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# CONTROL OF VECTOR-BORNE DISEASES IN A DOG SHELTER. EXPERIENCE FROM BULGARIA

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**ABSTRACT**

Vector-borne diseases present a constant concern in both veterinary and human medicine especially in the context of climate change. Prophylaxis is the one most important prerequisite for effective management and the role of animal care units should be recognized. The paper describes clinical experience and protocols from a dog shelter in Sofia, Bulgaria. It deals with some commonly identified pathogens namely *Dirofilaria immitis*, *Dirofilaria repens*, *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Anaplasma platys*, *Borrelia burgdorferi*, *Leishmania infantum* that can be accessed by rapid in-clinic test kits. Treatment and prevention strategies are further discussed.

**Key words:** dog, shelter, vector-borne, control.

## Introduction

Dog shelters are often viewed as a place where homeless or unwanted dogs can be left and kept away from society until they are washed, treated, vaccinated, dewormed, spayed/neutered and made good looking for adoption. However, this is only the façade of a hard working mechanism that often addresses questions of global importance such as diagnostics and control of diseases. Free roaming dogs can be a source of pathogens with zoonotic potential. Therefore, the existence of the above-mentioned facilities is crucial for the prevention of infection distribution within the species or between species.

Stray dogs experience poor health and welfare due to cruelty or lack of resources (Tasker, 2007). Poor nutrition and stress may increase susceptibility to a variety of disorders including vector-borne diseases (ESCCAP, 2019). The situation is further complicated by the fact that animals accepted in shelters come from unknown areas and possess no medical history. Vector exposure and abundance of reservoir hosts which include dogs living outdoors, stray dogs, hunting dogs, animals from adoption and rehoming units can influence disease distribution (ESCCAP, 2019). Long-term, sustainable strategies should be developed to deal effectively with such populations especially in the context of their role in disease transmission.

Climate change is recognized as a major contributor for increased prevalence and distribution of vector-borne diseases; anthropogenic and ecological factors should also be taken into consideration (Pherez, 2007; Stuchin, 2016). Global warming and the consequent variability in weather threaten to undermine scientific progress against this group of pathogens (Campbell-Lendrum, 2015) as vectors manage to adapt and cover new territories. Ticks, mosquitos, sandflies are ectotherms; therefore their abundance, survival and feeding activity will be enhanced by increasing temperature, as will the rate of development of the pathogen within the vector (Rocklöv and Dubrow, 2020). Transportation especially from endemic to nonendemic areas presents another potential cause for disease spread (Otranto and Dantas-Torres, 2010).

Reports of increased prevalence have been sent from all parts of Europe. Knowledge of prevalence statistics can only confirm the fact that strict and timely control is central to prevent vector-

borne disease distribution. The role of shelters in a related human-animal approach will be described in the following discussion.

**Materials and methods**

The study was conducted in a dog shelter in Sofia, Bulgaria during a period of 3 years (2017-2019). Documental data was collected in order to estimate the protocols and management strategies concerning vector-borne diseases in the local population. Results from rapid in clinic tests for the common and/or endemic infections were analyzed, namely Dirofilariosis, Anaplasmosis, Ehrlichiosis, Lyme disease and Leishmaniasis. Immunodiagnostic assay included: Anigen Rapid CaniV-4 Test Kit, Anigen Rapid Leishmania Ab Test Kit (BioNote Inc., South Korea).

**Results and discussion**

Management and control of contagious diseases continues to be one of the challenges of shelter medicine. The risk for introduction of infectious agents is always present as animals may be in the clinically silent incubation period or signs may be noted during examination.

The number of animals present in the shelter ranges between 150 and 200 with optimal capacity of 150 to avoid overcrowding. Admission is carried out in a small size facility where the veterinary clinic and cages for new arrivals are situated. Puppies and adult dogs are placed at separated premises that are not physically connected. There animals pass through vaccination and deworming depending on their age and health status followed by spay/neuter. Only after they can be moved to the main building with cages and offered for adoption.

All dogs are examined on the very day of entry with medical data written in a personal paper record and a computer program called "Shelter manager". It is obligatory to check for a microchip or put one if not found in order to track animals during their stay and after rehoming. Testing for vector-borne diseases is a part of the medical protocol. Dogs that are above 7 months of age are tested with Anigen Rapid CaniV-4 Test Kit. Presence of fillarial larvae is identified by a drop of blood under the microscope. The border line of 7 months is set as antigen tests for heartworm disease may be false negative and microfilariae may not be seen under this age (ESDA, 2017). Testing dogs less than 7 months is indicated in the presence of specific clinical signs, laboratory changes or ticks found over the body. Leishmania antibody testing is performed to patients with dermatological conditions especially when disease pattern suggests possible leishmaniasis, there are no other reasons found for the present condition or the animal comes from an area with increased morbidity.

Veterinarians and supportive personnel are supposed to carry latex gloves and other protective means (mask, clothing) when necessary during handling of animals. After examination and testing are finished the admission room is thoroughly disinfected. Depending on results dogs are accommodated in the respective sector – infectious or non-infectious, adult or puppy. The following procedures depend on the clinical condition. However, all animals are subjected to quarantine of at least 2 weeks. During this period additional testing can be done.

Scientific work from Bulgaria has indicated the wide distribution of vector-borne pathologies. Occurrence among a dog population from Southern Bulgaria was determined to be 16.2% for Dirofilaria immitis, 21% for Ehrlichia canis, 30.5-46.1% for Anaplasma phagocytophilum, 2.4% for Borrelia burgdorferi (Pantchev et al., 2015). A study in stray dogs from Sofia between Jan 2018 and Dec 2019 demonstrated 28.57% seropositive results of which Anaplasma spp. – 16.29%, D. immitis

− 13.39%, Ehrlichia spp. 1.34% and B.burgdorferi – 0.67% (Manev, 2020a). However annual variations in disease seroprevalence can be demonstrated. The same author reported between 12.63% and 18.87% anaplasmosis seroprevalence among the shelter population from the same place but during different testing periods (Manev et al., 2019; Manev, 2020b). Prevalence of D. immitis was estimated at 11.26% in stray dogs from Sofia region in 2017 (Manev, 2020c). Animals captured for routine neutering campaigns between Dec 2017 and Feb 2018 in Sofia were 31.25 % heartworm positive (Stoyanova et al., 2019). Another study in stray dogs showed 15% seropositive cases of D. immitis and 18% of D. repens (Radev et al., 2016). The percentage among privately owned dogs from different regions of the country during 2017-2019 was expectedly lower – 13.04% HW positive (Rafailov, 2020). Investigation of E. canis in privately-owned dogs from Northern Bulgaria documented ranges between 25 to 60% positive tests in different regions (Tsachev et al., 2006).

Concerning leishmaniasis data is still scares. A study by Pantchev et al. (2015) failed to identify antibodies against L. infantum. Healthy household dogs tested for the same pathogen were also found negative (Tsachev et al., 2007). However, lack of recognition does not mean the infection is not active as human cases prove the contrary. The number of positive cases is constantly rising from 10 in 2004 to 14 in 2014 (Likov, 2015).

Having in mind the above mentioned statistics the risk to encounter vector-borne disease in a shelter can be estimated as significant to high. Following a strategy to diagnose and manage such cases represents the only reasonable way to ensure canine as well as human health.

*Ehrlichia canis* is the causative agent of canine monocytic ehrlichiosis (CME) (ESCCAP, 2019). It is not considered a zoonotic agent. Diseases is transferred by the vector *Rhipicephalus sanguineus*. According to shelter`s rules dogs that show positive antibody tests are quarantined and treated irrespective if they show clinical and/or laboratory abnormalities. A single positive result may reflect a past infection (Sainz et al., 2015) that has resolved; however, treatment is instituted for safety reasons. Supportive care is given to patients with manifested infection. The protocol is based on the oral application of doxycycline at 5 mg/kg twice daily for 4 weeks (Sainz et al., 2015; Mylonakis and Theodorou, 2017). This regime guarantees complete recovery in the majority of cases (McClure et al., 2010).

Approach to anaplasmosis is similar to that in Ehrlichia cases. There are two species that affect dogs – *A. phagocytophilum* and *A. platys* (ESCCAP, 2019). Transmission in Europe is accomplished through the *Ixodes ricinus* vector (Tsachev, 2009). Disease in shelter dogs has been recognized as early as 3-4 months of age and is often seen in adult and elderly patients. Treatment with doxycycline at 5 mg/kg twice daily for 4 weeks is instituted to every animal with a positive antibody test. Infection with A. phagocytophilum transmitted by ticks was observed in humans (ESCCAP, 2019). Direct transmission from canides has not been reported. However, animals with ticks over the body and blood products obtained for examination should be handled cautiously.

*Borrelia burgdorferi sensu lato* affects both dogs and humans; however, there is no interdependency in terms of transmission (ESCCAP, 2019). Vectors include several species of Ixodes ticks. Cases of Lyme disease have been rarely seen in the shelter. Not more than 1 to 4 positive tests have been documented per year. Manifestation is usually subclinical and there were only a few dogs with fever or lameness (polyarthritis) that could be attributed to infection. Animals are quarantined and treated with doxycycline at 5 mg/kg twice daily for 4 weeks as a first choice antibiotic. Oral or parenteral application of beta-lactams is also curative (Littman et al., 2018). Having in mind the protracted nature of disease, a long course of antibiotics is indicated (Wormser and Schwartz, 2009). Not all dogs manage to clear the infection (ESCCAP, 2019) and there can be relapses post treatment.

Again animals positive for Lyme disease should be handled carefully and retested if judged necessary.

Prevention of tick-borne diseases should cover the entire period when vectors are active (ESCCAP, 2018). This is accomplished through regular acaricidal treatment with topical fipronil spray and spot on products; insecticide impregnated collars are also used in dogs that tolerate them. Spraying of shelter area against ticks with cyhalothrin or other similar chemicals is done monthly during the warm season and vegetation is kept low.

Heartworm disease caused by *D. immitis* is often seen among shelter dogs. The parasite is transferred by mosquitoes (Culicidae). According to ESCCAP (2019) disease is endemic across southern Europe including Bulgaria. All dogs above 7 months of age are tested with Anigen Rapid CaniV-4 Test Kit and a drop of blood is examined under the microscope to exclude or confirm D. repens. The Knott test that is often recommended is rarely performed due to the busy shelter routine. Positive dogs are isolated and started on a regime of ivermectin 6 micrograms/kg per os every month and doxycycline at 10 mg/kg daily for 4 weeks. Meanwhile patients are brought to referral veterinary clinics for thoracic X-rays and echocardiography. CBC and biochemistry testing are run every 2 weeks to check the general condition of affected dogs. Treatment is decided after staging of disease severity has been completed. Melarsomine dihydrochloride is the only effective drug available for treating adult heartworm infections in dogs (ESCCAP, 2019). Adulticide is applied at a dose of 2.5 mg/kg body weight by deep intramuscular injection in the lumbar muscles (Bowman and Atkins, 2009; ESDA, 2017). This has been accompanied by the administration of heparin and a corticosteroid. A two-step regime (American Heartworm Society, 2020) is preferred in the shelter to reduce the risk of pulmonary thromboembolism. The first injection is given one month after doxycycline treatment has finished. Melarsomine is repeated at least one month later with two doses applied 24 hours apart. A one-step approach with only two doses in an interval of 24 hours has been practiced when there were financial constrictions. This protocol is less popular but has proven to be successful in young adults with no concomitant disorders. Cage rest during the whole treatment period and especially around adulticide application is strictly followed. Surgical intervention has been performed in cases of caval syndrom as a life-saving procedure. Dogs are transferred to specialized units for adulticide or surgical treatment and being taken care of during the rest of the period. Follow up continues even after adoption. Retesting for dirofilariasis is done 6–9 months post treatment to reduce the risk of failure (American Heartworm Society, 2020). Exercise restriction is observed for 6 to 8 weeks after the last melarsomine injection. Owners are advised to visit a veterinarian for a health check at least once a year.

Suspicion of *D. repens* can be raised when antigen tests turn negative with microfilariae present in peripheral blood samples especially when there are obvious skin lesions. Positive dogs are put under quarantine and treated monthly with moxidectin + imidacloprid spot-on (Advocate®) (ESDA, 2017).

Humans can be accidental hosts of Dirofilaria spp. when they are exposed to infected mosquito (ESDA, 2017). D. immitis can be localized in the lung, mesentery and dura mater and mimic a neoplastic process (ESCCAP, 2019). It is still rarely diagnosed probably due to lack of awareness by physicians. D. repens is the most important species responsible for zoonotic infections in Europe (McCall et al., 2008; ESCCAP, 2019). The parasite may cause a larva migrans syndrome and form subcutaneous lesions (Capelli et al., 2018). Most human cases are asymptomatic and diagnosis is reached after the surgical removal of a nodule containing worms. The prevalence of microfilaraemia

can be correlated to emergence of disease in people; therefore prophylaxis of owned and especially shelter dogs is substantial for the control of disease distribution.

Measures against mosquito-borne infections include monthly *per os* administration of ivermectin at 6 micrograms/kg to shelter dogs more than 3 months of age. The current recommendation is to carry out prevention all year round, even in winter, due to urban heat islands, where mosquitoes manage to survive (ESDA, 2017).

Canine leishmaniosis is endemic in southern Europe with prevalence rates reaching 60% in exposed populations (ESCCAP, 2019). Disease is caused by *Leishmania infantum* with vectors the blood-sucking flies of the genus Phlebotomus (Reguera et al., 2016). Bulgaria together with other neighbouring countries falls in the area with increased risk of morbidity. The first case of canine visceral leishmaniasis was confirmed by Tsatchev et al. (2010) in two Rottweilers from the city of Petrich.

According to shelter protocol rapid tests are the first step to be taken in cases when leishmaniasis can be suspected. Anigen Rapid Leishmania Ab Test is performed to dogs with dermatological conditions especially those demonstrating skin lesions like alopecia, nodules, ulcers, hyperkeratosis, intense exfoliative dermatitis, mucocutaneous lesions and onychogryphosis (ESCCAP, 2019). These can be combined with enlargement of single or multiple lymph nodes, weight loss, anorexia and weakness in chronic cases. Amastigote stages can be visualized in Giemsa or Diff-Quick stained smears obtained from superficial lymph nodes or bone marrow aspirates (ESCCAP, 2019). IFAT (indirect fluorescent antibody test) and ELISA (enzyme linked immunosorbent assays) can be used as a confirmation when results from in clinic testing turn out to be positive. Samples are sent to Laboklin, Germany and results return within a week. PCR testing is also possible in some laboratories (Maia et al., 2009).

According to local legislation positive cases should be reported to authorities and subjected to euthanasia as these are reservoirs of infection. However, this strategy differs from measures imposed in other European countries where disease is also widely distributed like Italy and Spain. Culling of seropositive dogs in endemic areas have been shown to be ineffective in reducing Leishmania transmission (ESCCAP, 2019). Dogs are treated and retested in order to keep the infection subclinical and minimize the chance of transmission. Application of chemotherapy against leishmaniasis is expensive and covers extended periods with possible reactivation of disease. Different protocols have been proposed with single use or combination of allopurinol, meglumine antimoniate, miltefosine and other substances (Denerolle and Bourdoiseau, 1999; Noli and Saridomichelakis, 2014; Reguera et al., 2016). Repellents like insecticides impregnated collars that act against vectors should be used following producer recommendations. Another form of prevention can be the use of immunostimulant spot-on products (Reguera et al., 2016).

The shelter in question has not come across any case of leishmaniasis although testing conscientiously in clinic or sending samples abroad. This can be explained by the location of the facility that is away from regions with increased frequency of disease.

The review of available literature suggest the highest prevalence of anaplasmosis, ehrlichiosis and dirofillariosis in stray dogs that can be confirmed by observations from the shelter in Bulgaria. Therefore these are the pathogens that can be expected during admission in shelters. Borreliosis was less often identified and information about leishmaniasis is even more obscure. However, these infections should be reevaluated as clinical manifestation may be more challenging compared to other vector-borne diseases and cases may remain misdiagnosed.

Dogs and cats housed in kennels and catteries or animals living outdoors may be subjected to increased risk of acquiring vector-borne diseases compared to individual animals living indoors (ESCCAP, 2019). They can pose a significant threat to human health through their role in disease transmission (Tasker, 2007). Therefore it is essential to protect humans from coming into contact with possible carriers as well as care for the health and welfare of the animals.

## Conclusion

Management of vector-borne diseases is important from both human and veterinary medicine standpoints as long as many of these pathogens are transmissible to humans and dogs which often live in close contact (Dantas-Torres and Otranto, 2015). Shelters can actually act as a barrier to control and prevent distribution from the population of stray animals that often present carriers and reservoirs of infection. Protocols to detect pathogens should be constantly improved to reduce the impact of vector-borne diseases on society. Effective and sustainable approaches to cope with infective agents, especially those that possess zoonotic potential, present a significant part of the contemporary "One Health" concept.

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