

Canine Leptospirosis in Georgia

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Canine Leptospirosis is widespread globally and represents a severe public-health burden for the countries where the disease is endemic. This cross-sectional study aimed to examine the disease distribution in unvaccinated dogs in different regions of Georgia. The study was conducted between 01/08/2020 and 01/10/2021. During this period, 480 randomly selected dogs from 16 veterinary clinics, and 4 animal shelters were examined and included in the study. The disease status was ascertained using SNAP Lepto Test (IDEXX) to detect *Leptospira* spp. antibodies in canine serum. The distribution of the disease across the regions was evaluated using interpolation analysis to identify geographic spikes of the disease and the Kruskal-Wallis test for detecting significant variation of the infection across the regions. The prevalence of Leptospirosis is 1.9% in Georgia and varies from 0% to 8% across the regions. The disease prevalence was significantly different across the regions (P=0.01) with two significant geographic spikes – one in East Georgia and one in West Georgia. Furthermore, dogs in urban areas have a substantially elevated risk of being infected by *Leptospira spp.* In conclusion, Leptospirosis is an endemic canine disease in Georgia and requires the development of preventive programs to alleviate the health burden of the disease in the country. Notably, special attention should be paid to dog vaccination in urban areas of the country. © *2021 Bull. Georg. Natl. Acad. Sci.*

Leptospira spp., Canine Leptospirosis, Leptospirosis

Leptospirosis is a widespread zoonotic disease, the reservoir of which is domestic and wild animals [1-4]. The disease is caused by a spirochete bacteria belonging to the genus Leptospira that infects a range of mammals, including humans, livestock, and domestic animals [3].

Dogs have been known to be hosts for pathogenic leptospires for over 80 years [5]. While infection was most commonly associated with the presence of antibodies to the serogroups Canicola and Icterohaemorrhagiae, it is now clear that dogs are susceptible to infection with a wide range of serovars. Based on the available antibody prevalence data, the major serogroups to which dogs in Europe seroconvert to be

Icterohaemorrhagiae, Grippotyphosa, Australis, Sejroe and Canicola [6].

Infection is usually transmitted by direct contact of the oral mucosa or nasal mucosa or damaged skin with contaminated urine or water, and dogs are at risk of infection from drinking contaminated water [7, 8]. Leptospirosis can cause severe clinical diseases in dogs, including acute liver and kidney failure. It can also cause chronic carrier status with idiopathic polyuria/polydipsia, which may not be preceded by the severe liver or kidney disease [8].

A recent meta-analysis study identified being male sex and urban dogs as major risks factors for leptospirosis [9]. Dog ownership has also been identified as a risk factor for human leptospirosis [10] suggesting transmission of Leptospira spp. from dogs to humans. Dogs could serve as an important sentinel species for human infection, as well as an indicator of the presence of leptospires in specific environments [11, 12].

The problem of leptospirosis has been studied insufficiently in Georgia. Until 2005, no proper attention has been paid to preventing, diagnosing, and treating leptospirosis in animals or humans in South Caucasus. Leptospirosis was considered a sporadic problem for the region, and few identified human cases were considered only occasional imported ones. Since 2006, there has been a sharp increase in the incidence of the disease. The highest recorded incidence rates were 2.4 per 100,000 population in 2016 and 5.5 per 100,000 population in 2018 [13, 14].

Little is known about the distribution of leptospirosis among animals and its public health burden in Georgia. The project - ISTC G-2101 - examined 218 bats for Lyssavirus, Coronavirus, Yersinia, Leptospira, and Brucella pathogens by PCR method and found that 25 (13%) of bats were positive for Leptospira [14].

The purpose of this project was to examine the prevalence and regional distribution of the infection in unvaccinated dogs.

Technical Approach and Methodology

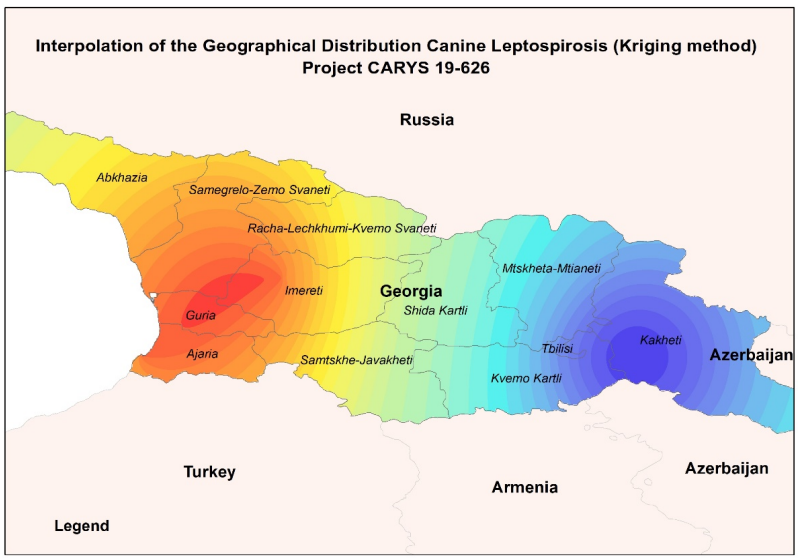
Study population and design. The cross-sectional study design was employed to collect animal information and blood samples from unvaccinated dogs. The project was carried out in veterinary clinics and animal shelters. Study subjects were randomly selected. Each animal was examined by a veterinarian, and information on animal age, sex, breed, vaccination status, and health status were collected.

Methods. Geographic coordinates of all positive cases were ascertained. Next, these coordinates were entered into geo-information systems for interpolation analysis.

Physical examination. For the preliminary diagnosis, a physical examination was carried out to identify common 10 clinical signs of canine leptospirosis: Lethargy; Arthralgia and myalgia; Polydipsia and polyuria; Oliguria or anuria; Altered hydration status (overhydration with oliguria/ anuria or dehydration with polyuria); Gastrointestinal abnormalities (decreased appetite, vomiting, diarrhea); Icterus; Bleeding tendency (petechia, melena, hematochezia, epistaxis); Tachypnea; Conjunctivitis.

Laboratory Testing. Blood was collected from the cephalic vein (5 ml) and stored in tubes with anticoagulant (e.g., EDTA) and frozen at the -20^{0}C for later processing for molecular and serological analysis. The SNAP Lepto Test (IDEXX) was used for the detection of *Leptospira* spp. antibodies in canine blood according to the manufacturer's instruction. Briefly, 3 drops of blood were dispensed into a sample tube, followed by 4 drops of the recombinant LipL32HRP conjugate and were mixed thoroughly by inverting 3–5 times. The entire contents of the sample tube were carefully added to the sample well of the SNAP device. Leptospira-specific antibodies in sera first bind to the LipL32-HRP conjugate to form immune

Map 1. Regional Distribution of Canine Leptospirosis.



Map 2. Interpolation Analysis of Canine Leptospirosis in Georgia.

complexes. The immune complexes flow across the result window and accumulate at the recombinant LipL32 on the sample spot of the test membrane. When the fluid flow reaches an activation window, the test then is subjected to wash solution and substrate reagents are released by snapping down the top of the device. The presence or absence of antibody was determined by visual interpretation after 10 minutes at ambient temperature. Color of the sample spot more intense than color of the background indicated that the sample was positive for Leptospira-specific antibodies, whereas a negative result was interpreted by the absence of color in the sample spot. The positive control spot on all tests indicated proper function of the test [15, 16].

Statistical 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. The study was conducted between 01/08/2020 and 01/10/2021. During this period, 480 randomly selected dogs from 16 veterinary clinics, and 4 animal shelters were examined and included in the study. The clinics and shelters were located in different regions of the country.

For the laboratory examination, only those dogs were selected who were not vaccinated and had clinical signs of leptospirosis. Blood samples were taken from 231 animals for lab analysis. Immuno-ferment analysis was conducted by the commercial tests – IDEXX Lepto Snap.

The case in the Tbilisi region had complications in terms of anaplasmosis. See Map 1 for the regional distribution of the positive cases.

The interpolation analysis showed that there are two geographic spikes of leptospirosis distribution in Georgia. See Map 2.

Conclusion

The study showed that leptospirosis is diagnosed in dogs with different characteristics (age, sex, and bread). Also, the disease is endemic for both West and East Georgia. Furthermore, the risk of infection is higher in urbanized areas compared to rural areas. The disease was most prevalent in one of the most urbanized areas of West Georgia – Kutaisi, Imereti.

Table. Regional Distribution of Examined Animals and Positive Cases

Region	Lab Invest. Animals	Invest. Animals	Number positive cases
Achara-Guria	23	34	2
Imereti-Racha	24	60	5
Kakheti	24	49	1
Kvemo kartli	25	62	0
Mtskheta-Tianeti	24	31	0
Samtskhe-Javakheti	23	26	0
Samegrelo	25	26	0
Shida kartli	23	37	0
Tbilisi	40	155	1
Total	**231**	**480**	**9**

All others were assumed to be infection-free. In total, 480 animals were examined by a licensed veterinarian to identify suspected leptospirosis cases (see Table).

In total, there were nine positive cases. The prevalence of leptospirosis is 1.9%. However, it varies across regions from 0% to 8%. The Kruskal-Wallis test showed that variation is statistically significant (P=0.01). The majority of positive cases (5) were from the Imereti region. The following highest number (2) of the cases were found in the Adjara region. Two other cases were found in Tbilisi (Capital Region) (one case) and Kakheti (one case).

The distribution of veterinary clinics and shelters is uneven across the country. Particularly, there is an urgent need for more veterinary clinics in Imereti-Racha, Shida Kartli, Samckhe-Javakheti, Kvemo Kartli, and Mtskheta-Mtianeti regions. There is also a need for developing preventive programs to reduce the disease burden.

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ბიომედიცინა

ძაღლის ლეპტოსპიროზი საქართველოში

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**საქართველოს სოფლის მეურნეობის მეცნიერებათა აკადემია, თბილისი, საქართველო
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ჩატარდა რეტროსპექტული კვლევა საქართველოს ყველა რეგიონში, ძაღლების ლეპტოსპიროზის შემთხვევების გამოვლენის მიზნით 2020 წლის 1 აგვისტოდან 2021 წლის 1 ოქტომბრის ჩათვლით. გამოკვლეულ იქნა 480 ძაღლი, შეფასდა მათი ჯანმრთელობის მდგომარეობა ლეპტოსპიროზისათვის დამახასიათებელი სიმპტომატიკის გამოვლენის მიზნით, მათგან 231 ცხოველი შესწავლილ იქნა ლეპტოსპიროზის სეროლოგიური დიაგნოსტიკის (IDEXX Lepto Snap) გამოყენებით. თითოეული ცხოველის შესახებ შეგროვდა შემდეგი ინფორმაცია: ცხოველის საცხოვრებელი არეალის კოორდინატები, ასაკი, სქესი, სტერილიზაციის სტატუსი, ჯიში, ფერი, თმის სიგრძე. დაავადების განაწილება რეგიონების მიხედვით შეფასდა კრუსკალ-უოლსის ტესტის გამოყენებით, ხოლო ინტერპოლაციური ანალიზის გამოყენებით დადგინდა დაავადების გეოგრაფიული პიკები. კვლევამ აჩვენა, რომ საქართველოში ძაღლის ლეპტოსპიროზის პრევალენტობა არის 1,9%, ხოლო სხვადასხვა რეგიონის მიხედვით პრევალენტობა მერყეობს 0%-დან 8%-მდე (P = 0,01), ორი მნიშვნელოვანი გეოგრაფიული პიკით – ერთი აღმოსავლეთ საქართველოში, ერთი დასავლეთ საქართველოში. გამოვლინდა, რომ ძაღლების დაავადების რისკი მჭიდროდ დასახლებულ ქალაქებში არის მნიშვნელოვნად მაღალი. გარდა ამისა, კვლევამ აჩვენა, რომ საქართველოში ძაღლის ლეპტოსპიროზი წარმოადგენს ენდემურ დაავადებას და მოითხოვს პრევენციული პროგრამების შემუშავებას ქვეყანაში დაავადების ტვირთის შესამსუბუქებლად. აღსანიშნავია, რომ განსაკუთრებული ყურადღება უნდა მიექცეს ძაღლების ვაქცინაციას ქვეყნის მჭიდროდ დასახლებულ ქალაქებში.

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