

## SURVEY ON CANINE EHRLICHIOSIS IN DOGS FROM CONSTANȚA COUNTY, ROMANIA

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**Abstract** Canine ehrlichiosis is a tick-borne disease of dogs caused by the pathogens from the genera *Ehrlichia* (*Ehrlichia canis*, *E. chaffeensis*, *E. ewingii*). *Ehrlichia canis* and *Ehrlichia ewingii* are common pathogens of domestic dogs that has a worldwide distribution. Canine monocytic and granulocytic ehrlichiosis are two important and potentially fatal diseases of dogs, and also have a public health significance.

The aim of our study is to complete the picture of *Ehrlichia canis* and *Ehrlichia ewingii* infections in dogs from Romania. Serum samples from 256 dogs living in 35 localities from Constanța county, located in South - Eastern part of Romania, were assayed through SNAP® 4Dx® Plus Test, IDEXX Laboratories. Overall, the results showed a percentage of 6.25% - 16 dogs from 256 were positive to *Ehrlichia canis* and *Ehrlichia ewingii*. The prevalence of *Ehrlichia canis* and *Ehrlichia ewingii* is very dependent on the distribution of their vectors. No associations ( $p > 0.05$ ) were found between the prevalence and age, gender, breed, origin, and lifestyle of the dogs.

**Key words:** *Ehrlichia canis*, *Ehrlichia ewingii*, dogs, diagnosis, Snap 4Dx Plus

### Introduction

Canine tick-borne diseases have a strong health significance for both animals and humans due to the sever clinical signs who lead to death and also because some of them are zoonoses. In the last few years their importance has increased, in the scientific world, due to climate and environment changes.

Canine monocytic ehrlichiosis (CME) is a tick-borne disease and is caused by a bacteria who is named *Ehrlichia canis*.

*Ehrlichia canis*, one of the agents who cause canine ehrlichiosis is a Gram – negative bacteria from the genera *Ehrlichia*. He is located obligatory intracellular in the white blood cells where forms microcolonies.

*Ehrlichia canis* has tropism for monocytes and macrophages.

In Europe, the main vector of the pathogens who cause canine ehrlichiosis is *Ixodes ricinus*, but the principal vector for *Ehrlichia canis* is *Rhipicephalus sanguineus*, who is worldwide (3).

In Romania a study done on tick species shows that *Rhipicephalus sanguineus* has 0.2% from the total number of ticks collected in the Delta of Danube, near Constanța county (1).

The disease has three phases: acute, subclinical and chronic. Rudoler and col., in 2015, published an article regarding the evaluation of acute phase proteins (APP) and oxidative marker responses in dogs vaccinated against CME with an

attenuated vaccine and subsequently challenged with a wild *E. canis* strain. The body of the dogs create an inflammatory response to *E. canis* infection which can include changes in certain acute phase proteins (APP) and in biomarkers of the oxidative status. APP responses are considered part of the innate immune response to CME (10).

The symptoms varies from each case and depends of the immune status of the host, age and breed, but often veterinarians clinicians can see: anorexia, epistaxis, weight loss, fever, lethargy. Gastrointestinal symptoms, ocular signs and respiratory problems are rare. In 1975, Smith and col. describe a case of *Ehrlichia canis* infection in a mixed dog who came from India and the symptoms observed were: epistaxis, anemia, leukopenia, hypoalbuminemia, and hypergammaglobulinemia. The necropsy was done after the dog's death and they observed: ecchymoses and petechiae on the gallbladder, kidneys, small intestinal mucosa, cerebrum, and cerebellum. Plasmacytosis and perivascular cuffing was evident in various organs, especially the cerebrum, cerebellum, and meninges (11). In Romania, in 2015, Morar and col. describe a case of *Ehrlichia canis* infection in a dog who came from a nonendemic region of Europe and support like this the geographical expansion of canine ehrlichiosis (8).

*Ehrlichia ewingii* is the causative agent of granulocytic ehrlichiosis in dogs and affects the neutrophils and rarely the eosinophils (3). Symptoms seen in the dogs diagnosed with *Ehrlichia ewingii* infection are: fever, lameness, lethargy, pain during walking and at the joint when the dog is palpated also due to the joint effusion. It could be possible to see neurological signs as: tremors, ocular symptoms (anisocoria) and an abnormal position of the head (5).

### **Materials and methods**

Blood samples were collected from 256 dogs with age between 1 and 13 years old. The dogs were from 35 localities from Constanța county, located in the South-Eastern part of Romania (figure 1).

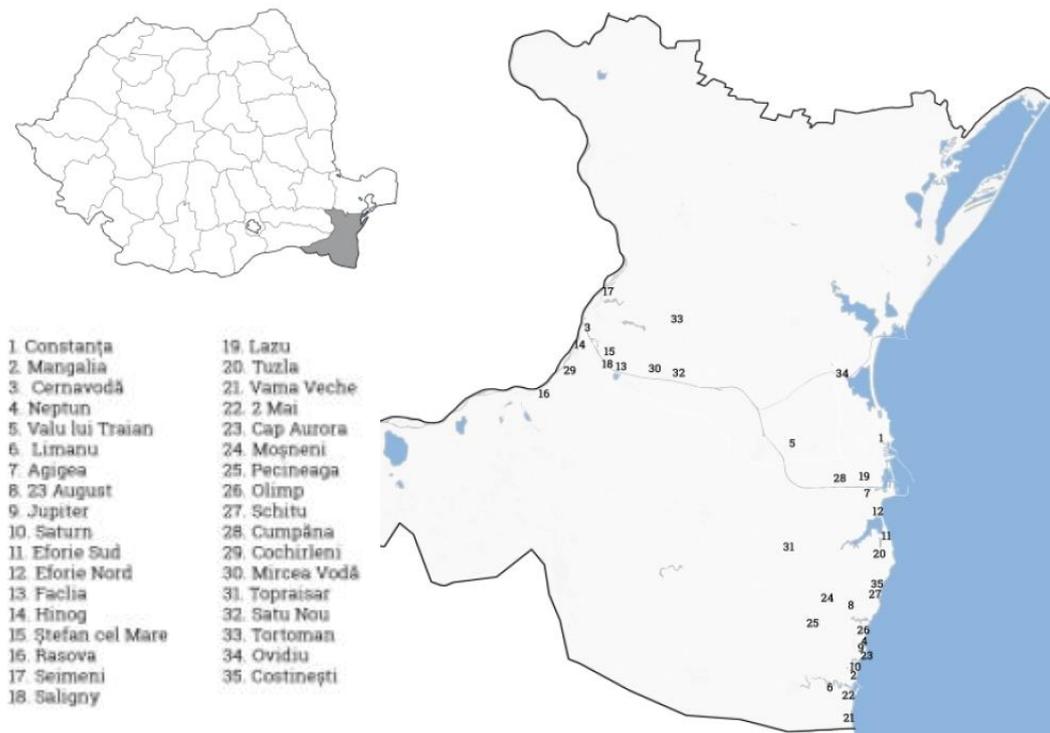


Figure. 1. Map of the localities from which were collected the samples (original)

The blood samples were collected in EDTA sterile vacutainers from the cephalic vein. By SNAP® 4Dx® Plus Test, IDEXX Laboratories, were tested all the blood samples.

This test is using an ELISA technique (figure 2) and is a quickly method who gives the results in 8 minutes. All the tests are stored into the fridge at 2-8°C. Before using they are took from the fridge and let at the room temperature to be properly use. After the test is ready to be used, we put 3 drops of blood sample and 4 drops of conjugate into a small plastic tube. This tube is gently inverted 4-5 times, to be sure that the sample is mixed. The entire contents of the tube 3 is put into the sample well of a SNAP device. The test device has a small area named the activation circle where sample migrate and confirm that the test can be pressed firmaly in the specific area of the device to be activated. After 8 minutes the result can be read on the device window and can be seen in the image below, figure 3 (14).

Prevalence of infectious agents among dogs and categorical risk factors (sex, breed) for infections were compared using the Microsoft Office Excel with one-way ANOVA and test.

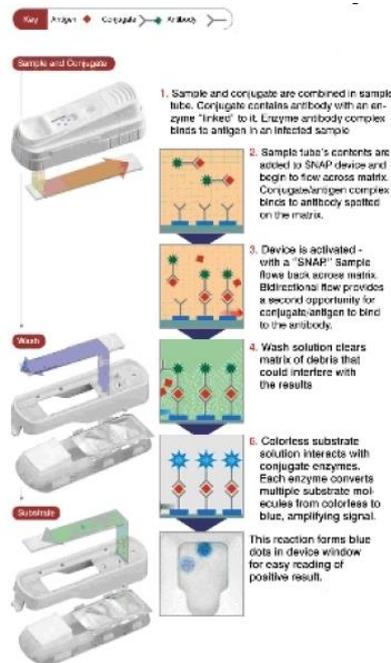


Figure 2. ELISA technique for SNAP 4DX Plus test (13)

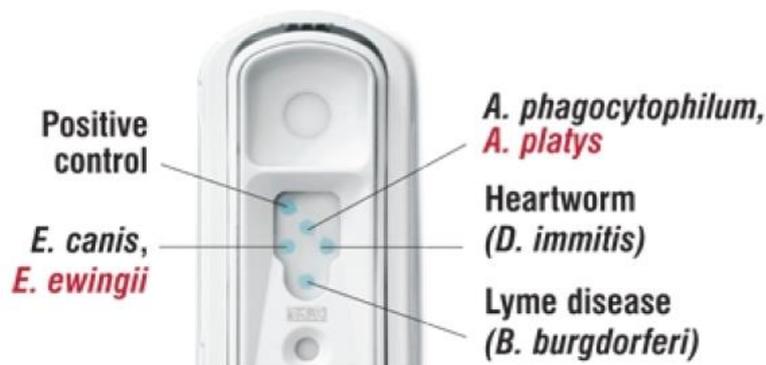


Figure 3. Result interpretation in SNAP 4Dx Plus test (14)

### Results and discussions

The result from our study showed that 6.25% (16/256) from all the dogs tested were positive to *Ehrlichia canis* and *Ehrlichia ewingii*. Three dogs from these were positive also to *Anaplasma phagocytophilum* and *Anaplasma platys*. The co-infection registered a percentage of 1.17% (3/256), figure 6. In the figure 4 Snap 4Dx Plus test has a negative result and in the figure 5, Snap 4 Dx Plus test has a positive result, which represent that the antibodies to *Ehrlichia canis* and *Ehrlichia ewingii* interacted with the corresponding antigens.



Figure. 4. Negative result (original)



Figure. 5. Positive result (original)



Figure 6. Positive: co-infection (original)

In the Table 1 are presented details about the dogs who were tested for antibodies to *Ehrlichia canis* and *Ehrlichia ewingii*: data regarding environment, rural and urban areas from Constanța county and the sex of the dogs.

Environment	Rural	Urban	Total
Female	125/256 (48.83%)	19/256 (7.42%)	144 (56.25%)
Male	87/256 (33.98%)	25/256 (9.77%)	112 (43.75%)

Table 1. Data of dogs from the study: sex and environment

The dogs had ages between 1 and 13 years old, both females, 56.25% (144/256) and males, 43.75% (112/256). All the dogs were included in 4 groups depending their age (figure 7).

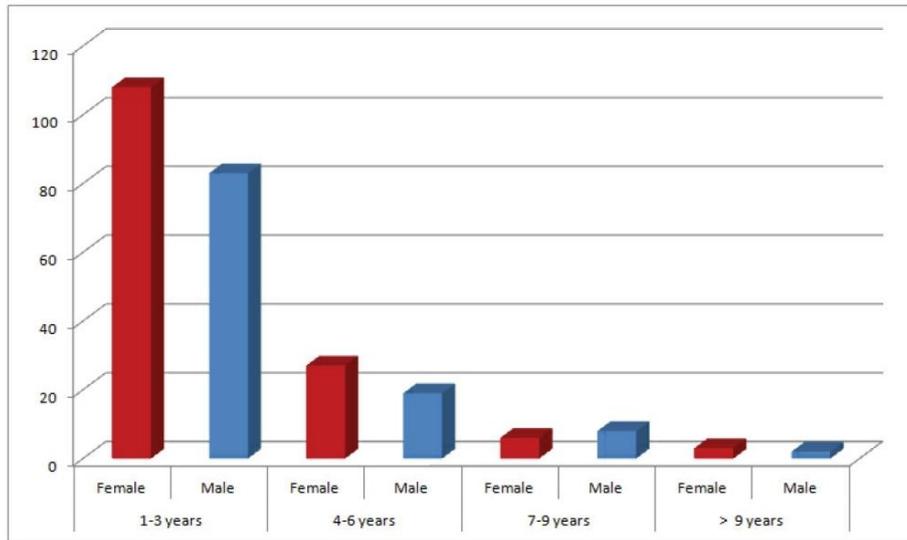


Figure 7. Diagram: group of age and sex of dogs from the study

The second table (table 2) include these groups. The representative group is the first one, dogs with age between 1-3 years old 74.60% (191/256) and the least representative group is > 9 years old, 1.95% (5/256).

Age	1-3 years		4-6 years		7-9 years		> 9 years	
Sex	Female	Male	Female	Male	Female	Male	Female	Male
	108	83	27	19	6	8	3	2
Total	191/256		46/256		14/256		5/256	
	74.60%		17.97%		5.47%		1.95%	

Table 2. Four groups of age for the dogs tested in the study

In our study, dogs were both from rural, 82.81% (212/256) and urban, 17.19% (44/256) areas from Constanța county (figure 8) and on their bodies we observed ectoparasites like fleas and ticks.

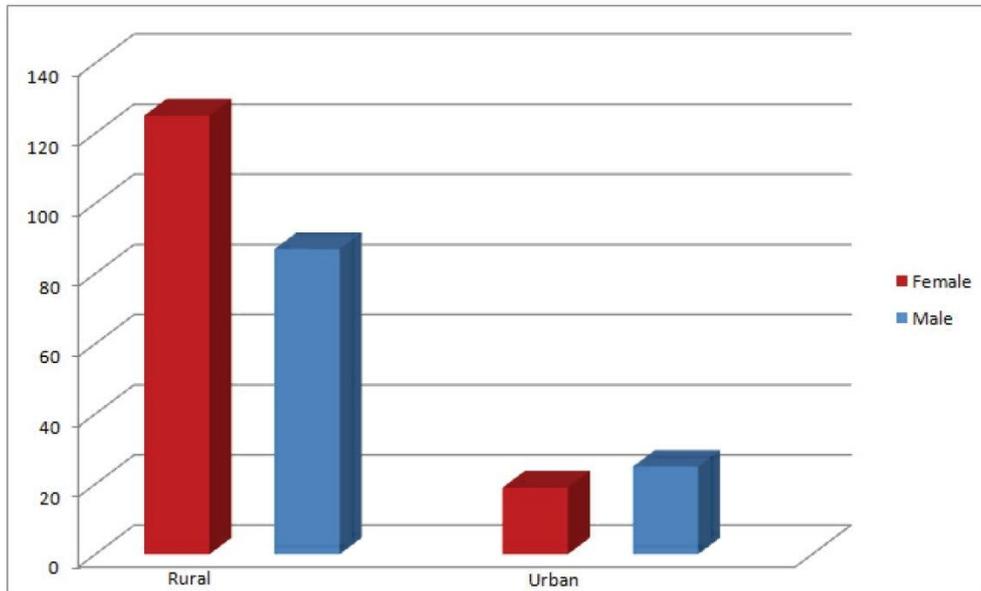


Figure 8. Diagram: lifestyle of the dogs from the study

The number of the dogs who had ticks on their body at the time we collected the blood samples was 49 (19.14%) from 256 dogs present in this study. Our results showed that even the ticks were not on the body surface of the dogs at the moment when we collected the blood samples, some of them were found positive to the antibodies to *Ehrlichia canis* and *Ehrlichia ewingii*. From the total number of dogs examined, the current study tested 11 males and 5 females who were positive for antibodies to *Ehrlichia canis* and *Ehrlichia ewingii*.

Fever was a clinical sign seen in a number of 29 dogs from a total of 256. In the others no fever was observed. Other clinical signs who were also observed in these 29 dogs were: lethargy, anorexia and weight loss. Only one male dog had epistaxis and he was a German Shepherd breed.

All the dogs tested were examined by groups of age. In the table 3 and figure 9 are observed the positive cases to antibodies of *Ehrlichia canis* and *Ehrlichia ewingii*, data referring to environment and to the presence of the ticks on the body of the positive dogs who were examined in this study.

Age	1-3 years		4-6 years		7-9 years		> 9 years	
	Female	Male	Female	Male	Female	Male	Female	Male
Sex	4 25%	7 43.75%	0	2 12.5%	1 6.25%	2 12.5%	0	0
Rural/ Urban	4/0	7/0	0	1/1	0/1	1/1	0	0

Presence of ticks on the animal body	1	3	0	2	1	1	0	0
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Table 3. Data referring to positive dogs tested for antibodies to *Ehrlichia canis* and *Ehrlichia ewingii*

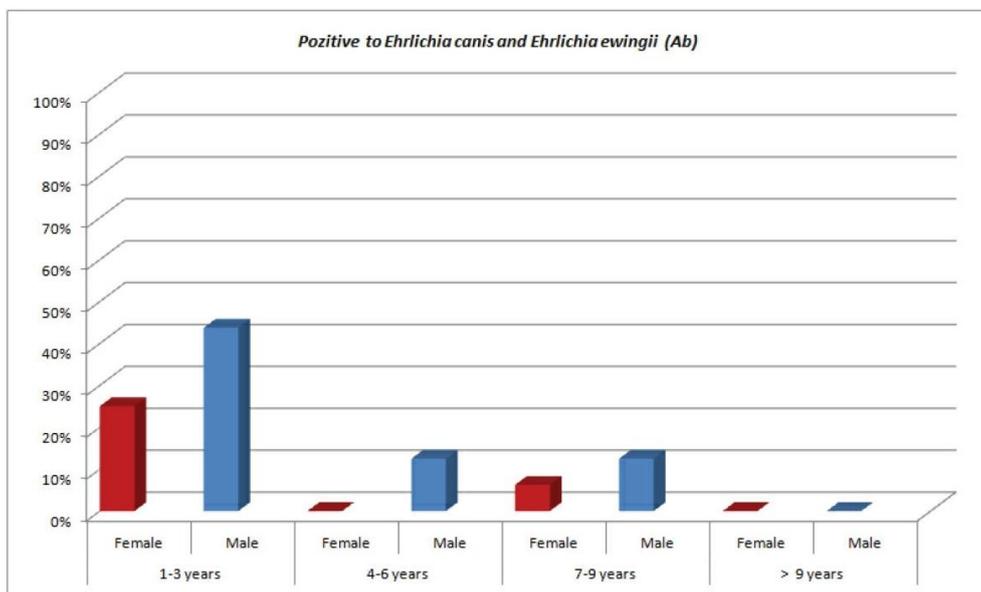


Figure 9. Diagram: positive dogs to *Ehrlichia canis* and *Ehrlichia ewingii* antibodies

The highest percentage, 68.75% (16/256), for positive dogs to antibody against *Ehrlichia canis* and *Ehrlichia ewingii* was obtained for the first group of age, 1 - 3 years old, both females (25%) and males (43.75%) and in the same time no positive case was found for the group of age, more then 9 years old (> 9 years). For the first group also were found 4 cases in which dogs had ticks on their bodies instead of the last group were no ticks were found.

Even the first group of age had more positive dogs to the antibodies against *Ehrlichia canis* and *Ehrlichia ewingii* than other groups, the results showed that there are no correlations between the age, gender and environment.

The infections is dependent of the spread of the vectors, ticks, which make possible the transmission of the pathogens. The presence of *Ehrlichia canis* and *Ehrlichia ewingii* is worldwide. This is confirmed by the studies done in several

countries. Our result obtained for the prevalence of *Ehrlichia canis* is in the limits (0.16%-62%) of other studies done in different countries (Ionița et. al., 2012, Mircean et. al., 2012, Farkas et. al., 2014, Perez et. al., 2014, McCowne et. al., 2014). In 2014 a study done by Starkey et. al., showed that dogs are at high risk of infection with *Ehrlichia ewingii* in many area of United States where ticks were common (12).

The result is high enough to worry us and to be considered also the infections with *Ehrlichia canis* and *Ehrlichia ewingii* in dogs from Romania.

### Conclusions

The presence of the antibodies to *Ehrlichia canis* and *Ehrlichia ewingii* in the blood samples collected from dogs involved in our study, from Constanța county, confirm the existence of canine infection with Ehrlichia spp.

The seroprevalence of *Ehrlichia canis* and *Ehrlichia ewingii* in dogs from Constanța county is 6.25%.

No associations were found between the prevalence and age, gender, origin, clinical signs, presence of ticks on the body surface and lifestyle of the dogs. Age can be considered an important risk factor for the infection with *Ehrlichia canis* and *Ehrlichia ewingii*.

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