



# Seroprevalence in Dogs Shows a High Incidence of Vector-Borne Diseases in Chiriquí Province, Panama

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**Abstract**

Between January 1, 2022, and December 31, 2023, 728 domestic canine who came to our hospital (HVHP) for consultation for different reasons and showed suspicion of a vector disease were evaluated. These patients were from 68 localities of the 14 districts of the Province of Chiriquí, Panama. Blood collections were performed, from the cephalic vein, for the SNAP 4Dx<sup>®</sup> rapid tests for detection of *Ehrlichia canis*/*Ehrlichia ewingii*, *Anaplasma platys*/*Anaplasma phagocytophilum* and *Borrelia burgdorferi* antibodies, and *Dirofilaria immitis* antigens in all patients. A whole blood hemogram was performed on 360 of the positive patients on a Mindray BJC2800VET, as well as a physical examination, temperature measurement, review of the status of the lymph nodes, a record of the reason for the consultation, age, and observation of key symptoms such as epistaxis or corneal edema. No blood count was recorded for patients who tested negative in the snap test. Patients were also grouped by district and month of the year in which they appeared at the hospital. 437 (60%) patients showed a positive result for at least one of target pathogens, and 291 (40%) showed a negative result. Of these, 415 were positive to *Ehrlichia* spp., 113 to *Anaplasma* spp., 29 to *D. immitis* and no reaction was evidenced to *B. burgdorferi*. In total, 321 dogs showed a single reaction to the exposed pathogens and 116 showed co-infections. May was the month that's showed the less incidence and December the highest. In the case of *Ehrlichia* spp., lack of appetite (26.8%), dermatitis (8.2%), weakness (5.9%), and convulsion (4.8%) were the more common symptoms. Lack of appetite was present in four patients (n=9; 44.4%) infected with *Anaplasma* spp. Cough was the only owner complaint symptom present in patients with dirofilariasis (n= 4; 40%). Only dogs with *Ehrlichia* spp. alone or in coinfection had a temperature equal to or greater than 40°C. Patients in Group III were those who presented the least alterations in the hematological values

Received: Oct 24 2024

Accepted: Nov 29, 2024

Published Online: Dec 06, 2024

Journal: Journal of Veterinary Medicine and Animal Sciences

Publisher: MedDocs Publishers LLC

Online edition: <http://meddocsonline.org/>

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**Keywords:** Vector-borne diseases; Dogs; Diagnoses; Symptoms; Serology; Whole blood hemogram; Panama.

**Abbreviation:** HVHP: Hospital Veterinario Healthy Pet; WBC: White Blood Cells; RBC: Red Blood Cells; HCT: Hematocrit; PLT: Platelets; VBD: Vector Borne Disease; PCR: Polymerase Chain Reaction.



of the red series. The number of platelets was the only hematological value that showed a statistically significant difference between the groups, with group III being the one that presented the highest values. Finally, understanding the importance of feral dogs as reservoirs of several zoonotic agents and stray dogs in maintaining the endemic cycles of VBD we will understand the expansion and distribution of these pathogens in Panama.

## Introduction

Vector-Borne Diseases (VBD) constitute a diverse group of viral, bacterial, and parasitic diseases transmitted for hematophagous arthropods to vertebrates, affecting both humans and animals [1-3]. In the case of veterinary medicine, diseases such as the Tropical Canine Pancytopenia (TCP, caused by *Ehrlichia canis*), Canine Cyclic Thrombocytopenia (CCT, caused by *Anaplasma platys*), Canine Babesiosis (caused by *Babesia* spp.), and dirofilariasis (caused by *Dirofilaria immitis*), are among the most relevant VBD in dogs worldwide [4,1,5]. Moreover, other diseases caused by protozoans as *Leishmania* spp. and *Trypanosoma* spp., or bacteria likes *Rickettsia* spp. and *Borrelia burgdorferi* s.l. bacteria, have been reported in dogs [1,6,7].

In many countries there are no studies on the incidence, prevalence, or presence of VBD in dogs, either because it circulates among animals without owners, animals are not taken to the doctor or due to lack of differential diagnosis [8,6]. On the other hand, fever, lethargy, cough, and other non-specific symptoms can be signs of a variety of diseases; therefore, the incidence of any of these diseases may go unnoticed in a certain region [9-11]. It is also possible for a single patient to present more than one causal agent of VBD, which is known as co-infection, increasing the risk of complications to the animal's health [12] and the probability of spray two or more diseases instead of one. Knowing which groups of pathogens circulate in dogs allows to understand potential risks of spreading diseases in a region, especially those that have a zoonotic connotation.

In Panama, TCP and CCT are among the most reported VBD in dogs [13], both transmitted by *Rhipicephalus sanguineus* s.l. ticks [14]. Although these diseases are mainly clinically diagnosed, other aspect are included to the discrimination, as such anamnesis, therapeutic diagnostic, or laboratory tests if the owner allows it; however, in most cases, any "tick fever" is diagnostic as TCP [8]. Moreover, in Panama rickettsial exposition has been reported in dogs [15], and there are one report of *R. sanguineus* s.l. infected with *Rickettsia rickettsii*, the pathogen that cause the most severe symptoms of rickettsiosis (*R. rickettsii* Spotted Fever (RRSF)), in a fatal case reported in City of Panama. In this sense, it is possible that dogs infected with *R. rickettsii* could go undetected as ehrlichiosis, in the absence of a differential diagnosis. In addition to TCP and CCT, canine leishmaniasis and filariasis (transmitted by Diptera, Nematocera) are other VBD in dogs of Panama and there are reports of *Trypanosoma cruzi* infesting dogs [16,17,2,7].

Since that VBD are not only of great importance to the owners because the health of their pets, but also some should be considered of zoonotic interest, the aim of this study was focused on serological detection of VBD in dogs with indistinguishable symptoms attended in Healthy Pet Veterinary Hospital (HVHP) in David city, Chiriqui province, Panama, during 2022-2023.

## Material and methods

**Sites:** The province of Chiriquí is in the western region of Panamá (Figure 1), delimited between the Pacific Ocean and the Talamanca Mountain Range, with locations ranging from sea level to 3,400 m altitude. It has an area of 6548 km<sup>2</sup> and a population of 462,056 inhabitants. The economic activities that occur in the province are tourism, agrobusiness, and commerce. The samples were collected during consultation at the HVHP in David city and came from the fourteen districts of the province. The general characteristics of these districts appear in Table 1.

**The study:** The samples correspond to January 1<sup>st</sup>, 2022, to December 31<sup>st</sup>, 2023, when dogs that were taken for consultation were treated in HVHP. All patients were evaluated for presence of fever, presence of swollen lymph nodes, as well pathognomonic clinical signs such as epistaxis, bilateral corneal edema, lethargy, low appetite, which taken as the inclusion criterion, along the consent of the owners to include laboratory tests. Data like age, origin and month of blood collect were recorded for each patient.

For the analyses, 1.0 ml of whole blood was taken from the cephalic vein, preserved on a EDTA tube for a whole blood hemogram using a Mindray BJC2800VET., and the SNAP 4Dx<sup>®</sup> rapid tests (IDEXX Laboratories, Inc., Westbrook, ME, USA) were performed. The SNAP 4Dx test was done following the manufacturer's instructions and consists of an enzyme immunoassay analysis that detects antibodies against *A. platys/Anaplasma phagocytophilum*, *E. canis/E. ewingii*, and *B. burgdorferi* s.l. from specific antigens; and female *D. immitis* body surface antigens from specific antibodies, with sensitivities ranging from 99.1%, 96.2%, 98.8% and 99.2%, respectively [18]. In positive patients to the target VBD, the clinical signs described above was re-evaluated and the hemogram data (White Blood Cell Count (WBC), Red Blood Cell Count (RBC), hematocrit % (HCT), and Platelet Count (PLT)) were recorded. Six seroprevalence groups were established to designate patients infected with *Ehrlichia* spp. (I), *Anaplasma* spp. (II), *D. immitis* (III), in addition to co-infections: *Ehrlichia* spp./*Anaplasma* spp. (IV), *Ehrlichia* spp./*D. immitis* (V), and *Ehrlichia* spp./*Anaplasma* spp./*D. immitis* (VI) triple co-infections.

**Statistical Analysis.** For statistical comparison, the Analysis Of Variance (ANOVA) and Tukey-Kramer test were used at the level of significance of 5%.

## Results

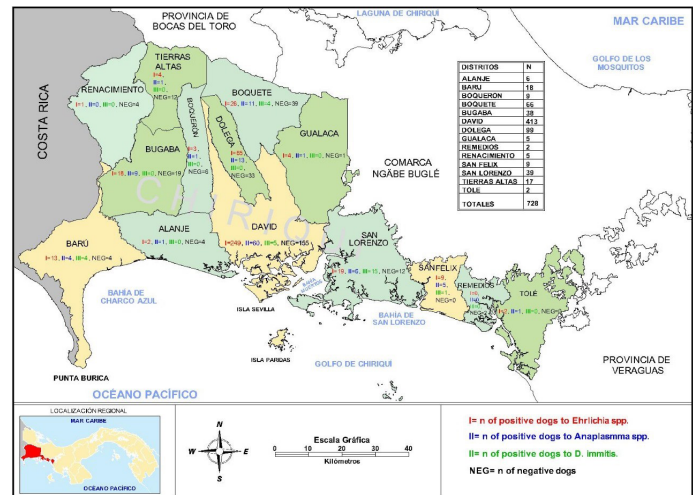
728 dogs ranged in age from two months to 17 years, coming from 68 localities belonging to the 14 districts of Chiriqui province, including Paridas Islands and Boca Brava Island that belong to the San Lorenzo District, were analyzed according to the inclusion criteria. Of these, 437 (60%) showed a positive result for at least one of the aforementioned VBD, and 291 (40%) showed a negative result. The distribution and incidence of positive samples by district can be observed in Table 2, Figure 1. David was the district with the highest number of patients and the highest number of seroprevalence (n= 413; 94%); while that Remedios district shows no positive dogs (n= 2; 0%). The variation of infections according to the months can be observed in Figure 2. May was the month that's showed the less incidence and December the highest. 174 (59%) and 263 (60%) positive dogs to at least one VBD were diagnosed in 2022 and 2023, respectively.

In total, 321 dogs showed a single reaction to the exposed pathogens and 116 showed co-infections. The most frequent reaction was to *Ehrlichia* spp. (*E. canis*/*E. ewingii*) antigens (415/437, 94%), which 299 correspond to single infection; 116 co-infections with other VBD (99 correspond to Group IV, 13 Group V, and four to Group VI). On the other hand, patients had 12 single reactions to *D. immitis*, and 10 patients reacted only to *Anaplasma* spp. Dual coinfection *Anaplasma* spp./*D. immitis* was not observed. No positive result was found for *B. burgdorferi* s.l.

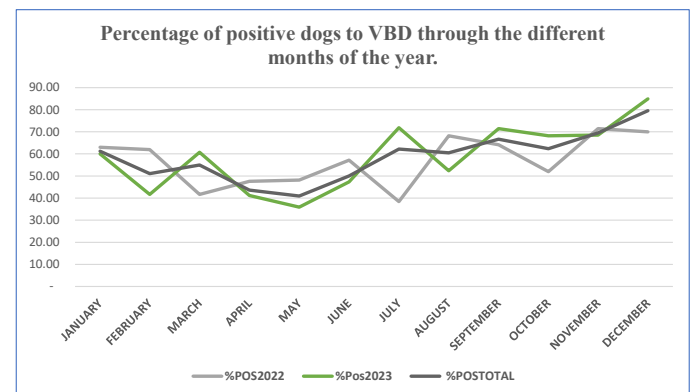
Of the patients physically evaluated, three patients of Group IV and one of Group I showed bilateral corneal edema. Three patients in Group IV and nine of Group I showed epistaxis. No negative patient showed epistaxis or bilateral corneal edema. One patient with *Ehrlichia* spp. infection alone presented generalized lymphadenopathy, while 100 showed lymphadenopathies of the prescapular lymph node (66 of Group I, two of Group II, 7 of Group III, 17 of Group IV, 7 of Group V and one of Group VI) (N=360).

The signs and reasons for consultation are summarized in Table 3. In the case of *Ehrlichia* spp., where a greater variety of clinical manifestations was observed, and of this, lack of appetite (26.8%), dermatitis (8.2%), weakness (5.9%), and convulsion (4.8%) were the more common symptoms. Of these, convulsion, dermatitis and epistaxis were observed only on dogs infected with *Ehrlichia* spp. It is important to mention that in 24 patients the clinical suspicion arose during a routine examination. Lack of appetite was present in four patients (n=9; 44.4%) infected with *Anaplasma* spp., and 24.1% of dogs with *Ehrlichia* spp./*Anaplasma* spp. coinfection. This coinfecting group also showed 8.6% dermatitis and 3.4% convulsion as reason for consultation. Cough was the only owner complaint symptom present in patients with dirofilariasis (n= 4; 40%). Tumors and cachexia were present as rare symptoms in concomitant infections of Group VI.

Regarding the 360 positive patients that underwent hemograms and temperature control, the results and statistical analysis of each Group are detailed in Table 4. Group VI with triple coinfection did not participate in the statistical analysis due to the sample size being very small (n=2). The percentage of positive dogs infected with the studied VBD, who presented hematological and temperature data outside the normal range, can be seen in Table 5. There was no significant difference between the averages of the five groups for the parameter's temperature, WBC, RBC and HCT at the 95% confidence interval (p>0.05); however, only dogs with *Ehrlichia* spp. alone or in coinfection had a temperature equal to or greater than 40°C. 19% of dogs from Group I and 33% of patients from Group IV showed leukopenia, whereas 66% and 55 % of these showed low red cells count, respectively. On the other hand, patients in Group III were those who presented the least alterations in the hematological values of the red series. The number of platelets was the only hematological value that showed a statistically significant difference between the groups evaluated (p<0.05), with group III being the one that presented the highest values and close to normal when compared to the other groups analyzed, this group being, once again, which less affected the hematological values related to thrombocytes. The other four Groups (I, II, IV, V) presented average platelets values below the level considered minimally normal (200 x 10<sup>9</sup>/L), being that 71% of the patients with just *Ehrlichia* spp. presented thrombocytopenia while 29% of these showed normal platelets values (Table 5).



**Figure 1:** Geographical position of Chiriquí province in Panama and Seroprevalence of Vector Borne Disease [*Ehrlichia* spp. (I). *Anaplasma* spp. (II). *Dirofilaria immitis* (III). *Ehrlichia* spp./*Anaplasma* spp. (IV). *Ehrlichia* spp./*Dirofilaria immitis* (V). *Ehrlichia* spp./*Anaplasma* spp./*Dirofilaria immitis* (VI)] in dogs from the 14 Districts of the province, during 2022-2023.



**Figure 2:** Percentage of positive dogs to Vector Borne Disease according to the different months of the year in Chiriquí province, Panamá between 2022-2023. (N. 437) (2022 n.174)(2023 n.263).

**Table 1:** Geographic and population characteristics of the 14 Districts of Chiriquí province, Panamá.

DISTRICS	AREA (km <sup>2</sup> )	POPULATION (≈)	ELEVATION (m)
Alanje	443	16 500	36 (6-77)
Barú	595	55 775	53 (0-706)
Boquerón	295	19 865	706 (29-3422)
Boquete	488	21 370	1096 (188-3313)
Bugaba	879	78 208	133
David	868	172 000	156 (-1 – 2478)
Dolega	250	25 102	622 (52-2590)
Gualaca	625	9 750	649 (15-2232)
Remedios	166	4 052	98 (-4 – 689)
Renacimiento	529	20 524	930 (2-2930)
San Félix	218	6 304	52 (-2 – 523)
San Lorenzo	647	7 507	65 (-1 – 1273)
Tierras Altas	365	22 885	1461 (492-3427)
Tolé	482	11 885	183 (-4 – 830)

**Table 2:** Seroprevalence of VBD [*Ehrlichia* spp. (I). *Anaplasma* spp. (II). *Dirofilaria immitis* (III). *Ehrlichia* spp./*Anaplasma* spp. (IV). *Ehrlichia* spp./*Dirofilaria immitis* (V). *Ehrlichia* spp./*Anaplasma* spp./*Dirofilaria immitis* (VI)] in dogs from the 14 Districts of Chiriqui province, Panama, during 2022-2023.

DISTRICTS	ANALYTES						Positives	Negatives	n
	I	II	III	IV	V	VI			
Alanje	2	1	0	1	0	0	2	4	6
Barú	13	4	4	4	3	0	14	4	18
Boquerón	3	1	0	1	0	0	3	6	9
Boquete	26	11	4	9	1	2	27	39	66
Bugaba	18	9	0	8	0	0	19	19	38
David	249	60	5	53	3	0	258	155	413
Dolega	65	13	0	12	0	0	66	33	99
Gualaca	4	1	0	1	0	0	4	1	5
Remedios	0	0	0	0	0	0	0	2	2
Renacimiento	1	0	0	0	0	0	1	4	5
San Félix	9	5	1	4	0	1	9	0	9
San Lorenzo	19	6	15	5	6	1	27	12	39
Tierras Altas	4	1	0	0	0	0	5	12	17
Tolé	2	1	0	1	0	0	2	0	2
<b>TOTAL</b>	<b>415</b>	<b>113</b>	<b>29</b>	<b>99</b>	<b>13</b>	<b>4</b>	<b>437</b>	<b>291</b>	<b>728</b>

**Table 3:** Symptoms that are a reason for the owner to bring his pet for consultation in canines positive to *Ehrlichia* spp. (I). *Anaplasma* spp. (II). *Dirofilaria immitis* (III). *Ehrlichia* spp./*Anaplasma* spp. (IV). *Ehrlichia* spp./*Dirofilaria immitis* (V). *Ehrlichia* spp./*Anaplasma* spp./*Dirofilaria immitis* (VI) in Chiriquí province, Panamá. In bold the percentage of most prevalent symptoms.

Groups (N)	Reason	n	%
I. (N= 269)	Epistaxis	6	2.2
	Under weight	7	2.6
	Threw up	9	3.3
	convulsion	13	4.8
	Diarrhea	8	2.9
	Dermatitis	22	8.2
	Decline/Weakness	16	5.9
	<b>Lack of appetite</b>	72	<b>26.8</b>
	Routine review	24	8.9
	Cough	12	4.5
	Pain	5	1.8
	Others	75	27.9
II. (N= 9)	<b>Lack of appetite</b>	4	<b>44.4</b>
III. (N= 10)	<b>Cough</b>	4	<b>40.0</b>
IV (N= 58)	Dermatitis	5	8.6
	Epistaxis	3	5.2
	<b>Lack of Appetite</b>	14	<b>24.1</b>
	convulsion	2	3.4
	Cough	5	8.6
V. (N= 12)	<b>Cough</b>	5	<b>41.7</b>
VI (N= 2)	Cachexia	1	50
	Tumors	1	50

**Table 4:** Analysis of hematological and temperature data of dogs infected with VBD [*Ehrlichia* spp. (I). *Anaplasma* spp. (II). *Dirofilaria immitis* (III). *Ehrlichia* spp./*Anaplasma* spp. (IV). *Ehrlichia* spp./*Dirofilaria immitis* (V). *Ehrlichia* spp./*Anaplasma* spp./*Dirofilaria immitis* (VI)] from Chiriqui province, Panama.

GROUPS	I	II	III	IV	V	VI
<b>TEMPERATURE</b>						
Min	37.20	38.50	37.80	37.00	37.80	38.60
Media	39.05	39.04	38.81 a	38.88	38.93	39.30
Max	41.10	39.70	39.40	40.50	40.00	40.00
<b>WHITE BLOOD CELLS [x 10<sup>9</sup>/L (6-17)]</b>						
Min	0.80	3.60	8.40	0.10	7.70	8.20
Media	11.11	10.13	14.96	11.33	12.99	13.35
Max	50.10	22.40	26.70	40.70	16.40	18.50
<b>RED BLOOD CELLS [x 10<sup>12</sup>/L (5.5-8.5)]</b>						
Min	0.91	2.60	3.40	1.80	2.60	2.70
Media	4.73	5.20	5.44	4.63	4.56	4.75
Max	8.50	8.00	7.30	7.00	5.90	6.80
<b>HEMATOCRIT [ (37-55)]</b>						
Min	6.50	16.60	24.80	11.60	10.90	17.60
Media	30.60	33.68	35.40	30.42	28.71	30.75
Max	61.30	46.30	47.60	47.60	36.80	43.90
<b>PLATELETS [x 10<sup>9</sup>/L (200-900)]</b>						
Min	9.00	23.00	107.00	10.00	46.00	134.00
Med	160.91 a	123.22 a	318.40 b	146.91 a	172.33 a	199.50
Max	582.00	429.00	753.00	623.00	313.00	265.00



**Table 5:** Percentage of positive dogs infected with VBD [*Ehrlichia* spp. (I). *Anaplasma* spp. (II). *Dirofilaria immitis* (III). *Ehrlichia* spp./*Anaplasma* spp. (IV). *Ehrlichia* spp./*Dirofilaria immitis* (V). *Ehrlichia* spp./*Anaplasma* spp./*Dirofilaria immitis* (VI)] in Chiriquí province, Panamá, who presented hematological and temperature data outside of the normal range.

	I (%)	II (%)	III (%)	IV (%)	V (%)	VI (%)
Temperature (39.4 – 39.9°C)	16.0	33.3	10	12.1	8.3	0
Temperature (≥ 40°C)	11.1	0	0	12.0	8.3	50
Leukopenia (≤ 5.9 x 10 <sup>9</sup> /L)	19.7	33.3	0	24.1	0	0
Lymphocytosis (≥ 17.4 x 10 <sup>9</sup> /L)	13.0	11.1	30	17.2	8.3	50
RBC (≤ 5.4 x 10 <sup>12</sup> /L)	66.5	55.5	60	75.8	91.6	50
HCT (≤ 36.9%)	71.4	55.5	60	82.7	100	50
Thrombocytopenia (≤ 199 x 10 <sup>9</sup> /L)	71	100	30	82.7	58.3	50

## Discussion

This is the first study of the incidence of VBD in dogs in Chiriquí province, using a serological analysis that confirms the circulation of three of the four types of target infectious agents. As expected, this work identified some biases that could vary the general panorama of VBD in dogs in this province. For example, our data included patients who attended the same clinic-hospital (HVHP) in David city, which could vary the incidence in some of the locations if the number of patients were increased. This was also evident in the number of patients, which were significantly more in the David city (n= 413) than in places like Tolé or Remedios, with two samples each. Moreover, the diagnostic method was not expanded to other potential pathogens reported in Panama and that could be present in the province of Chiriquí, such as *Trypanosoma* spp., *Leishmania* spp., or *R. rickettsii* [16,17].

During this investigation, patients with TCP represented (415/728, 57%) of the diagnosis, including TCP patients with multiple infections. This disease was found in 13 of the 14 districts of the province; in addition, CCT was the second most prevalent VBD (113/728, 15.5%), occurring in 12 of the 14 districts. On the other hand, the low number of patients from districts such as Remedios or Tolé could explain why these pathogens were not found; then, a greater number of cases could demonstrate the circulation of both diseases in this district. Along to CCT, TCP appear is the most common VBD on dogs in Panama, which corroborates previous studies in City of Panama, including the incidence of the co-infections among *E. canis* and *A. platys*. In this sense, a study carried out in a hospital clinic, 10% (n= 1452) of dogs attended with TCP clinic diagnostic and confirmed with blood smears. Moreover, a molecular screening developed in 10 veterinary clinics show DNA in 157/201 samples, corresponding to 129 (64%) *E. canis*, 43 (21%), *A. platys*, and 15 (7%) showed DNA from both species [13]. Another study that included 104 dogs that attended a veterinary clinic for routine check-up, preoperative screening or clinical illness, showed that 56 (53%) presented parameters compatible with VBD, and antibodies against TCP 14 (25%) dogs, CCT in two (3%), and three (5%) with coinfection [8]. In this case, the patients came from rural and urban areas of the provinces of Panama, Panama Oeste and Colón. It is important to highlight that *E. canis*-*A. platys* co-infections with incidences between 2.5-7% have also been found in Mexico [19,1,12].

The pathogens that cause TCP and CCT are transmitted by *R. sanguineus* s.l., a highly synanthropic tick species that is widely distributed in the province, especially in towns below 1000 m.a.s.l. [15,20]. In Chiriquí, the incidence of dogs parasitized with *R. sanguineus* s.l. in towns of Boquete (+1100 m.a.s.l.) or

Tierras Altas (+1500 m.a.s.l.) is considerably lower than those that come from towns between 0-500 m.a.s.l. [20]; even so, both pathogens could be found in these sites, which could be due to an expansion in their distribution due to urbanization in those areas [15,20]. *Rhipicephalus sanguineus* s.l. is considered as a “poli-vector” because its implication on the transmission of *Babesia canis*, *Babesia vogeli*, *R. rickettsii*, and, for ingestion is associate with *Hepatozoon canis* infections [1,21]. Although humans are considered as sporadic host of *R. sanguineus* s.l. [22,23], in Panama its relevance as vector of zoonotic diseases has been implicate in a human case of ehrlichiosis in Chiriquí [24], and RRSF in City of Panama.

Clinic manifestations of TCP and CCT are well described and reviewed worldwide with data that summarizes its distribution, epidemiology, clinical symptoms, diagnostic, treatment and zoonotic potential [25-30]. In general, TCP is reported to be a widely occurring disease in Brazilian pet clinics [29], being a multisystemic pathology that occurs acutely, sub-clinically and chronically, causing fever, lack of appetite, weight loss, lymphadenomegaly, bleeding, leukopenia and thrombocytopenia [27]. In a Brazilian review of Canine Ehrlichiosis, the authors conclude that this disease should be a focus of study since it could serve as a parameter for the knowledge of human Ehrlichiosis [26] whereas, in a review of India the authors conclude that the disease can be managed well with the correct and on time diagnoses and treatment [28]. Furthermore, in a review of anaplasmosis caused by *A. phagocytophilum* in United States the authors cited that patient showing variations of temperature between 39.2 and 41.4°C, added to lack of appetite in 47-88% of the cases, 90% showed thrombocytopenia and a large majority also presented lymphopenia and fever [25]. In a systematic review carried out more recently in the United States on the epidemiology and clinicopathology of *A. phagocytophilum* in dogs, the authors conclude that there is a lack of information in some parts of the world about its prevalence, the role of the dog as a reservoir, the diversity of its vectors and the possibility that the genetic variation of the bacteria diversifies its pathogenicity [30]. Which is why we consider phylogenetic studies in populations from different parts of the world to be of vital importance to try to better understand their evolution and adaptation in time and space. Despite the anterior, there is with few data that describe its pathogenicity in Latin American and its severity when act as coinfection.

In Panama, a study of molecular diagnosis of infections by *Ehrlichia* spp. in 201 dogs in Panama, was related with anemia (74.7%), thrombocytopenia (81.9%), and leukopenia (6.3%); while that these same clinical manifestations in infections caused by *A. platys*, varied to anemia (62.5%), thrombocytopenia

nia (75%), and no patient with leukopenia [13]. Moreover, [8] found in a sample of 104 animal patients from 38 urban and 18 rural settlements that 53.84% (n=56) shows parameters compatible with tick-borne disease, although only 22 owners allowing the immunochromatographic tests. Of these, *E. canis*/*E. chaffeensis* was found in blood of 14 patients, two reacted to *A. platys*/*A. phagocytophilum*, three shows co-infections *Ehrlichia* spp. *Anaplasma* spp., and three did not reacted to the immunochromatographic test. A relevant aspect of this work was the finding of fever, depression, anorexia and diarrhea in 100% of cases with co-infections, vomit and hematuria in 66,66% of cases, petechiae and epistaxis in 33,33% of cases; furthermore, the effectivity of the treatment was evident in 91.66%, and one patient died, possibly due to bone marrow aplasia from chronic ehrlichiosis.

In general, both studies presented similar results to ours, with variations of percentages although the associations of anaplasmosis with leukopenia only was found in our work.

In United states an experimental infection and co-infection of dogs with *E. canis* and *A. platys* showed that the group infected with *E. canis* (N=6) and co-infected with *E. canis*/*A. platys* (N=6) had the lower hematocrit when compared with the group infected just with *A. platys* (N=6), thrombocytopenia was also observed in all three groups [31]. Three years later in Mexico, 152 canines blood samples from six veterinary clinics and two shelters were analyzed using the ELISA Snap4Dx, Idexx, and observed 74.5% (113/152) of dog's positive for *E. canis*, and of these positive patients 103(91.2%) presented fever, 98(86.7%) anorexia, 80(70.8%) epistaxis and 106(93.8%) thrombocytopenia [32]. Also in Mexico, blood from 384 canines from different veterinary clinics and a shelter were analyzed by PCR for the diagnosis of *E. canis* and *A. phagocytophilum*, resulting in 103 (26.8%) of the animals positive for *E. canis* and none for *A. phagocytophilum*. At the same time, it was observed in these positive patients that 49 had hematocrit less than 37% and 6 showed thrombocytopenia less than  $180 \times 10^9/L$  [33]. Still and more recently in this North American country, 5,469 canine blood samples, from 30 days age, regardless of breed or sex, were evaluated by hemograms and SNAP4Dx Idexx ELISA in three veterinary diagnostic laboratories in Baja California and reported 262 (4.79%) dogs positive for *Ehrlichia* spp., 54 of these being co-infected with *Anaplasma* spp., 35% and 36% hematocrit, and  $136 \times 10^9/L$  and  $62 \times 10^9/L$  thrombocytopenia in patients with *Ehrlichia* spp. and in co-infection with *Ehrlichia* spp./*Anaplasma* spp., respectively [34].

In Central America, in Nicaragua a randomized study in dogs showed anemia in 70% of cases (n=27) of *E. canis* by immunochromatographic test [35]. In the north of Trinidad, 92 stray dogs were sampled by an indirect immunofluorescent *E. canis* antibody test, and 41 (44.6%) were found positive, observing a close relationship between thrombocytopenia and these animals [36]. Studies in Costa Rica found that infections by *A. platys* are related to lethargy, fever and poor body condition, while *E. canis* was related to fever and alopecia in 146 dogs, and it is concluded that infections and coinfections are associated with severe anemia [1]. Moreover, *E. canis* in Costa Rica show clinical anomalies that were observed in a minority of the affected patients, with weakness in 0.2%, temperature above 39.5°C in 19.5%, weight loss in 4.5%, lymphadenomegaly in 2.0%, while that, the most common complaints from owners were bleeding and weight loss [37].

On the other hand, in Peru in 2021, when determining the frequency of *Anaplasma* spp. and *Ehrlichia* spp. in canines from three districts, the incidence was of 30% *Ehrlichia* spp, 6%, *Anaplasma* spp., and 55% coinfection between both. It was observed that 83% and 60% of patients with *Ehrlichia* spp. and *Anaplasma* spp. respectively, presented anorexia higher values than those found here. In relation to hematological values, the study demonstrated WBC within normality for both, RBC of 4.1 and 4.0; HCT of 28.8 and 28.6; PLT of 106 and 112, respectively (N. 100) [38]. In Argentina, seven dogs diagnosed with *Ehrlichia* spp. presented hematological alterations with average HCT values of 28.7, RBC of 4.51, PLT of 119.2 and normal WBC values [39], which is like our results. These authors indicate that the association between thrombocytopenia and clinical signs compatible with the disease such as anorexia, fever, weight loss, weakness and seizures could be an indicator of the presence of the bacteria in canines [39]. In this sense, it is important to highlight that debilitating, febrile symptoms associated with lymphadenopathy, chronic dermatitis and/or seizures may also be related to *Ehrlichia* spp.

*Dirofilaria immitis* was found in five of the 14 districts of Chiriqui, up to an altitude of among 300-800 m.a.s.l. Dogs positive to heartworm was coming from Barú (Puerto Armuelles), Boquete (Calderas), David (David), San Felix (Las Lajas Beach) and San Lorenzo (Boca Chica, El Manzanal Beach, Paridas Islands, Boca Brava Island) (Table 2). Comparing with previous findings [2,7], this is the first report of *D. immitis* from David, San Felix and Boquete districts, which could mean that it is a more widespread pathology than previously considered. The relevance of dirofilariasis as a zoonosis in the world and the damage caused to dog's organs is implicit in the literature [40-42,4]. The discussion of the symptoms and hematological data of dirofilariasis becomes limited since there are a few specific references in the literature for the symptoms and hematological alterations caused exclusively by *D. immitis* in Central America [2,43].

The first report of this VBD in Panama was done in 2021 when the authors related three cases, one of *D. immitis* alone and the other two coinfecting with *E. canis*. In the first case was observed enlarged prescapular lymph node, weight loss and low temperature 37.8°C, showing also hematological alterations like 26.3 WBC, 2.6 RBC, 10.9 HCT and 115 PLT. In the other two coinfecting cases the authors reported normal temperature and 39.5°C fever, enlarged prescapular lymph nodes, corneal edema and severe dermatitis, with no alterations on WBC (8.0 and 8.6), mild alteration on RBC and HCT of one dog (5.2 and 31.1%, respectively), and lower platelets levels on both cases (73 and 162) [2]. In a study reporting embolism and lung damage due to *D. immitis* in a canine in Panama, it was observed that in a patient with disease solely due to heartworm, it was observed cough, lack of appetite, fatigue and normal temperature. The whole blood hemogram of this case showed worse alterations than in the present study, with normal value of WBC (10.0), but low RBC, HCT and PLT (4.3, 25.6 and 107.0, respectively) [43].

In a study carried out in Pereira, Colombia, using SNAP 4Dx Idexx and PCR, 100 canine patients were analyzed in search of vector borne infections and a prevalence of 28% for *D. immitis* was observed, it was reported too, without specifying what the specific etiological agent was, that the animals positive for the VBD found all presented anemia and 70% of them had thrombocytopenia [44]. Also in Colombia, when evaluating blood from 173 dogs from the metropolitan Caribbean area, using

immunochromatography and PCR, a frequency of infection for *D. immitis* of 60.1% (104/173) was observed, being reported as the highest frequency found in the country until now [45]. Already in Brazil, when carrying out clinical and hematological observations in 34 canines naturally infected with *D. immitis*, it was reported that the most common reason for consultation among owners was cough 14.7% (5/34) and that thrombocytopenia was observed in 26.5% (9/34) of patients [46]. Still in Brazil in the same year, when the impact of *D. immitis* infection in canines was studied, it was observed that of 26 canines analyzed, 11 (42.3%) were positive for the nematode, with cough also being the main symptom cited by the owners [47].

The lack of diagnosis of *B. burgdorferi* s.l. in this study was to be expected, since despite the clinical or serological suspicion of the presence of the Lyme disease complex in animals and humans from Central America [48,12], until now there is no definitive evidence from indigenous cases to support this. Although there are three strains of *B. burgdorferi* s.l. were recently identified in *Ixodes* cf. *boliviensis* ticks from the Panama Highlands, its pathogenic capacity is unknown [23]. In Panama, relapsing fever *Borrelia* is the only borreliosis confirmed [49].

Finally, the circulation of several pathogens in different parts of the province of Chiriquí demonstrates the need to expand diagnostic coverage in patients who present with fever, lethargy, weakness or laboratory parameters such as thrombocytopenia, since some pathogens could be of zoonotic interest, both in urban and rural environments. On the other hand, feral dogs are reservoirs of several zoonotic agents, and an evaluation of the risk related to these kinds of animals is also necessary [6]. It is worth highlighting the importance of stray dogs in maintaining the endemic cycles of VBD and their movement within and between provinces, which may be favoring the expansion and distribution of these pathogens in Panama.

**Funding:** This project was financed by Healthy Pet Veterinary Hospital, David, Chiriquí.

**Author's contribution:** **SCC**, **PGC** and **SB**: conceived the study. **SCC**, **PGC**: performed the laboratory work and analyses. **SCC** and **SB**: drafted the manuscript. All authors contributed to reviewing the manuscript and read and approved the final version.

**Conflict of interests:** The authors declare no conflicts of interests.

**Acknowledgements:** We thank Susan Kelley for correcting the English of the manuscript.

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