CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

The Occurrence and Detection of Tick-borne Pathogens in Dogs on Aruba

MASTER'S THESIS

Prague 2022

Author: Bc. Martina Čelakovská

Chief supervisor: RNDr. Jiří Černý, Ph.D.

Second (specialist) supervisor: MVDr. Daniela Lukešová, CSc.

Declaration

I hereby declare that I have done this thesis entitled “The Occurrence and Detection of Tick-borne Pathogens in Dogs on Aruba” independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague on the 21st of April 2022

.....

Martina Čelakovská

Acknowledgments

The realization of this thesis would not be possible without the support and guidance of my supervisor RNDr. Jiří Černý, Ph.D. and his around-the-clock consultations.

I would also like to thank

- My second supervisor, MVDr. Daniela Lukešová, Csc. for the assistance in the project setting and the official arrangements of the internship on Aruba;
- The Faculty of Tropical AgriSciences and EDUCA o.p.s. for providing financial support of the internship during which the samples were collected;
- The Veterinary Dienst Aruba for the possibility of carrying out this research as well as all the veterinary clinics, foundations and the Animal Shelter on Aruba for cooperation in tick collection and gathering information about the dogs and Lemond S. for facilitating the communication with the clinics;
    - A big thanks goes to Cindy Tatiana Riaño Calderón, DVM for tireless and thorough tick collection, veterinary consults and support during my stay on Aruba;
- Associate Professor Saima Naz and Paulina Lesiczka for help with scientific procedures and consultations for this research and Adam Smetana and Helena Dobešová for consultations of statistical analyses and the e4 group students for recommendations and advices;
- My entire family, who was me from the very beginning until the last moment providing around-the-clock emotional, technological and financial support;
- My friend Anthony Williams, BBA for being my accountability partner and help to stay focused;
- My friends and classmates from Class of 2020/2021 who made the studies enjoyable despite all sorrows;
- And last but not least, my Mauka, for everyday emotional support.

Abstract

Research on tick-borne diseases in the Caribbean is very scarce. On Aruba, the Dutch Caribbean, in particular, some research reports were issued by the Utrecht Center of Tick-borne Diseases. However, there is no scientific publication in a peer-reviewed journal with impact factor on the presence of different tick species and the prevalence of tick-borne diseases. For example, the presence of *Borrelia burgdorferi* sensu lato has not been studied at all. The aim of this thesis was hence to provide information about local tick species and tick-borne pathogens to the local Public Health Department.

Samples of ticks collected between 2019 and 2020 from different locations on the island were morphologically identified and subsequently screened for presence of *Anaplasma platys* and *Borrelia burgdorferi* sensu lato using 16S rRNA and flagellin PCR tests, respectively. Additionally, important information about dogs, from which the ticks were collected, was recorded and thereafter statistically analyzed. Morphological identification distinguished three different species of the genus *Rhipicephalus* among 300 screened ticks. 168 ticks were identified as *Rhipicephalus sanguineus* species, 111 ticks as *Rhipicephalus turanicus,* and 21 ticks as *Rhipicephalus appendiculatus*. A total of 325 ticks were screened for *Anaplasma platys* with 0 % prevalence and *Borrelia burgdorferi* sensu lato with a prevalence of 8.9 %. Morphological identification of tick species and differentiation of particular borrelia species found should be confirmed by genetic sequencing in the future. The results of this thesis suggest that street dogs and crossbreed dogs were the most susceptible to tick infestation and thus also borrelia infection compared to pet and purebred dogs. Those findings should cause concern about the possible transmission of this pathogen to humans or other animals on the island.

Keywords: *Anaplasma*; *Borrelia*; Caribbean; PCR; tick; vector-borne disease; zoonotic disease

Contents

List of tables

List of figures

List of the abbreviations used in the thesis

ABCs – Aruba, Bonaire and Curaçao

PCR – polymerase chain reaction

1. Introduction and Literature Review

1.1. Lesser Antilles - Caribbean Islands

Caribbean islands are divided into two categories: The Greater Antilles and the Lesser Antilles. The Lesser Antilles (see Figure 1) is an area with an approximate amplitude of 940 km, from the British and US Virgin Islands in the north to Trinidad in the south, and an approximate breadth of 1140 km from Aruba in the west to Barbados in the east (Britannica 2021; Allen 2017). The islands vary in their geological formations (sedimentary, volcanic islands) and climatic conditions and are further accordingly divided into three groups - the Leeward Islands, the Windward Islands, and the Leeward Antilles (Allen 2017).

Figure 1. Map of Lesser Antilles. Modified picture (Jochim 2017).

1.1.1. Leeward Antilles

The Leeward Antilles comprise five islands belonging to the Kingdom of the Netherlands and several Venezuelan islands along the southern Caribbean Plate and the

northern coast of South America. Aruba, Bonaire, and Curaçao (often mentioned as the ABCs) are the three biggest Dutch islands of the archipelago (Allen 2017).

This group of islands has many similarities in their climate, geomorphology, and history. Falling outside the hurricane belt, tropical storms are very rare in this area during this problematic season. The islands have a very dry climate, they are mostly limestone with large dunes occurring mostly on Aruba (Kohsiek et al. 1987).

The ABCs have, since ever, had a close connection with South America. It was the mainland from which it was the easiest to reach the islands, particularly Aruba. For this reason, many waves of immigration had been occurring for centuries, particularly from today's Venezuela. However, they also have Dutch colonial history and therefore a strong connection with the slave trade, particularly Curacao and Bonaire (Allen 2017).

1.1.2. Aruba

Aruba is an island located around 30 km away from the coast of Venezuela. The climate is very dry, with a very low average precipitation of 471.1 mm (Kohsiek et al. 1987; Oduber & Ridderstaat 2016; Allen 2017). It belongs to the **Bsh** climatic category according to Köppen's classification (see Figure 2). Category **B** denotes arid and semiarid climates, subcategory **Bsh** is classified as hot semiarid steppes with dry summers (Kottek et al. 2006). The average temperature is 29.7°C with a constant wind coming mostly from the northeast and the southeast (Oduber & Ridderstaat 2016). Parts of mainland Colombia and Venezuela have the same climatic conditions as seen in Figure 2.

Arid grasses and cactuses are the predominant vegetation (Berschauer & Ros 2014) and the main local land fauna species described in local literature (Boer 2001) are listed below (Table 1). More information on particular species of bats and rodents can be found in Bekker (1996) and birds in Lepage (2022). The marine fauna is different from the neighboring islands due to the shallow waters around Aruba as it is the closest to the Venezuelan mainland, the depth being almost 10 times shallower than the waters around Curaçao and Bonaire (Berschauer & Ros 2014). The island has three landscapes, the oldest central lava formation rocks, and batholith rocks, both surrounded by limestone formations (Allen 2017).

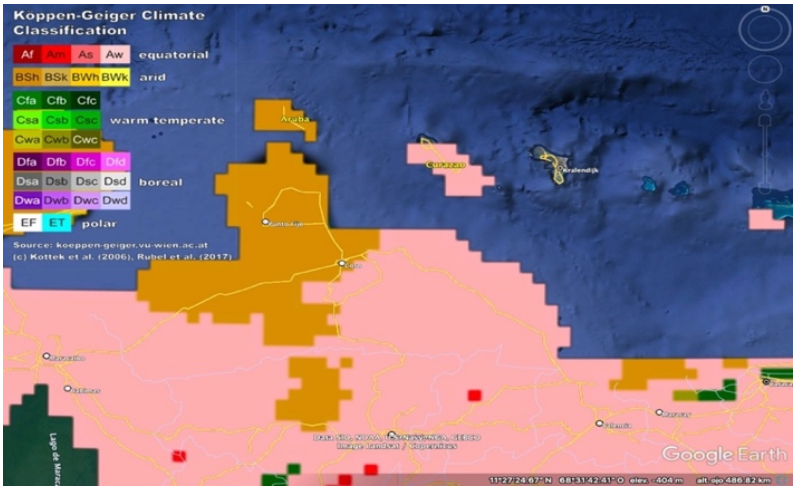

Figure 2. Köppen-Geiger Climate Classification for the ABCs and Venezuela. From Climate Change and Infectious Diseases Group (2007).

Table 1. List of the most common fauna species on Aruba. Based on Boer (2001).

LAND MAMMALS	
Latin name	**Common name**
Capra aegagrus hircus	Goat
Chiroptera order	Bats
Equus asinus asinus	Feral Donkey
Mus musculus	House Mouse
Sus scrofa	Feral Pig
Sylvilagus floridanus nigronuchalis	Cottontail
LAND REPTILES	
Latin name	**Common name**
Ameiva bifrontata	Cope's Ameiva
Anolis lineatus	Tree Lizard, Anole
Boa constrictor	Boa Constrictor
Chelonoidis carbonarius	Red-footed Tortoise
Cnemidophorus arubensis	Whiptail Lizard
Cnemidophorus lemniscatus	Rainbow Whiptail
Crotalus durissus unicolor	Aruban Rattlesnake
Gonatodes antillensis	Antilles Gecko
Gonatodes vittatus	Wiegmann's Striped Gecko
Gymnophthalamus laevicaudus	Spectacled Teju
Hemidactylus mabouia	Tropical House Gecko
Iguana iguana	Green Iguana
Leptodeira annulata bakeri	Cat-eyed Snake
Phyllodactylus julieni	Aruba Leaf-toed Gecko
Phyllodactylus martini	Dutch Leaf-toed Gecko
Thecadactylus rapicaudus	Smooth Gecko
Trachemys scripta	Common Slider

1.2. Ticks and Tick-borne Diseases in the Caribbean

1.2.1. Ticks

The Caribbean is, as previously mentioned, a very vast and international area, having encountered significant migration and trade exchanges at both intercontinental and inter-insular levels throughout its entire history. This phenomenon is logically connected with animal movements and therefore the introduction and spreading of new pathogens. This occurs mostly by the legal or illegal import of infested domestic animals (e.g., dogs, farm animals) and by the influx of migratory birds from the Americas. The Caribbean does, thus, record both endemic and imported species of ticks (Basu & Charles 2017, Gondard et al. 2017).

In the Caribbean 56 tick species from 10 genera and 2 families - Argasidae and Ixodidae were described (Gondard et al. 2017). Soft ticks (Argasidae) are represented by the genus *Ornithodoros* with 15 different species mostly to be found in Cuba, Jamaica, Puerto Rico, and Trinidad and Tobago (Basu & Charles 2017). Hard ticks (Ixodidae) found across the Caribbean are more widespread, including 17 species of *Amblyomma*, 3 species of both *Rhipicephalus* and *Ixodes*, 2 species of *Haemaphysalis*, and one species of *Aponomma quadricavum* and *Anocentor nitens* (Basu & Charles 2017, Gondard et al. 2017). The most widely distributed ticks are, according to Basu & Charles (2017), *Rhipicephalus microplus*, *Rhipicephalus sanguineus,* and *Anocentor nitens*; the occurrence of those ticks is interpreted in a map below (Figure 3).

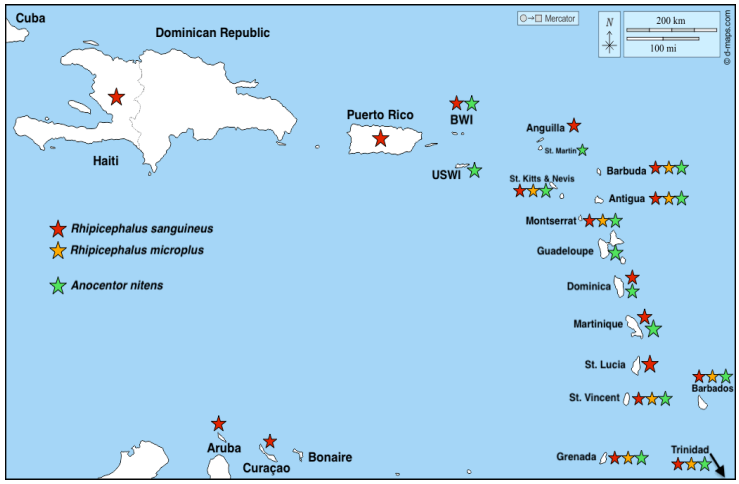

Figure 3. Presence of the main tick species in the Caribbean. Based on Basu & Charles (2017) and Gondard (2017).

1.2.1.1. Aruba

On Aruba, no scientific research has been published in peer-reviewed journal on the occurrence of tick species, nevertheless, three existing research reports from the Utrecht Centre of Tick-borne Diseases state that all the collected ticks in those studies were identified as *Rhipicephalus sanguineus.* In a study from 2008, Straten collected 4237 ticks from 100 neglected dogs (Straten 2008). In 2010 two studies were reported: Vugteveen collected ticks from 117 dogs from a veterinary clinic and 40 dogs from the kill cage, but the total number of ticks collected remains unknown (Vugteveen 2010). The same applies to Shuit's research, who collected ticks from 129 dogs from a veterinary clinic and 40 dogs from the kill cage (Shuit 2010).

1.2.1.2. *Rhipicephalus sanguineus* Latreille, 1806

Brown dog tick, tropical dog tick, or kennel tick, as called in different anglophone countries, *Rhipicephalus sanguineus* is a very important vector of several tick-borne diseases. Its distribution is cosmopolitan and it feeds mostly on dogs, however, it can also be found on domestic, wild animals, and humans (Walker et al. 2003; Dantas-Torres 2007; Basu & Charles 2017).

This vector has significant importance in the veterinary and medical fields since it can transmit and act as a reservoir of many pathogens (Dantas-Torres 2007). In dogs, the transmission of many pathogens had been reported, namely parasitic *Babesia vogeli, Babesia canis, Babesia gibsoni,* and *Hepatozoon canis,* and bacteria such as *Anaplasma platys, Ehrlichia canis,* and *Rickettsia rickettsia* or *Rickettsia conorii conorii*. In cattle then *Babesia bigemina,* and in humans many *Rickettsia* species and *Coxiella burnetti* (Walker et al. 2003; Dantas-Torres 2007; Basu & Charles 2017). Although transmission has not exactly been proven, those tick species were found infected by *Leishmania* spp. therefore it is possible that this tick could potentially be capable of transmitting such a parasite (Coutinho et al. 2005). Transmission of pathogens to humans has also been reported although it rarely parasites on humans (Palmas et al. 2001).

The genus *Rhipicephalus* is of African origin as it was described by Latreille in 1806 (Pegram et al. 1987). The different species in this genera can be hard to distinguish morphologically. The guide for species identification can be found in Walker et al. (2003).

This tick species has four developmental stages (egg, larva, nymph, adult) and is a three-host parasite (Figure 4). Tick in each of those stages feeds once to realize the transformation. After egg incubation, the hatched larvae start feeding for up to 10 days. Nymphs then feed for up to 11 days before they develop into an adult. An adult female tick can feed for up to three weeks before it drops from the host, lays eggs, and dies. They can lay around 4000 eggs (Nuttall 1915; Walker et al. 2003; Sonenshine & Roe 2014).

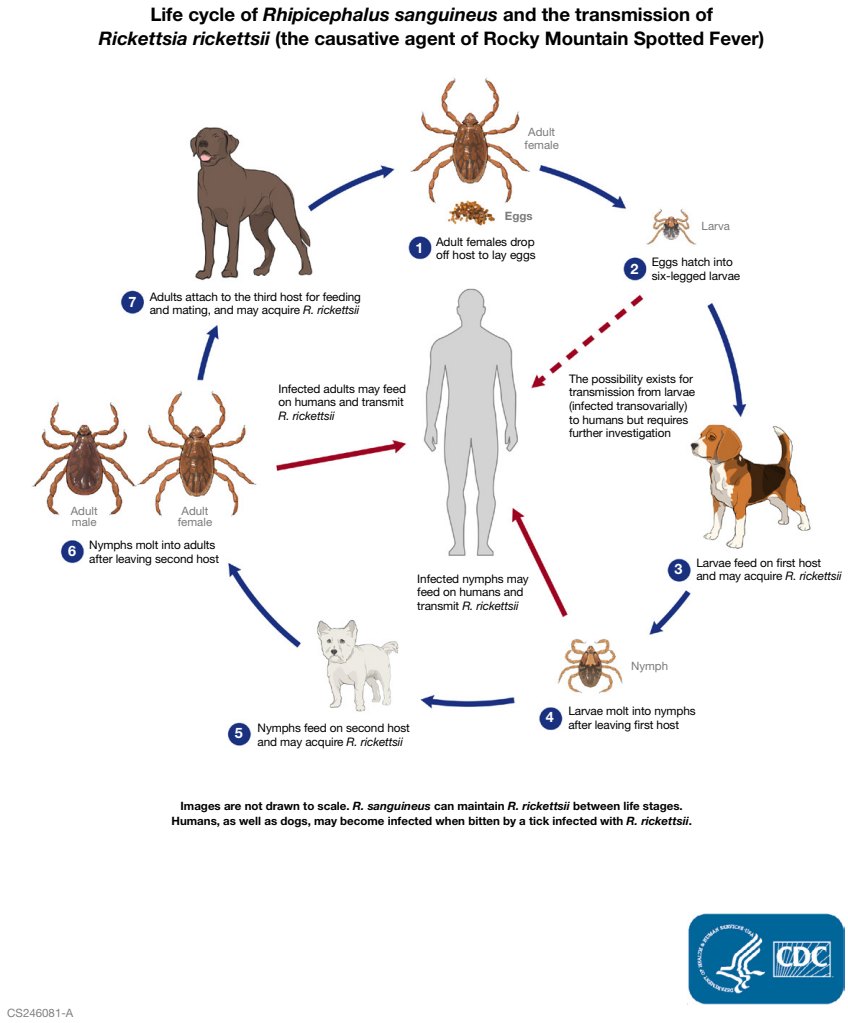

Figure 4. The life cycle of *Rhipicephalus sanguineus.* CDC (2022).

Rhipicephalus sanguineus has very good host-seeking skills since it can actively hunt. The primary host of the Brown dog tick is a dog. First developmental stages can be found in small mammals such as rodents while the adults choose larger mammals and even can parasite on humans when the tick population is very abundant (Walker et al. 2003; Dantas-Torres 2007).

1.2.2. Tick-borne Diseases in the Caribbean

According to Maggi & Krämer (2019), the most important tick-borne diseases that affect both dogs and cats on the Caribbean islands are babesiosis, bartonellosis, and ehrlichiosis. In addition, dogs are also commonly affected by anaplasmosis and hepatozoonosis. Humans, however, do not usually suffer from those diseases. Table 2 was created based on complex literary research from several resources and shows the presence of the most significant dog tick-borne diseases on different Caribbean islands.

Table 2. Diseases reported in dogs on different Caribbean islands. ✓ - reported in dogs, x - not reported in dogs, or no research done specifically on dogs. Based on: Bool & Sutmoller (1957); Davoust et al. (1999); Georges et al. (2008); Straten (2008); Yabsley et al. (2008); Moreta (2009); Schuit (2010); Vugteveen (2010); Klarenbeek (2010); Rodriguez et al. (2012); Spruit (2012); Westra (2012); Kelly et al. (2013); McCown et al. (2013); Li et al. (2015); Silva et al. (2016); Starkey et al. (2016); Grochowska et al. (2021); Morshed et al. (2021).

Disease/ Country	Babesiosis	Hepatozoonosis	Anaplasmosis	Bartonellosis	Borreliosis	Ehrlichiosis	Rickettsiosis
Aruba	✓	✓	x	x	x	✓	x
Cuba	x	x	✓	x	x	x	x
Curaçao	x	x	✓	x	x	✓	x
Dominica	✓	x	x	x	x	x	x
Grenada	✓	✓	✓	✓	x	✓	x
Haiti	✓	✓	✓	x	x	✓	x
Martinique	x	x	x	✓	x	x	x
Montserrat	✓	x	x	x	x	x	x
Puerto Rico	x	x	✓	x	x	✓	x
St. Kitts & Nevis	✓	✓	✓	x	x	✓	x
Trinidad	✓	✓	✓	x	x	✓	x

Silva et al. (2016) analyzed 100 dog blood samples and 431 ticks collected on Cuba. All of the ticks were identified as *Rhipicephalus sanguineus* and were pooled into 49 samples for PCR (polymerase chain reaction) analyses. Five of those pools (9.8 %)

tested positive for *Anaplasma platys*. The prevalence of *Anaplasma platys* in dog blood samples was 16 %.

Starkey et al. (2016) conducted research on Haiti using PCR for pathogen detection in dog blood samples. The prevalence was 6.3 % for *Anaplasma platys*, 0 % for *Anaplasma phagocytophilum*, 7.7 % for *Babesia vogeli*, 7.2 % for *Ehrlichia canis,* and 19.3 % for *Hepatozoon canis*. Snap 4Dx Plus test (IDEXX Laboratories, Inc., Westbrook, ME) was also used to assess the seroprevalence of *Anaplasma* spp., *Ehrlichia canis,* and *Borrelia burgdorferi* with results: of 17.6 %, 32.9 %, and 0 %, respectively.

In 752 dog blood samples collected around Puerto Rico, none tested positive for *Borrelia burgdorferi*. The overall prevalence of diseases was 6 % for *Ehrlichia canis*, 1 % for *Anaplasma phagocytophilum,* and 5 % for coinfection of *Ehrlichia canis* and *Anaplasma phagocytophilum* (McCown et al. 2013).

Kelly et al. (2013) performed similar research on St. Kitts using blood samples from 372 dogs with a PCR prevalence of 19 % for *Ehrlichia canis*, 12 % for *Babesia vogeli*, 10 % for *Babesia gibsoni*, 11 % for *Anaplasma platys,* and 6 % *Hepatozoon canis.*

In a study conducted on Grenada (Yabsley et al. 2008) the PCR prevalence for *Ehrlichia canis* was 24.7 %, 19.2 % for *Anaplasma platys*, 7 % for *Babesia canis vogeli*, 7 % for *Hepatozoon canis* and 1.4 % for *Bartonella* spp. in a total of 73 blood samples collected from dogs. Snap 3Dx tests (IDEXX Laboratories, Inc., Westbrook, ME) were performed to assess the antibodies' presence with a resulting prevalence of 49.3 % for *Ehrlichia canis* and 20.5 % for *Anaplasma* spp. There were no significant differences between males and females. 2 dogs were positive for *Borrelia burgdorferi*, but both of them had travel history in the USA, therefore it could not be considered acquired on Granada.

Georges et al. (2008) found the following prevalence in 348 canine blood samples on Trinidad: 0.6 % for *Anaplasma, Ehrlichia, Babesia* and *Theileria* nonspecific analyses; 1.7 % for *Theileria* and *Babesia* only, 2.3 % for *Theileria* and *Babesia* together with *Babesia canis vogeli*. Nonspecific *Anaplasma* and *Ehrlichia* only had prevalence of 4.3 %. *Anaplasma, Ehrlichia* plus *Anaplasma platys* was 2.3 % and *Anaplasma, Ehrlichia* and *Ehrlichia canis* was 13.2 %.

Rodriguez et al. (2012) discussed the possible presence of *Borrelia burgdorferi* on Cuba using the ELISA (enzyme-linked immunoabsorbent assay) analyzing human sera and the presence of typical clinical signs for Lyme disease.

1.2.2.1. Curaçao

Spruit conducted research in 2012 to evaluate the tick species present in wildlife fauna as well as in domestic animals all over Curaçao. There were 56 dogs examined and 1071 ticks were found and collected. The average number of ticks per dog was 19.1 and all were identified as *Rhipicephalus sanguineus.* Ticks of genus *Amblyomma* were found on 4 (out of 27 examined) local turtles *Geochelone pardalis* and on 14 iguanas *Iguana iguana*. No ticks were found on other examined wild and domestic animals: 3 tiger pythons, 30 equids including horses, ponies, mules, and donkeys, 175 goats and sheep, 22 cattle, 36 pigs, and 4 rabbits (Spruit 2012).

The first dog-specialized research was done by Klarenbeek (2010) who collected 1314 ticks from 129 dogs coming to a veterinary clinic; another 71 examined dogs were tick-free. The average number of ticks per dog was 6.6 and all of the collected ticks were of *Rhipicephalus sanguineus* species. This study also focused on the dog's history of tick-borne canine ehrlichiosis (reported in 27 % of purebred and 19.8 % of crossbreed dogs). Tick control products were reported to be used by only 5 % of dog owners.

Westra (2012) did similar research to the previous one, collecting ticks and diagnosing tick-borne diseases on the basis of clinical signs and results of Snap 4Dx Plus tests (IDEXX Laboratories, Inc., Westbrook, ME). In a total of 120 dogs that were examined for ehrlichiosis, 115 were positive (37 % purebred, 62 % crossbreed). The Snap 4Dx Plus test also shows the presence of antigens for three other pathogens, where 9 % of dogs showed positivity for heartworm disease caused by *Dirofilaria immitis*, 27 % were positive for *Anaplasma* spp., and 1.7 % positive for *Borrelia burgdorferi*. The *Borrelia* result was discussed as a false positive in this study due to the belief that this bacterium is only transmitted by the tick species *Ixodes ricinus* which has never been found on Curaçao. However, this tick is not its strict vector and many other species were found to be carrying *Borrelia burgdorferi* e.g., *Ixodes pacificus, Ixodes angustus,*

Dermacentor reticulatus and *Rhipicephalus sanguineus* (Grochowska et al. 2021, Morshed et al. 2021).

Also, 976 ticks were collected from 55 dogs, the average number of ticks per dog being 18. All of them belonged to *Rhipicephalus sanguineus* species. 944 ticks resulted positive for *Ehrlichia canis* using PCR method (Westra 2012).

1.2.2.2. Veterinary Conditions Overview of Aruba

According to Pan American Health Organization (2012, 2017), there were no cases of zoonoses reported on Aruba between 2006 and 2017. Anecdotal records of babesiosis and hepatozoonosis were reported by Bool & Sutmoller (1957) but analyses for those diseases have not been renewed since then. *Ehrlichia canis* has been repeatedly reported (Straten 2008; Moreta 2009; Schuit 2010; Vugteveen 2010). According to the statements of local veterinarians, dogs were the most common patients coming to the clinics with the most common tick-borne disease reported being ehrlichiosis and anaplasmosis. Snap 4Dx Plus tests method is the majorly used procedure in detecting those diseases in the veterinary clinics on Aruba.

There are six veterinary clinics on the island:

- Veterinaire Klinieken Aruba in Wayaca and in Noord;
- Animal Care Clinic in Paradera;
- Animal Health Hospital in Noord;
- Contreras Veterinary Services in Noord with a small subdivision in Savaneta.

1.2.3. Tick-borne Diseases in Latin America

The most important tick-borne parasitic diseases in Latin America are babesiosis, hepatozoonosis, and bacterial anaplasmosis, bartonellosis, borreliosis, ehrlichiosis, and rickettsiosis. According to Maggi & Krämer's review article (2019), these pathogens were recorded in many countries (Table 3). Only one case of Lyme disease was reported in a dog from Costa Rica (Montenegro et al. 2017). For the purpose of this thesis, only countries with direct access to the Caribbean Sea were chosen, as it is meaningless to

compare island climate with countries that abound predominantly with mountain ranges.

Table 3. The diseases reported in Caribbean countries of Latin America. Based on Maggi & Krämer (2019).

Disease/ Country	Babesiosis	Hepatozoonosis	Anaplasmosis	Bartonellosis	Borreliosis	Ehrlichiosis	Rickettsiosis
Colombia	✓	✓	✓	✓	x	✓	✓
Costa Rica	✓	✓	✓	x	✓	✓	✓
Honduras	x	x	x	x	x	✓	x
Nicaragua	✓	✓	✓	x	x	✓	✓
Panama	x	x	✓	x	x	✓	✓
Venezuela	✓	✓	✓	x	x	✓	x

Studies conducted across Latin America found that certain factors play a role in the assessment of risk factors for infection with different pathogens. Two studies from Brazil and Costa Rica showed that disease prevalence was higher in male dogs, older dogs, and in mixed breed dogs (Toledo Vieira et al. 2018; Barrantes-Gonzalez et al. 2018). Louly et al. (2009) also suggest that the English Cocker Spaniel breed is more likely to be heavily infested by *Rhipicephalus sanguineus* ticks than Beagles.

1.2.3.1. Colombia

Colombia, and in particular its Caribbean region, has highly suitable environmental and socioeconomic conditions to support the spread of pathogens (Pesapane 2019). There is a high potential for comparing the different tick-borne pathogens with those from Aruba, since the climatic conditions are very similar. The immigration from Colombia could also play an important role in disease and pathogen introduction (personal experience, 2020).

According to McCown et al. (2014) in a study conducted in Barranquilla "Exposure to tick-borne pathogens was the highest in shelter animals and military working dogs: more than 90 % of the samples were seropositive or PCR positive for one or more organisms as compared to 51 % in client-owned animals." Results (see Table 4) show that *Ehrlichia canis* and *Anaplasma platys* are important pathogens and can even occur quite often in coinfection. Neither *Anaplasma phagocytophilum* nor *Borrelia burgdorferi* was confirmed in this study. It can be also noticed that not all seropositive samples were positive using PCR analysis.

Table 4. Pathogens PCR prevalence in Barranquilla. Based on McCown et al. (2014).

Pathogen/ tested samples	*Ehrlichia canis*	*Anaplasma platys*	Coinfection *E.canis/A.platys*	*Borrelia burgdorferi*	*Anaplasma phagocytophilum*
Total DNA samples [n]	**218**	**218**	**218**	**218**	**218**
PCR positive out of all samples [n]	62	35	16	0	0
[%]	28.4	16.1	7.3	0	0
Seropositive out of all samples [n]	163	116	101	0	2
PCR positive out of seropositive samples [n]	60	26	10	N/A	0

In 2015, McCown et al. published another study on pathogens prevalence in three different cities (Barranquilla, Medellín, and Cartagena) this time using the Snap 4Dx Plus test. The seroprevalence of analyzed *Ehrlichia canis* and *Anaplasma phagocytophilum* is shown in the table below (Table 5). *Borrelia burgdorferi* was not detected in any of the analyzed samples. The city of Medellín with its high altitude was chosen in this study as a counterpart to the tropical wetland cities - Barranquilla and Cartagena (McCown et al. 2015).

In a study carried out during 2017 (Pesapane et al. 2019), 170 samples of dog blood from Santa Marta and Ciénaga cities, Colombia, were analyzed with PCR. The results are found in Table 5. They were compared to the results from McCown et al. 2014 since these Caribbean cities have similar tropical weather to the previously studied Barranquilla and Cartagena. *Ehrlichia canis* infection prevalence here, however, still remained the lowest.

Lyme disease (*Borrelia burgdorferi* infection) had been detected in Colombian people, but not in research focused on dog patients (Maggi & Krämer 2019).

Table 5. Pathogens reported in different Colombian cities. The sign "- "means that the selected pathogen was not analyzed in the given city.

Pathogen prevalence [%]/ Colombian city	Barranquilla	Baranquilla	Medellín	Cartagena	Ciénaga	Santa Marta	Santa Marta
Total samples [n]	**218**	**223**	**175**	**100**	**34**	**136**	**38**
***Ehrlichia canis* [%]**	28	82	30	80	0	19.1	-
***Anaplasma platys* [%]**	16	-	-	-	5	23.8	-
***Anaplasma phagocytophilum* [%]**	0	40	11	51	-	-	-
Coinfection *E.canis/ A.platys* [%]	7	-	-	-	0	8	-
Coinfection *E.canis/ A.phagocytophilum* [%]	0	40	6	49	-	-	-
***Borrelia burgdorferi* [%]**	0	0	0	0	-	-	0
***Babesia* spp. [%]**	-	-	-	-	-	-	10
***Anaplasma marginale* [%]**	-	-	-	-	-	-	52
***Coinfection Babesia/ A. marginale* [%]**	-	-	-	-	-	-	5
***Coxiella* spp. [%]**	-	-	-	-	-	-	89
Reference	McCown 2014	McCown 2015	McCown 2015	McCown 2015	Pesapane 2019	Pesapane 2019	Cotes-Perdomo et al. 2020

1.2.3.2. Venezuela

Venezuela, as well as Colombia, has similar climatic conditions to the ABCs and there is also a high level of immigration to Aruba (personal experience, 2020).

Huang et al. (2005) conducted research to assess the occurrence of two *Anaplasma* species in Venezuela using the PCR. Analyses were done on ticks, the blood of military dogs, and their owners. Twelve collected ticks were identified as *Rhipicephalus sanguineus* species and none of them were infected with *Anaplasma platys*. Seven out of 43 samples (16 % prevalence) of dog blood resulted positive for *Anaplasma platys*, but not for *Anaplasma phagocytophilum*. None of the human samples (n=25) were tested positive for any of the pathogens.

Lyme borreliosis had so far been reported in humans only, no research on dogs has been done (Maggi & Krämer 2019).

1.3. Studied Pathogens

1.3.1. *Anaplasma* spp.

A disease called anaplasmosis has two main causative agents - *Anaplasma phagocytophilum* and *Anaplasma platys*. They both cause different forms of anaplasmosis: granulocytic anaplasmosis and thrombocytotrophic anaplasmosis, respectively (Sykes & Foley 2014). The general distribution is found in Figure 5.

Anaplasma phagocytophilum causes disease in dogs, cats, humans, horses, camelids, and in Europe also in ruminants. The first three mentioned hosts are only accidental since the pathogen's reservoir is wildlife. For the transmission to the host to happen, the tick must be feeding for at least 36 - 48 hours (Carrade et al. 2009). This form of anaplasmosis is mostly asymptomatic, but some dogs can develop symptoms like fever, lethargy, low appetite, and arthritis with lameness (Egenvall et al. 1998). A previous study done by Egenvall et al. (1997) showed that Golden Retrievers had a higher predisposition for this infection. It is usually spread by a tick of the *Ixodes* genus and can often be found in co-infection with *Borrelia burgdorferi* (Granick et al. 2009). It can also infect humans, causing human granulocytic anaplasmosis, therefore it acts as a zoonosis (Sykes & Foley 2014).

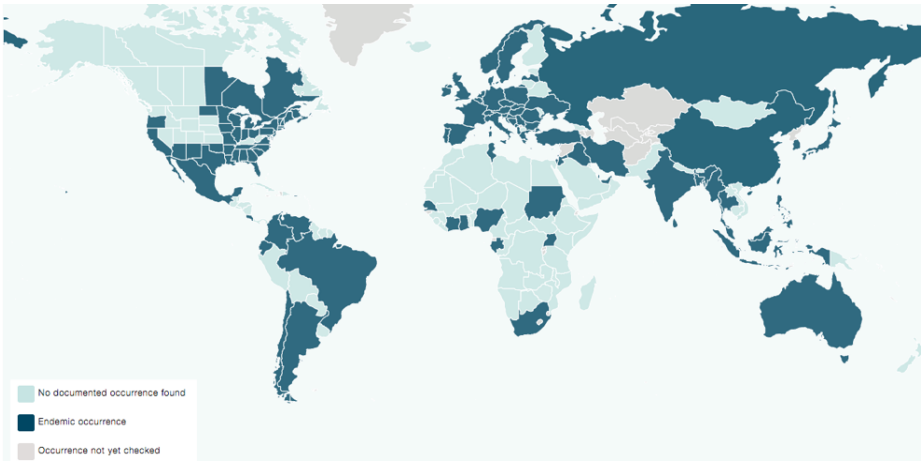

Figure 5. Distribution of anaplasmosis in the World. CVBD (2021).

Anaplasma platys causes canine cyclic thrombocytopenia in dogs only. No case of infection in a human has been reported. Commonly asymptomatic disease, fever, and

lethargy may sometimes appear. Coinfection with *Ehrlichia canis* is frequent. It is present on all continents and frequently found in *Rhipicephalus sanguineus* ticks (Sykes & Foley 2014), but it was also recorded in *Rhipicephalus turanicus* e.g., in a study from Israel (Harrus et al. 2011).

Infection by both of these pathogens can only be prevented by avoiding ticks and frequent use of an acaricide. Both forms of anaplasmosis can be treated by doxycycline broad-spectrum antibiotic (Sykes & Foley 2014).

1.3.2. *Borrelia burgdorferi* sensu lato

Spirochetes from *Borrelia burgdorferi* sensu lato complex, a group of about 20 different genospecies, are causative agents of Lyme disease, sometimes also called Lyme Borreliosis. It is present in Europe, Asia, and parts of North and South America (Figure 6). De facto, most of the *Borrelia burgdorferi* s.l. complex is present in temperate zones of the Earth; therefore, it is not believed to occur in tropical areas. However, *Rhipicephalus sanguineus* can also transmit *Borrelia* spp., and is an important vector of this pathogen in Brazil (Spickler 2021).

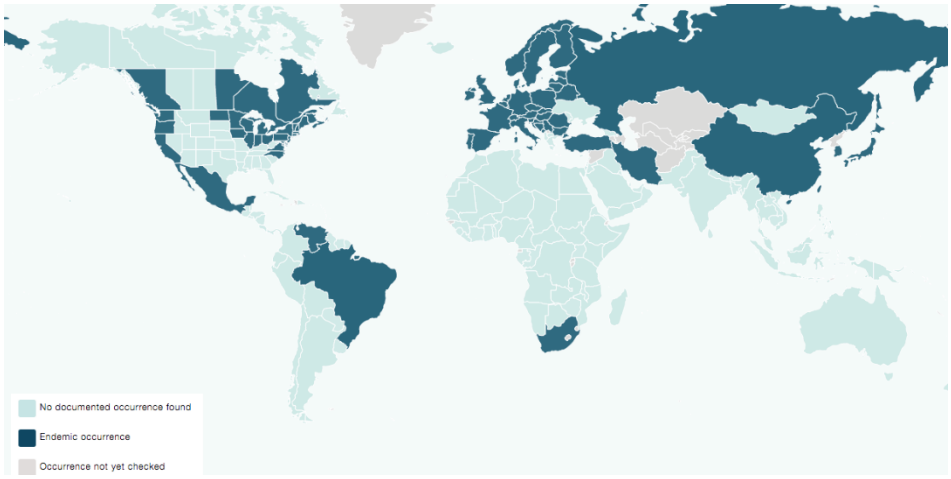

Figure 6. Distribution of Borrelia burgdorferi in the World. CVBD (2021).

Wildlife serves as its reservoir; hence dogs are only accidental hosts. For the transmission to occur, the tick needs to be feeding on its host for at least 24 – 48 hours (Spickler 2021).

Lyme disease in dogs seems to be rather asymptomatic, i.e., no specific symptoms or signs. However, some 5 % of infected animals develop the disease and clinical signs may most commonly include specific arthritis of carpal joints with lameness shifting from leg to leg, unspecific fever, anorexia, and lethargy (Spickler 2021).

Around half of the bacteria present in *the Borrelia burgdorferi* s.l. complex can affect humans and it is an important zoonosis in countries affected by the occurrence of this pathogen. The illness in dogs can be treated with antibiotics, e.g., Amoxicillin or Doxycycline (Spickler 2021).

2. Aims of the Thesis

The aims of this diploma thesis were to obtain current data on the presence of tick species and prevalence of selected tick-borne pathogens affecting dogs on the island of Aruba. The prevalence of the pathogens confirmed by the PCR method was compared with previous findings from the island as well as selected neighbor islands and mainland countries. Information collected about the dog patients used for this study and the ticks found on them were statistically analyzed to assess risk factors and impacts on tick infestation and pathogen transmission.

Hypotheses:

1. H_{01}: The only tick species on Aruba is *Rhipicephalus sanguineus.*

   H_{A1}: There are also other tick species on Aruba.

2. H_{02}: All dogs on Aruba have the same risk of getting infested by ticks.

   H_{A2}: There are specific risk factors that presuppose tick infestation.

3. H_{03}: *Borrelia burgdorferi* is not present on Aruba.

   H_{A3}: *Borrelia burgdorferi* is present on Aruba.

These aims and hypotheses were established in cooperation with the Department of Public Health, Veterinaire Dienst Aruba. There is no scientific research published about tick-borne diseases on Aruba hence the importance of this study for the local authorities and veterinarians to provide important information about animal health and the zoonotic potential of the researched pathogens.

3. Methods

3.1. Sample Collection

Ticks were collected from dogs on Aruba during two sampling periods, one in March 2019 and the second between August 2020 till January 2021. Ticks were taken from pet dogs during appointments hours at the veterinary clinics on Aruba (Veterinaire Klinieken Aruba – Wayaca and Noord; Animal Care Clinic; Contreras Veterinary Services; Animal Health Hospital), from abandoned dogs in governmental facility Centro di Control di Cacho) further mentioned as the kill cage), and from feral dogs rescued by Aruba Animal Shelter and other local foundations (Crijojo Trappers, Sergeant Pepper's Friends, Luna Foundation, Nine Lives). The main sampling points on the island are shown below (Figure 7).

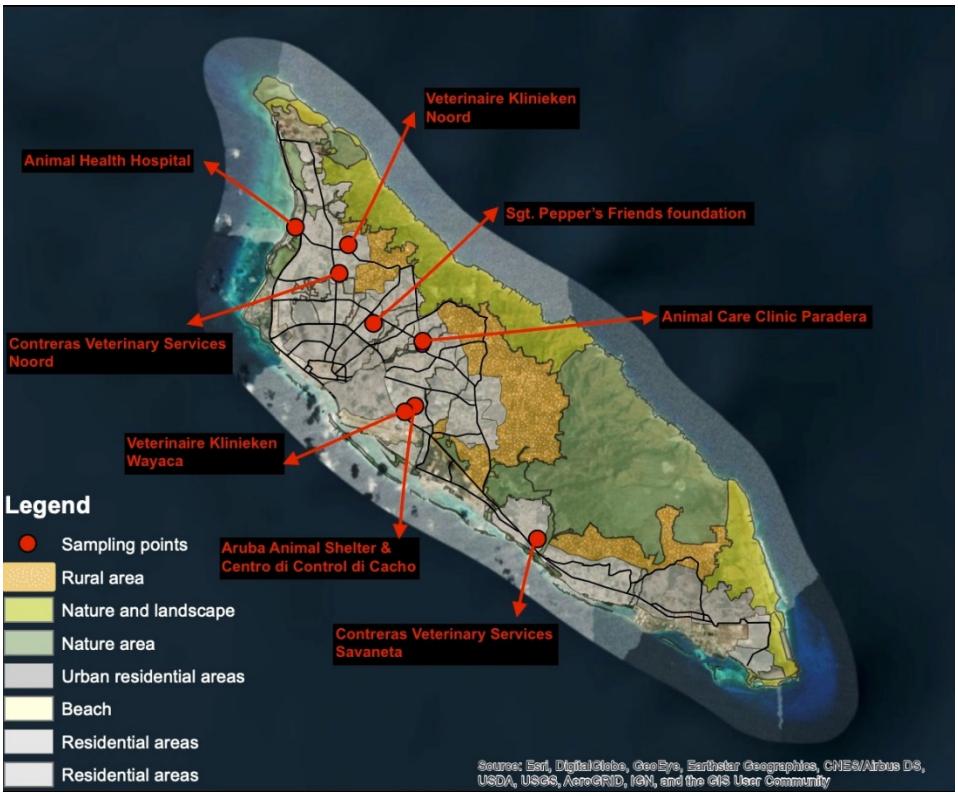

Figure 7. Location of veterinary clinics and animal facilities used as sampling points on Aruba.

Ticks were taken alive in the majority of cases, crawling on a dog's body or feeding. All larvae, nymphs, and adults were collected. Samples were fixed in 96 % ethanol for transportation to the Czech Republic where the laboratory analyses took place. Every time a tick was collected from a dog, information about this dog and tick was written down. Once a week between August 2020 and January 2021, information was collected from dog patients coming to the Wayaca Veterinary Clinic, recording both patients with ticks and without ticks.

3.2. Sample Database Creation

During the collection of the ticks, important information about them and about their canine hosts was taken with the help of a questionnaire that is attached in the appendix (Appendix 1: Questionnaire). A sample database was created and later statistically analyzed.

Collected information about the dogs was: name, sex, breed, age, veterinary clinic affiliation, provenance as neighborhood, status (pet/street dog), current disease symptoms, health history, use of tick control, and alternatively date of the last dose of tick control. This information was important mostly for the local veterinarians to track back the dogs that may test positive for some of the researched pathogens.

Dogs were divided into different age groups with a help of an experienced veterinarian: from 0 to 7 months, from 7 months to 2 years, 2-5 years, 5-10 years and older than 10 years, and labeled: puppies, juniors, young adults, mature adults, and seniors, respectively. The division of different provenances of the dogs was kept identical to the six official regions of Aruba – Noord, Oranjestad, Paradera, Santa Cruz, Savaneta, and San Nicolas. To avoid a bias in the later analyses, one extra category had to be created (Oranjestad foundation/shelter) since most of the animal foundations and the animal shelter are found in the capital city of Oranjestad, and dogs from all over the island are brought there.

Information collected about the ticks was: the location of the tick on the dog's body, whether the tick was feeding or only crawling on the dog's body, the number of

ticks collected from the dog, and the total number of ticks present on the examined patient. Later on, the genus, species, sex, and developmental stage of the ticks were identified.

On 22 different dates, once a week, between August 2020 and January 2021, it was possible to record information of examined canine patients that did not have any ticks. Data collected about those dogs were: name, sex, breed, age, and provenance.

3.2.1. Statistical Analysis

A database with information about ticks and their hosts was created in Microsoft Excel, where the graphic figures were subsequently created. Further statistical analyses were also performed in Excel. The correlation was used for the dependence of age interval and tick infestation and a two-sample t-test to test the average number of ticks in male/female dogs and in street/pet dogs. ANOVA was used for testing average tick count in different age categories, different dog breeds, individual localities, and lastly to assess differences in tick infestation depending on the last administration of acaricide. Univariate analysis was used to assess potential risk factors for tick infestation and for *Borrelia burgdorferi* infection.

3.3. Identification of Tick Specimens

All tick specimens were identified before the DNA extraction. The identification was performed by determining unique morphological features under the binocular microscope with the help of Associate Professor Saima Naz, an experienced veterinary entomologist, using a guide to the identification of species by Walker et al. (2003).

3.4. DNA Extraction

DNA extraction was done using Genomic DNA Mini Kit (Tissue) (GENEAID). Extraction was performed according to the manufacturer's instructions. The user manual is attached in the appendix (Geneaid manual for DNA extraction).

3.5. PCR

This diploma thesis is based on the PCR method by amplifying segments of DNA of selected bacteria genomes (16S rRNA for *Anaplasma platys* and flagellin for *Borrelia burgdorferi*) using specific primers. For both reactions nested PCR was required.

3.5.1. Used Primers

The pathogen-specific primers used for this study are listed in Table 6.

Table 6. Primers used for selected protozoan parasites and bacteria.

Pathogen	*Anaplasma platys*	*Borrelia burgdorferi* OUT	*Borrelia burgdorferi* IN
Forward primer	5'-AAGTCGAACGGATTTTTGTC-3'	5'-GCATCACTTTCAGGGTCTCA-3'	5'-CTTTAAGAGTTCATGTTGGAG-3'
Reverse primer	5'-CTCTCCCGGACTCTAGTC-3'	5'-TGGGGAACTTGATTAGCCTG-3'	5'-TCATTGCCATTGCAGATTGT-3'
Amplicon size [bp]	504	503	447
Primer annealing temperature [°C]	60	55	58
Reference	Fernandes 2017	Wills et al. 2018	Wills et al. 2018

3.5.2. Reaction mixture

DNA was mixed with a 2x PPP mastermix (Top-Bio, Prague, Czech Republic) including Taq polymerase, specific primers, and filled with pure PCR grade water. The reaction-specific mastermix compositions are listed in Table 7. For *Anaplasma platys*, both reactions were carried out with remained the same reaction composition.

Table 7. Mastermix reaction composition for *Anaplasma platys* (a) and *Borrelia burgdorferi* (b).

a)

Anaplasma platys	10 µl
PPP mastermix	5 µl
Forward primer	1 µl
Reverse primer	1 µl
PCR grade water	2 µl
DNA template	1 µl

b)

Borrelia burgdorferi	OUT 20 µl	IN 20 µl
PPP mastermix	10 µl	10 µl
Forward primer	1 µl	1 µl
Reverse primer	1 µl	1 µl
PCR grade water	5 µl	3 µl
DNA template	3 µl	5 µl

3.5.3. Conditions of PCR reaction

PCR reaction was carried out in T100 Thermal Cycler (BioRad) in the following order. Initial denaturation at 95°C for 15 minutes, followed by 40 cycles of DNA amplification (denaturation at 95°C for 30 seconds, primer annealing temperature specific for each pathogen for 30 seconds, and 72°C for 1 minute for DNA amplification), and the final elongation at 72°C for 10 minutes. The annealing temperature for *Anaplasma platys* is 53°C, and for *Borrelia burgdorferi* 55°C and 58°C.

Positive samples for selected pathogens acquired from the Institute of Parasitology, Biology Centre CAS, and Faculty of Veterinary Medicine, University of Veterinary Sciences, Brno, were used as positive controls. Negative controls were created with PPP Mastermix without the addition of a DNA template.

3.6. Gel Electrophoresis

Gel electrophoresis was used to visualize the PCR results. 1.5 g of agarose was dissolved in 100 ml of TBE buffer (1.5 % gel) and colored by 2 µl of ethidium bromide. TBE – (Tris/Borate/EDTA) buffer was used as a solution for the DNA environment. Electrophoresis was run at 120 V using PowerPac (BioRad) power source and HU13 (Scie-Plas) electrophoresis pool. The voltage gradient between electrodes was 5 V/cm. The electrophoresis was run for approximately 1 hour. Afterward, the separated DNA molecules were visualized by UV transilluminator, G:Box Chemi XRQ (Syngene) and photographed. The pictures were analyzed on a computer.

4. Results

4.1. Tick Identification

The total number of dogs examined for ticks and where ticks were found was 404. A total of 1508 ticks were collected during the sampling. Due to limited time capacity, it was possible to identify the genus of only 330 ticks. All of them were identified as *Rhipicephalus* spp. by morphological determination. Only 300 samples were identified to the level of species. Three species were identified: *Rhipicephalus sanguineus* (n=168), followed by *Rhipicephalus turanicus* (n=111), and lastly *Rhipicephalus appendiculatus* (n=21). The sex distribution is elaborated in Table 8 although it was not possible to assess the sex in non-adult ticks (mentioned as N/A in the table).

Table 8. Basic results in numbers.

a)

	[n]
Total dogs examined	404
Total ticks collected	1508
Ticks identified for genus	330
Ticks identified for species	300

b)

Tick	[n]	Females	Males	N/A
Rh. appendiculatus	21	15	2	4
Rh. sanguineus	168	93	53	22
Rh. turanicus	111	63	35	13
Total	300	171	90	39

4.2. Statistical Analyses

On 22 different dates during the sample collection, it was possible to gather information about dog patients that presented at the clinic both with and without ticks. The following chart shows the difference in the number of dogs with and without ticks on different dates between August 2020 to January 2021 (Figure 8). The data about these patients were for the most part collected by veterinarians, nurses, and assistants, and due to the lack of instructions among different workers, not all the information was gathered from every patient. That data is therefore missing in the table and could not

serve for statistical analyses. The total number of dogs examined was 484 from which 41 had ticks (8.5 %) and 443 (91.5 %) were tick-free.

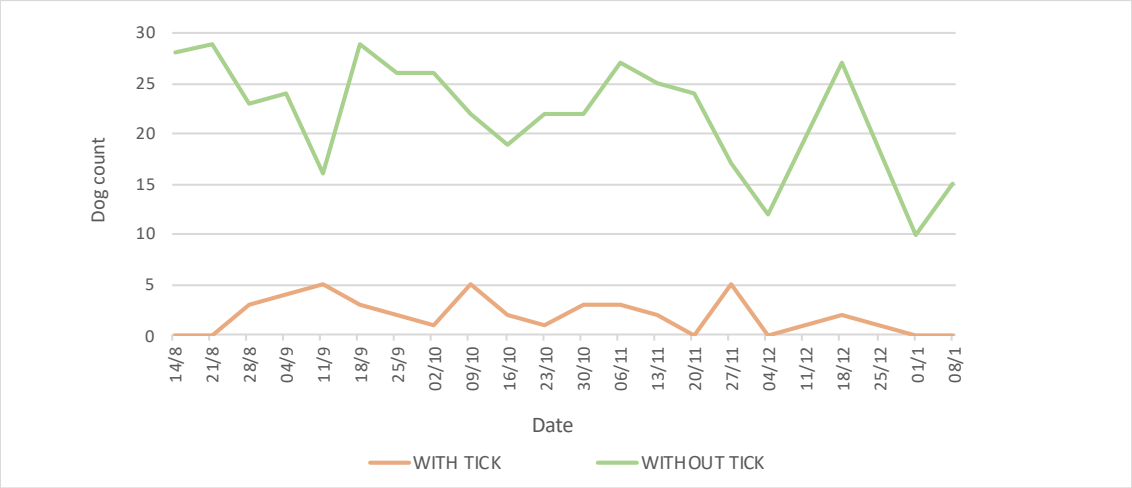

Figure 8. Dog count with and without tick on different dates during sample collection.

Some of the patients came to the clinic repeatedly but each sampling was counted as one event. The total number of samplings on patients that visited the clinic was therefore 509. Due to some missing information about certain patients, the total number of patients in each statistical analysis was different.

All the analyses were performed with significance assigned at p≤0.05. There was no statistically significant difference found between the sex of the dog (p=0.0608) shown in Figure 9a. A significant difference was found between pets and street dogs (p=0.0408) (Figure 9b).

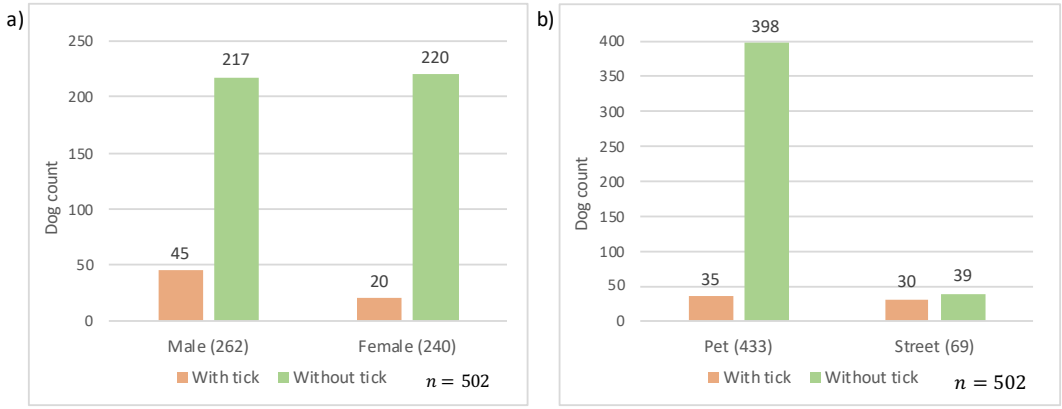

Figure 9. Dog count related to the dog's sex (a) and status (b).

No statistically significant differences were found neither between age categories with p=0.291 (Figure 10) nor between different patient's residence locations reaching from p=0.171 to p=0.563 (Figure 11). The examined dog breeds were subsequently grouped into two categories – purebred dogs and crossbred dogs (Figure 12). There was a significant difference found with p=0.0004.

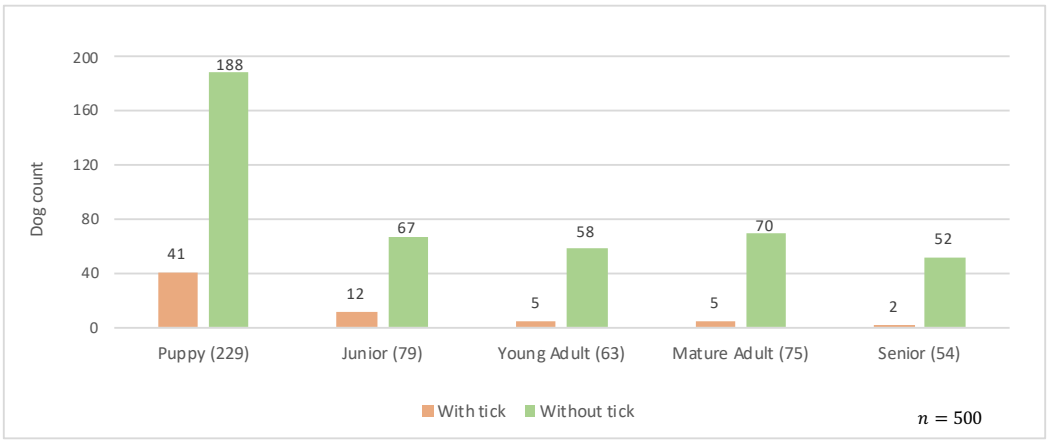

Figure 10. Dog count related to dog's age interval.

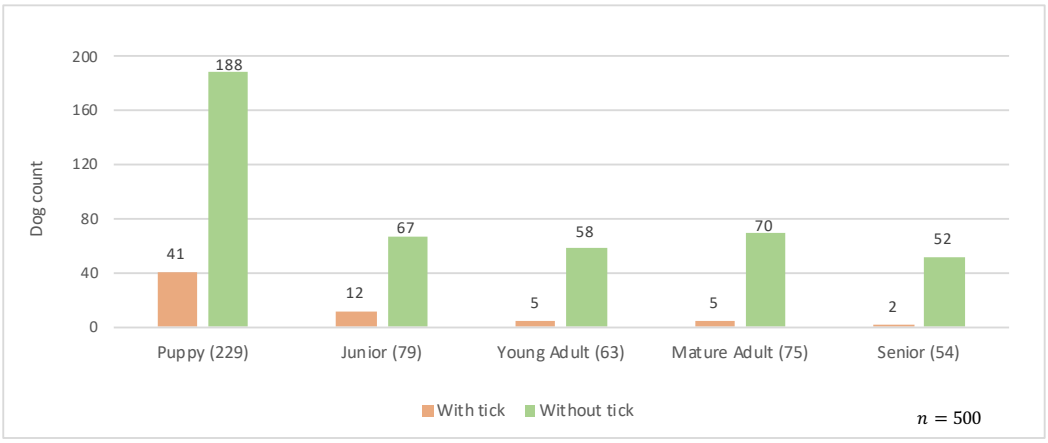

Figure 11. Dog count related to dog's provenance.

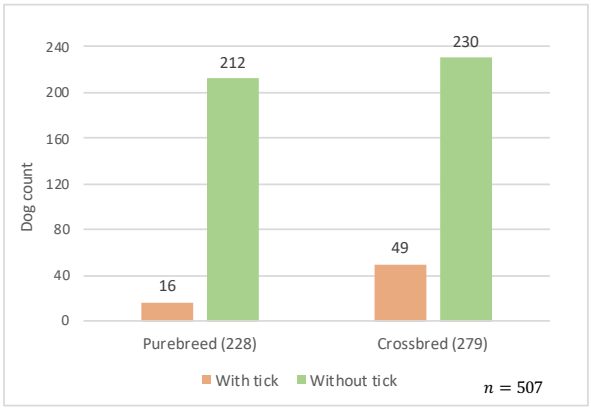

Figure 12. Dog count related to pure or crossbred group.

The purebred category was eventually split into different dog breeds (Figure13). No significant difference was found in dogs with/without ticks between different breeds (p=1).

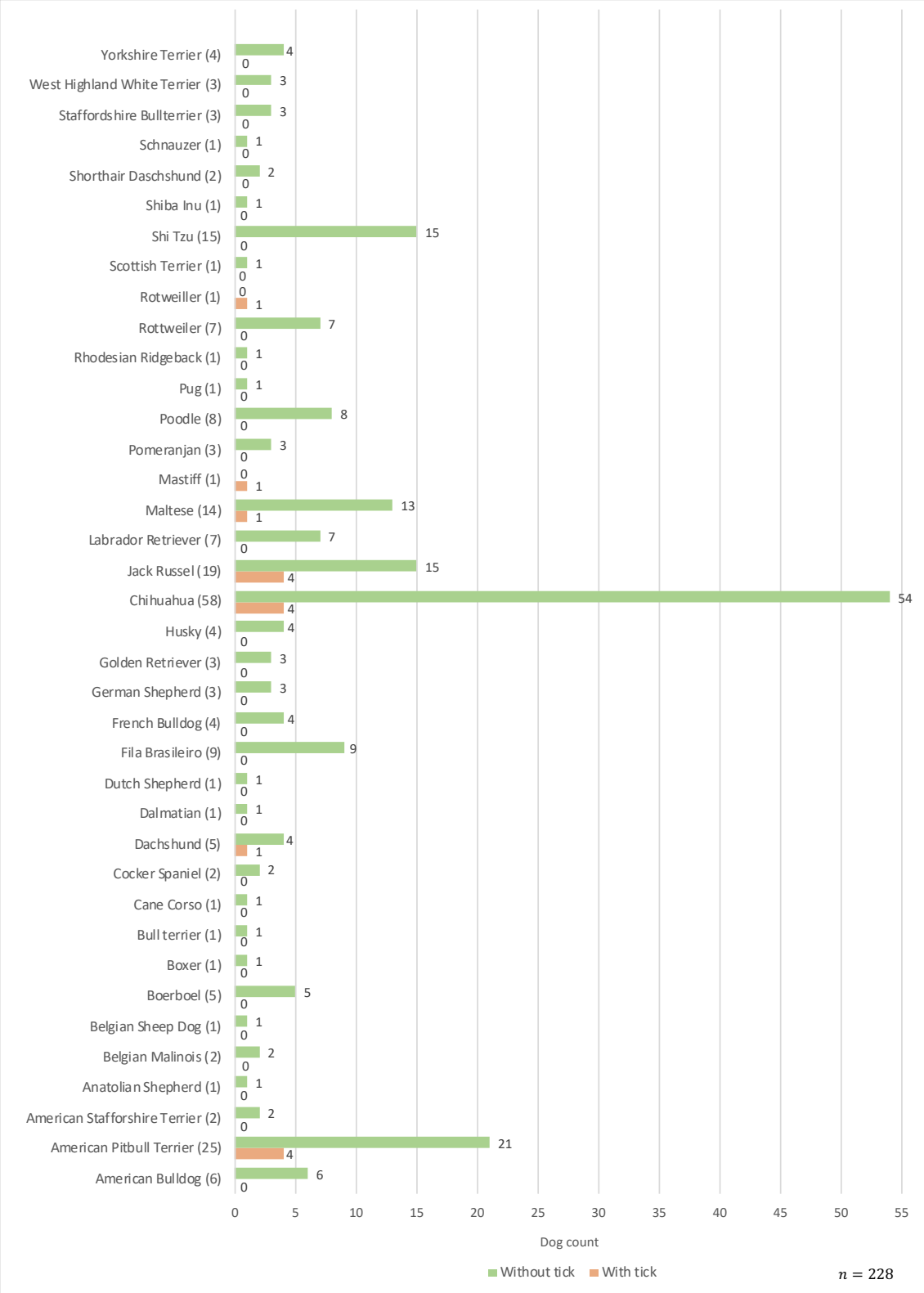

Figure 13. Dog count related to each pure breed.

A second database was created out of all sampled dogs with ticks (a total of 576 samples from 404 dogs). The statistical analyses were carried out on the basis of the average number of ticks collected from each patient. The dogs´ sex distribution is shown in Figure 14. According to the chart, males were found to be slightly more infested by ticks, but no statistically significant difference was found with significance assigned at p≤0.05 with p=0.3565. The sex of the other dogs was unknown.

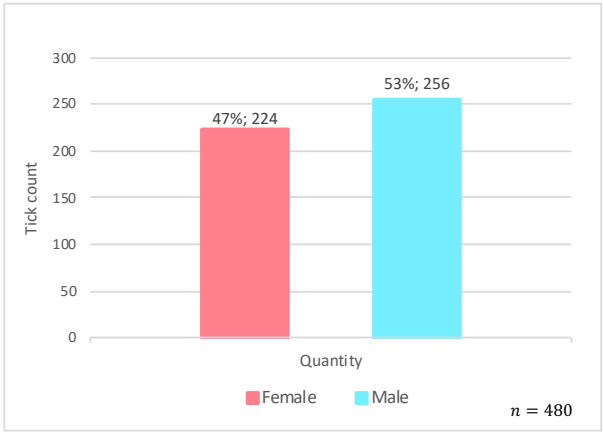

Figure 14. Tick count related to the sex of the dog.

Next statistical analyses were performed to assess the possible differences between the average tick count in dogs of different age groups with p=0.676 (Figure 15), in different locations of their provenance with p=0.592 (Figure 16), and between different dogs' breeds with p=0.9707 (Figure 17). As for the provenance of the dogs, the highest average can be seen in the San Nicolas region. Mixed breed, American Pitbull Terriers and Jack Russel Terriers were, on average, most infested by ticks, respectively. However, none of these differences was statistically significant.

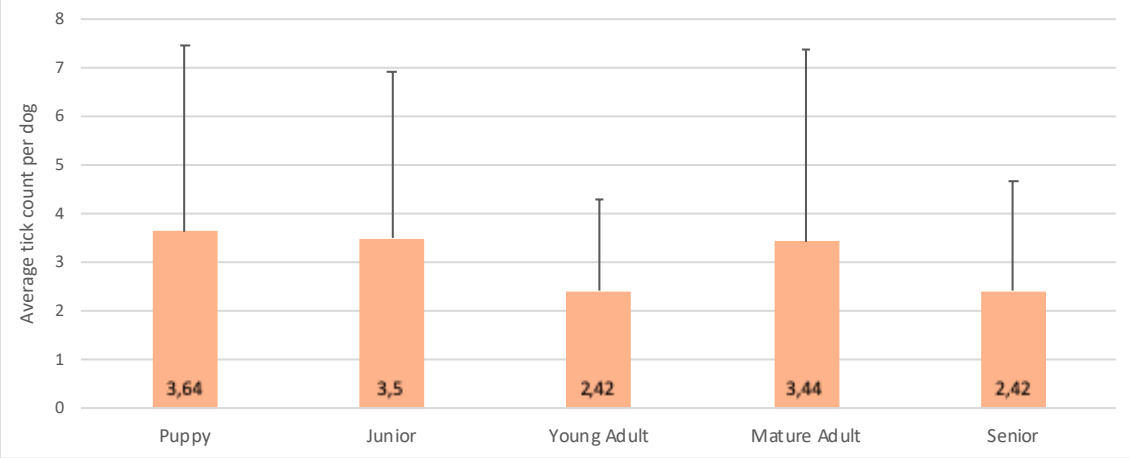

Figure 15. Average tick count related to the age interval of the dog.

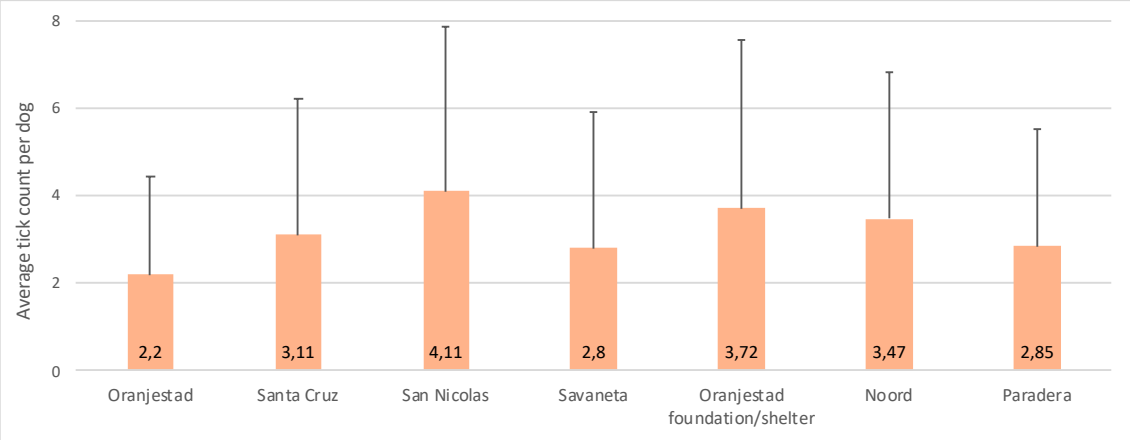

Figure 16. Average tick count related to the provenance of the dog.

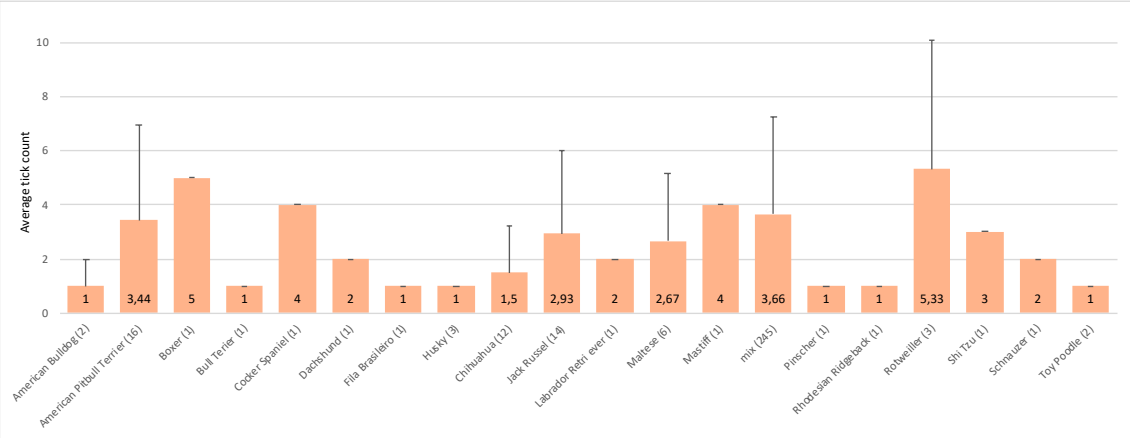

Figure 17. Average tick count related to the breed of the dog.

In several dogs, information about the last dose of acaricide was recorded (Figure 18). It shows that there is no difference in tick infestation regardless of when they received the product. Accordingly, there was no statistically significant difference with a result of p=0.616. Surprisingly, it seems that dogs that were given this product in less than one month and dogs that have never received any product against ticks have the same tick infestation.

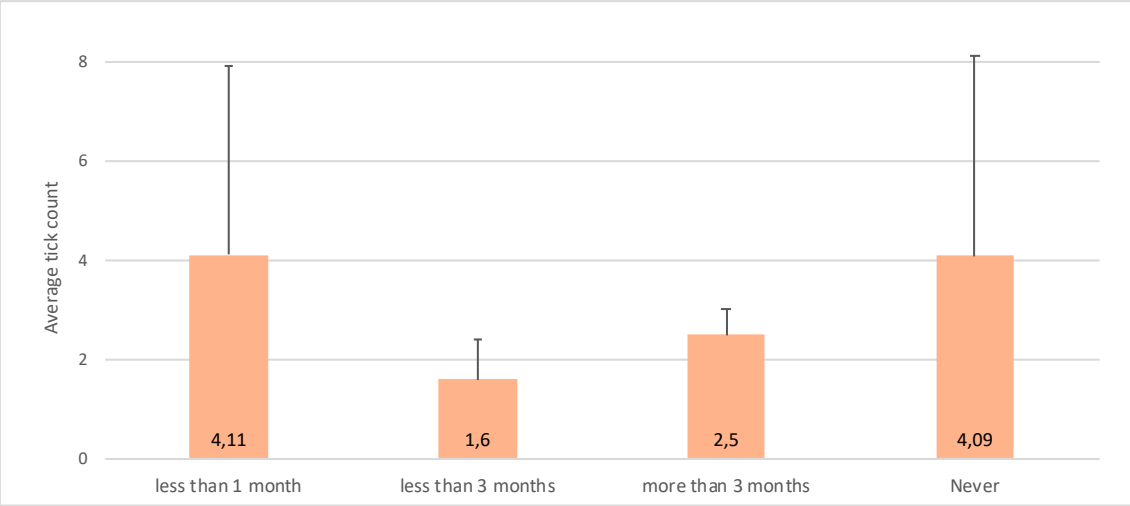

Figure 18. Average tick count related to the last dose of acaricide given to the dog.

4.3. PCR Analyses

A total number of 323 ticks were analyzed for the presence of *Anaplasma platys*. Seven other ticks were unfortunately lost during the handling of the samples. None of those tested samples came out positive.

The same number of tick samples was tested for the presence of *Borrelia burgdorferi*. Twenty-nine samples resulted positive, that is 8.9 % prevalence. An example of positive samples on gel agarose is shown in Figure 19.

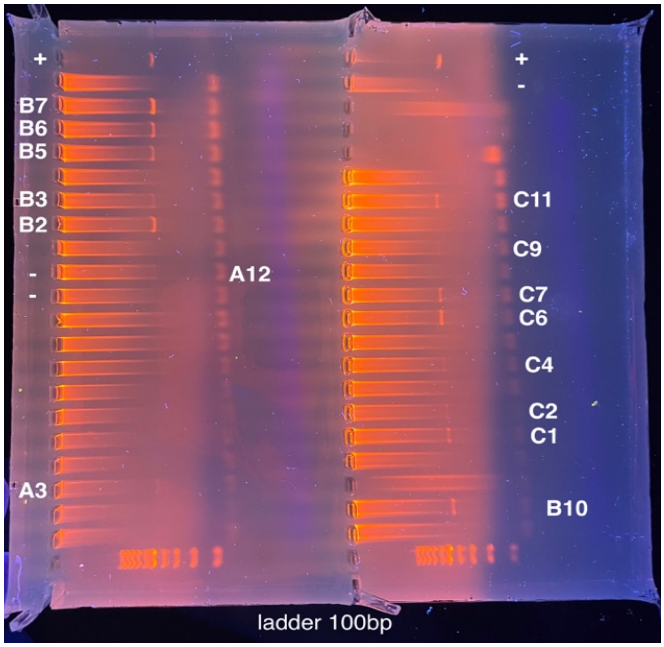

Figure 19. **Samples positive for *Borrelia burgdorferi*** have a glowing band. The "+ "marks positive control.

4.3.1. Statistical Analyses of *Borrelia* Positive Samples

Those samples were taken from 19 different dogs with the following distribution in age groups (Figures 20 and 21). No ticks were positive in the Mature Adult and Senior age groups.

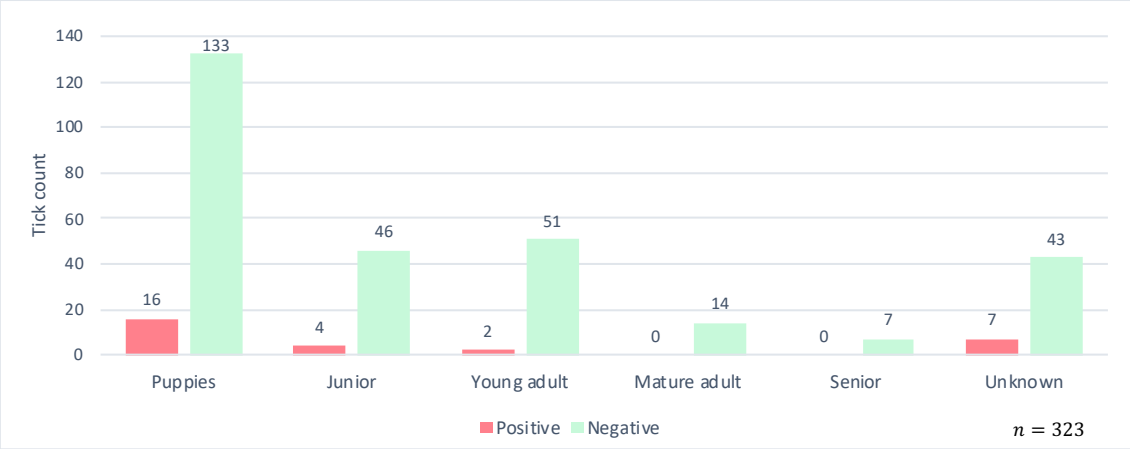

Figure 20. Distribution of positive and negative ticks analyzed for *Borrelia burgdorferi* in different age groups.

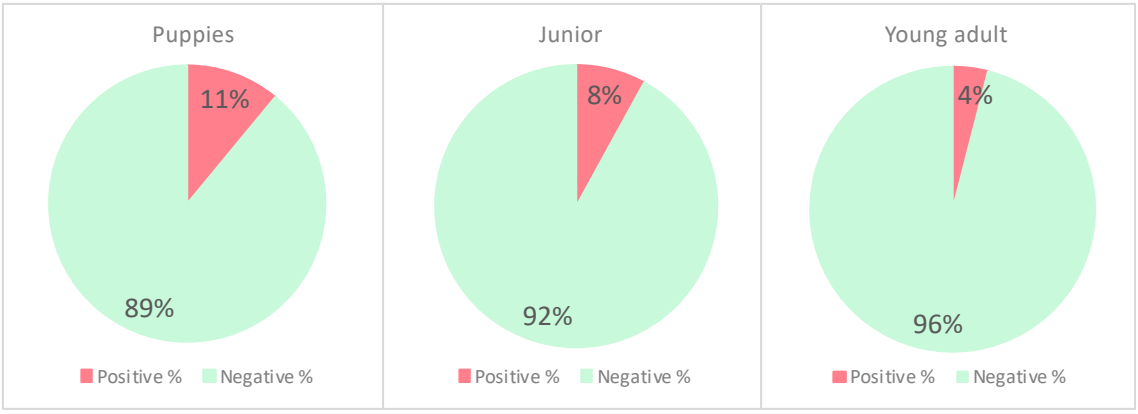

Figure 21. Percentage of positive and negative ticks analyzed for *Borrelia burgdorferi* in age groups that had at least one positive sample.

The following figures show the number of positive ticks in the total number of every tick species (Figure 22) and the percentage in every species (Figure 23). The species of other 23 ticks was not identified. The highest percentage of ticks positive for borrelia was found in *Rhipicephalus turanicus* however it was not statistically significant.

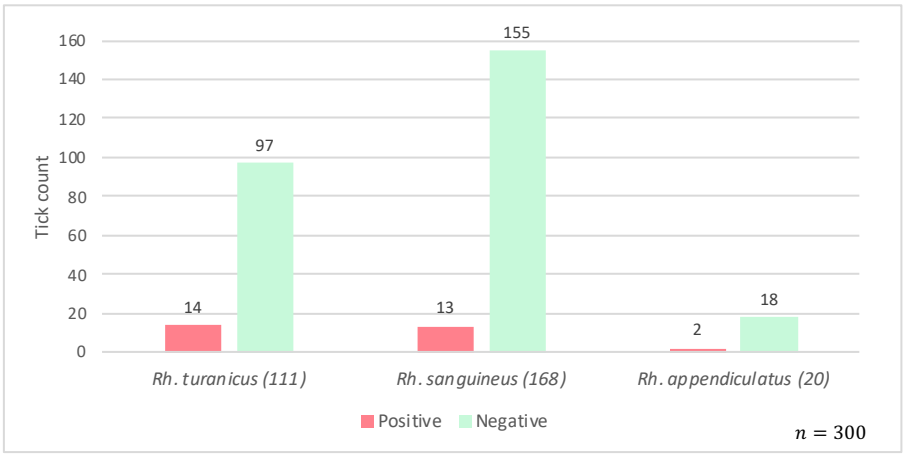

Figure 22. Distribution of positive and negative ticks analyzed for *Borrelia burgdorferi* in different tick species.

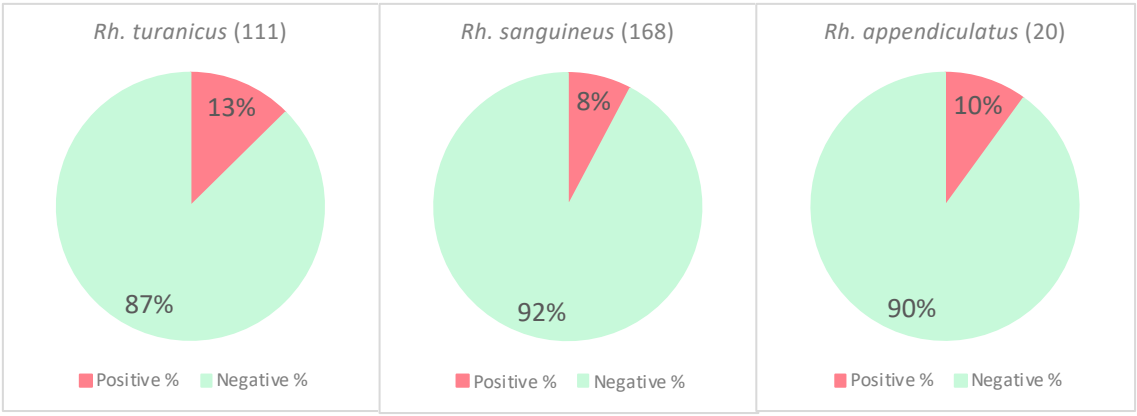

Figure 23. Percentage of positive and negative ticks analyzed for *Borrelia burgdorferi* in different tick species.

Figure 23 shows positive ticks in pet and street dogs. There were some dogs whose status was unknown and they were not included in this statistic. There was a statistically significant difference found with p=0.0084.

Figure 24. Distribution of positive and negative ticks analyzed for *Borrelia burgdorferi* according to the status of the dog.

The overall results show that street dogs and crossbred dogs are significantly more infested by ticks and also that street dogs have a higher probability to be infected by borrelia. Differences between all other categories were not found to be statistically significant.

5. Discussion

There are no other data about tick-borne pathogens from Aruba published in peer-reviewed journals, therefore it is difficult to compare the current results with past results from research on the island.

Rhipicephalus sanguineus species was the most widely identified tick and it has also been the only tick found in some previous research on Aruba and Curaçao and is reported to be very widespread all over the Caribbean islands. The other two species found on Aruba in this study, *Rhipicephalus turanicus* and *Rhipicephalus appendiculatus,* differ highly from the past results as well as from the results from other islands and Latin America. The theoretical presence of *Rhipicephalus turanicus* tick has been discussed in a study on livestock (Li et al. 2015) however, it has not been scientifically confirmed. The distribution of *Rhipicephalus appendiculatus* has been reported on the African continent (Spickler 2009; Njaa 2017). To confirm the presence of the two novel tick species found on Aruba, molecular sequencing should be carried out.

The only research that collected information about dogs with ticks, as well as dogs without ticks, was Klarenbeek (2010) in a study conducted on Curaçao. There were 129 dogs with ticks and 71 dogs without ticks. That is 64.5 % of dogs with ticks and 35.5 % of dogs without a tick. In this study, results showed that 8.5 % had ticks and 91.5 % were tick-free. In both studies, the examined dogs were patients of a veterinary clinic, therefore it is unclear why there can be such an important difference. However, these studies were conducted more than 10 years apart from each other, hence we can suggest that the general knowledge about acaricides has improved and the frequency of their usage has increased over the past years.

One statistically significant difference was found in tick presence between pets and street dogs. This result was rather expected as street dogs are usually severely neglected compared to pet dogs that receive regular treatment from their owners, including the administration of acaricide.

Another statistically significant difference was found in tick presence when the dogs were divided into two groups: purebred and crossbred ones. This could be

influenced by the dog's genetics although it is also apparent that, unlike the purebred dogs, crossbreeds are more likely to be street dogs and therefore have a higher susceptibility to ticks. No other statistically significant differences were found in the next categories (between male and female dogs, different age intervals, locations, and dog breed) although we expected to find some, at least between the regions of the island. Each region has different socio-economic conditions and in certain areas the level of animal care is low, which could suggest a higher risk for tick infestation and hence also pathogen transmission (Pesapane 2019). San Nicolas is the region considered the most socially disadvantaged and abounding in free-roaming dogs. Also, our artificially created category "Oranjestad foundation/shelter" was expected to have higher rates of tick infestation because dogs brought to those facilities are very recently taken from the streets of the entire island, regrettably often sick and highly infested with ticks. Compared to the study of Louly et al. (2009) who suggest that the Cocker Spaniel breed was more infested by ticks than other breeds, there was no difference in tick infestation between dog breeds examined on Aruba. However, it is important to mention that although this study analyzed a broad range of breeds, each of them was represented by low number of individuals.

No statistically significant difference was found either between the categories of last administration of acaricide product. It would have been expected that dogs that have never received any product against ticks would have a higher tick infestation, unlike dogs that receive their treatment regularly and their last dose was administered in less than 3 months from the sample collection. Our results may seem confusing, but there is an important information that should be mentioned – it is unknown if the owners have administered the correct dose of the product to their dog. Products against ticks tend to be very expensive for many dog owners which could cause them to buy a less expensive product that is meant for a smaller dog breed or divide one tablet between several dogs. This could result in an underdose of the active substance and hence not diminish the number of ticks found on the dog. Also, some dogs were administered the product on the same day on which they visited the clinic and they were automatically put in the category of dogs who received the product in less than one month. It was not possible to track back the exact information and therefore it is

possible that the product has not yet started working and thus possibly diminish the number of ticks feeding on the dog.

Contrary to most of the results found in the Caribbean area, no sample in this study tested positive for *Anaplasma platys*. I did not have a positive control containing the *Anaplasma platys* strain from this area, therefore I cannot say (based on experimental results), that the PCR reaction I used is able to detect the strains of *Anaplasma platys* which circulates in the Caribbean. Nevertheless, the reaction was prepared following a protocol by Fernandes (2017) which worked well. It would be rather expected to find at least some positive samples for this bacterium since there is quite high prevalence on the other islands and mainland countries. The PCR results showed a prevalence of 9.8 % in ticks from Cuba (Silva et al. 2016) but 0 % in ticks from Venezuela (Huang et al. 2005). Other prevalence from dog blood samples analyzed by PCR was 6.3 % in Haiti, 11% in St. Kitts, 16 % in Cuba, 16 % in Venezuela, 19.2 % in Grenada, and reaching up to 24 % in certain cities in Colombia (Huang et al. 2005; Yabsley et al. 2008; Kelly et al. 2013; Silva et al. 2016; Starkey et al. 2016; Pesapane 2019). On Curaçao, the prevalence was reported to be 27 % using Snap 4Dx tests only (Westra 2012). Although most of those analyses were done on blood samples, considering the above-mentioned prevalence, it is quite implausible that it would be 0 % on Aruba after analyzing 300 tick samples. However, my results could be justified by the similar result of Huang et al. (2005). This analysis should be repeated using an eligible positive control sample (that we would like to acquire from the studied area).

Twenty-nine ticks of *Rhipicephalus* species were found to be positive for *Borrelia burgdorferi* sensu lato. This tick is not known to be a common vector of Lyme disease; however, it has been reported by Morshed et al. (2021). As *Borrelia* occurrence is not limited only to the temperate zones, as it is often believed, it could be very possibly present on Aruba. No official reports on Lyme disease in humans on Aruba were found, therefore it becomes difficult to assess its real presence on the island. The Snap 4Dx test, which is widely used, shows the presence of *Borrelia* antibodies, but it has never been recorded to show positive results at veterinary clinics (personal communication with the veterinarians and nurses at the clinics, 2020). The particular borrelia species from these positive samples should be determined by sequencing in the future.

The finding of 29 ticks positive for *Borrelia burgdorferi* is a very interesting result considering the zoonotic potential of this pathogen. Dogs are the brown dog tick's primary host; however, the ticks can easily feed on humans as those two tend to live close together. The whole Caribbean area seems to be rather Lyme disease-free (McCown et al. 2013; McCown et al. 2014; McCown et al. 2015; Starkey 2016; Maggi & Krämer 2019; Cotes-Perdomo et al. 2020). Nevertheless, Rodriguez et al. (2012) discussed its possible presence in people from Cuba and there were also some cases of positive findings in dogs, e.g., Montenegro et al. (2017) found one positive dog in Costa Rica and Yabsley et al. (2008) found two positive dogs on Grenada. However, those had an important travel history that suggests a denial of autochthonous infection. The travel history of the Arubian dogs whose ticks were positive for *Borrelia* is currently unknown but is in the process of investigation for pet dogs. The probability that the street dogs with positive ticks have traveled outside Aruba is highly unlikely. Westra (2010) states that two borrelia positive dogs found on Curaçao are a false-positive result, due to the belief that this bacterium is only transmitted by the tick species *Ixodes ricinus* which has never been found on Curaçao. I decline this assertion as this tick is not the only borrelia vector and many other species were found to be carrying *Borrelia burgdorferi,* e.g., *Ixodes pacificus, Ixodes angustus, Dermacentor reticulatus,* and *Rhipicephalus sanguineus* (Grochowska et al. 2021; Morshed et al. 2021).

Toledo Vieira et al. (2013) and Barrantes-Gonzalez et al. (2018) suggested that disease prevalence was higher in male dogs, older dogs, and mixed breed dogs. Results of my study on Aruba showed a higher disease prevalence only in street dogs. These results can also be confirmed by a study conducted in Barranquilla (McCown et al. 2014), in which it was found that 90 % of street dogs were positive for some pathogen compared to only 51 % positivity in pet dogs.

As for the hypotheses, we can accept all alternative hypotheses.

1. H_{01}: ~~The only tick species on Aruba is *Rhipicephalus sanguineus.*~~

   H_{A1}: There are other tick species on Aruba.

2. H_{02}: ~~All dogs on Aruba have the same risk of getting infested by ticks.~~

   H_{A2}: There are specific risk factors that presuppose tick infestation.

3. H_{03}: ~~*Borrelia burgdorferi* is not present on Aruba.~~

   H_{A3}: *Borrelia burgdorferi* is present on Aruba.

Despite all the mentioned studies, the research on tick-borne pathogens, particularly *Borrelia burgdorferi*, in dogs in the Caribbean remains very scarce and should not be underrated.

6. Conclusions

Three different tick species were found on Aruba in this study using morphological identification, namely, *Rhipicephalus sanguineus, Rhipicephalus turanicus* and *Rhipicephalus appendiculatus*. The two latter species are a novel finding as they have not been reported to feed on dogs in the Caribbean before. As mentioned in the Discussion, a molecular confirmation using sequencing is needed to confirm the exact tick species identification.

Spirochetes of *Borrelia burgdorferi* sensu lato were found by PCR method in 29 ticks collected from 19 different dogs. This pathogen is not commonly reported in the Caribbean, therefore more testing should be done for borrelia presence in dogs as well as humans. The particular borrelia species from these positive samples should be determined by genetic sequencing.

Street dogs and mix breed dogs were found significantly more infested by ticks than pet and purebred dogs. Ticks collected from crossbred dogs had significantly higher chance to be infected by borrelia compared to those from purebred dogs. Differences between all other monitored parameters were not statistically significant.

These results can serve as an important baseline for the Arubian authorities and veterinarians for the assessment of the current situation of occurrence of ticks and the tick-borne diseases and their zoonotic potential as this issue had not been previously addressed and investigated.

7. References

- Allen CD. 2017. World Geomorphological Landscapes: Landscapes and Landforms of the Lesser Antilles. Springer International Publishing AG, Salt Lake City.
- Barrantes-Gonzalez AV, Jimenez-Rocha AE, Romero-Zúñiga JJ, Dolz G. 2018. Serology, molecular detection and risk factors of *Ehrlichia canis* infection in dogs in Costa Rica. Ticks and Tick-borne Diseases **7**: 1245–1251.
- Basu AK, Charles RA. 2017. Ticks of Trinidad and Tobago – An Overview. Academic Press, London.
- Bekker JP. 1996. The Mammals Of Aruba (Mammalia: Chiroptera, Rodentia, Lagomorpha). Vereniging voor Zoogdierkunde en Zoogdierbescherming (VZZ), Veere.
- Berschauer DP, Ros LG. 2014. A survey of mollusks collected in Aruba. The Festivus **45** (3): 54-62.
- Boer BAd. 2001. Nos Bestianan – Onze Dieren – Our Animals. Curaçao, Bonaire, Aruba. Stichting Dierenbescherming Curaçao, Curaçao.
- Bool PH, Sutmoller P. 1957. *Ehrlichia canis* infections in dogs on Aruba (Netherlands Antilles). Journal of the American Veterinary Medical Association **130**: 418–420.
- Britannica, The Editors of Encyclopaedia. 2020. Lesser Antilles. Encyclopedia Britannica. Available from https://www.britannica.com/place/Lesser-Antilles (accessed in December 2021).
- Carrade DD, Foley JE, Borjesson DL, Sykes JE. 2009. Canine granulocytic anaplasmosis: a review. Journal of Veterinary Internal Medicine **23**: 1129-1141.
- CDC. 2022. Life cycle of *Rhipicephalus sanguineus* and the transmission of *Rickettsia rickettsii* (the causative agent of Rocky Mountain Spotted Fever). Available from https://www.cdc.gov/ticks/pdfs/Rhipicephalus-sanguineus-LifeCycle.pdf (accessed in March 2022).

• Climate Change and Infectious Diseases Group. 2006. World Map of the Köppen-Geiger climate classification updated. High resolution map and data for Google Earth [software], June 2006, updated version March 2007. Available from http://koeppen-geiger.vu-wien.ac.at/present.htm (accessed in November 2021). System requirements: Windows/ Mac OS.

• Cotes-Perdomo AP, Oviedo A, Castro LR. 2020. Molecular detection of pathogens in ticks associated with domestic animals from the Colombian Caribbean region. Experimental and Applied Acarology **82** (1): 137-150.

• Coutinho MT, Bueno LL, Sterzik A, Fujiwara RT, Botelho JR, De Maria M, Genaro O, Linardi PM. 2005. Participation of *Rhipicephalus sanguineus* (Acari: Ixodidae) in the epidemiology of canine visceral leishmaniasis. Veterinary Parasitology **128**: 149–155.

• CVBD Maps Elanco. 2021. Occurrence maps. Available from https://cvbd.elanco.com/cvbd-maps (accessed in March 2021).

• Dantas-Torres F. 2007. The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): From taxonomy to control. Veterinary Parasitology **152**: 173-185.

• Davoust B, Drancourt M, Boni M. 1999. Survey of seroprevalence of *Bartonella vinsonii, Ehrlichia canis* and *Coxiella burnetii* in dogs in southeast France, French Guyana, Martinique, Senegal, Ivory Coast and Sudan. Abstract 232B in EUWOG-ASR Joint Meeting, Marseille, France.

• Egenvall AE, Bjoersdorff A, Lilliehook L, Engvall EO, Karlstam E, Artursson K, Hedhammar A, Gunnarsson A. 1998. Early manifestations of granulocytic ehrlichiosis in dogs inoculated experimentally with a Swedish *Ehrlichia* species isolate. Veterinary Record **143**: 412-417.

• Egenvall AE, Hedhammar AA, Bjoersdorff AI. 1997. Clinical features and serology of 14 dogs affected by granulocytic ehrlichiosis in Sweden. Veterinary Record **140**: 222-226.

• Fernandes M. 2017. Identificação molecular da *Ehrlichia canis*, *Babesia* spp., *Anaplasma platys* e *Hepatozoon* spp. em cães anêmicos atendidos no Hospital Veterinário da UFPB [MSc. Thesis]. Universidade Federal da Paraíba, Areia.

• Georges K, Ezeokoli CHD, Newaj-Fyzul A, Campbell M, Mootoo N, Mutani A, Sparagano OAE. 2008. The application of PCR and reverse line blot hybridization to detect arthropod-borne hemopathogens of dogs and cats in Trinidad. Animal Biodiversity and Emerging Diseases **1149**: 196-199.

• Gondard M, Cabezas-Cruz A, Charles RA, Vayssier-Taussat M, Albina E, Moutailler S. 2017. Ticks and Tick-Borne Pathogens of the Caribbean: Current Understanding and Future Directions for More Comprehensive Surveillance. Frontiers in Cellular and Infection Microbiology **7**: 490. DOI: 10.3389/fcimb.2017.00490.

• Granick JL, Armstrong PJ, Bender JB. 2009. *Anaplasma phagocytophilum* infection in dogs: 34 cases (2000-2007). Journal of the American Veterinary Medical Association **234**: 1559-1565.

• Grochowska A, Dunaj J, Pancewicz S, Czupryna P, Majewski P, Wondim M, Tryniszewska E, Moniuszko-Malinowska A. 2021. Detection of *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum* and *Babesia* spp. in *Dermacentor reticulatus* ticks found in the city of Bialystok, Poland – first data. Experimental and Applied Acarology **85** (1): 63-73.

• Harrus S, Perlman-Avrahami A, Mumcuoglu KY, Morick D, Eyal O, Baneth G. 2011. Molecular detection of *Ehrlichia canis*, *Anaplasma bovis*, *Anaplasma platys*, *Candidatus midichloria mitochondrii* and *Babesia canis vogeli* in ticks from Israel. Clinical Microbiology and Infection **17**: 459-463.

• Huang H, Unver A, Perez MJ, Orellana NG, Rikihisa Y. 2005. Prevalence and molecular analysis of *Anaplasma platys* in dogs in Lara, Venezuela. Brazilian Journal of Microbiology **36**: 211-216.

• Jochim MJ. 2017. Leeward Islands #118 (1949). A Stamp a Day. Available from https://stampaday.wordpress.com/2017/03/26/leeward-islands-118-1949/ (accessed in January 2022).

• Kelly PJ, Xu CH, Lucas H, Loftis A, Abete J, Zeoli F, Stevens A, Jaegersen K, Ackerson K, Gessner A, Kaltenboeck B, Wang CH. 2013. Ehrlichiosis, Babesiosis, Anaplasmosis and Hepatozoonosis in Dogs from St. Kitts, West Indies. PLoS ONE 8 (e53450) DOI: 10.1371/journal.pone.0053450.

• Klarenbeek MM. 2010. *Rhipicephalus sanguineus, Ehrlichia canis* and current tick control methods on Curaçao [Research report]. Utrecht Centre of Tick-borne Diseases, Utrecht.

• Kohsiek LHM, Hulsbergen CH, Terwindt JHJ. 1987. Beach erosion along the west coast of Aruba, Netherlands Antilles. Journal of Coastal Research **3** (1): 37–53.

• Kottek M, Grieser J, Beck C, Rudolf B, Rubel F. 2006. World Map of the Köppen-Geiger climate classification updated. Meteorologische Zeitschrift **15**: 259-263.

• Lepage D. 2022. Checklist of the birds of Aruba. Avibase, the world bird database. Available from https://avibase.bsc-eoc.org/checklist.jsp?region=AW (accessed in January 2022).

• Li J, Kelly P, Zhang J, Xu CH, Wang CH. 2015. Development of a pan-Babesia FRET-qPCR and a survey of livestock from five Caribbean islands. BMC Veterinary Research **22**: 246 (2015). DOI: 10.1186/s12917-015-0560-0

• Louly CCB, Soares S, Silveira D, Neto O, Silva A, Borges L. 2009. Differences in the susceptibility of two breeds of dogs, English cocker spaniel and Beagle, to *Rhipicephalus sanguineus* (Acari: Ixodidae). International Journal of Acarology **35**: 25–32.

• Maggi RG, Krämer F. 2019. A review on the occurrence of companion vector-borne diseases in pet animals in Latin America. Parasites & Vectors **12:** 145 (2019). DOI: 10.1186/s13071-019-3407-x.

• McCown ME, Alleman A, Sayler KA, Chandrashekar R, Thatcher B, Tyrrell P, Stillman B, Beall M, Barbet AF. 2014. Point prevalence survey for tick-borne pathogens in military working dogs, shelter animals, and pet populations in northern Colombia. Journal of Special Operations Medicine **14**: 53-57.

• McCown ME, Monterroso VH, Cardona W. 2015. Monitoreo de *Ehrlichia canis, Anaplasma phagocytophilum, Borrelia burgdorferi y Dirofilaria immitis* en perros de tres ciudades en Colombia. Revista CES Medicina Veterinaria y Zootecnica **10**: 224-231.

• McCown ME, Opel T, Grzeszak B. 2013. Vector-Borne Disease Surveillance in Puerto Rico: Pathogen Prevalence Rates in Canines – Implications for Public Health and the U.S. Military – Applying the One Health Concept. Journal of Special Operations Medicine **13** (2): 58-62.

• Montenegro VM, Bonilla MC, Kaminsky D, Romero-Zúñiga JJ, Siebert S, Krämer F. 2017. Serological detection of antibodies to *Anaplasma* spp., *Borrelia burgdorferi* sensu lato and *Ehrlichia canis* and of *Dirofilaria immitis* antigen in dogs from Costa Rica. Veterinary Parasitology **236**: 97–107.

• Moreta V. 2009. Examination of the Diagnostic and Therapeutic Effectivity among Dogs Suspected of an *Ehrlichia canis* infection on the Island of Aruba [Research report]. Utrecht Centre of Tick-borne Diseases, Utrecht.

• Morshed MG, Lee MK, Boyd E, Mak S, Fraser E, Nguyen J, Dykstra EA, Otterstatter M, Henry B, Galanis E. 2021. Passive Tick Surveillance and Detection of *Borrelia* Species in Ticks from British Columbia, Canada: 2002-2018. Vector Borne Zoonotic Diseases **21** (7): 490-497.

• Njaa BL. 2017. The Ear. Pages 1223-1264 in Zachary JF, editor. Pathologic Basis of Veterinary Disease (Sixth Edition). University of Illinois, Illinois.

• Nuttall GHF. 1915. Observations on the biology of Ixodidae. Parasitology **7**: 408–456.

• Oduber M, Ridderstaat J. 2016. Impacts of Cyclic Patterns of Climate on Fluctuations in Tourism Demand: Evidence from Aruba. Journal of Tourism Research & Hospitality **5**: 2 (e1000159) DOI: 10.4172/2324-8807.1000159.

• PAHO. 2012. Aruba. Health in the Americas, 2012 Edition: Country Volume. Pan American Health Organization. Available from: https://www3.paho.org/salud-en-las-americas-2012/index.php?option=com_content&view=article&id=19:aruba&Itemid=130&lang=en (accessed in January 2020).

• PAHO. 2017. Health in the Americas+, 2017 Edition. Summary: Regional Outlook and Country Profiles. Pan American Health Organization, Washington, D.C.

• Palmas C, Bortoletti G, Conchedda M, Contini C, Gabriele F, Ecca AR. 2001. Study on immunobiology in ectoparasites of public health interest: *Rhipicephalus sanguineus*. Parassitologia **43**: 29–35.

• Pegram RG, Clifford CM, Walker JB, Keirans JE. 1987. Clarification of the *Rhipicephalus sanguineus* group (Acari, Ixodoidea, Ixodidae). I. R. sulcatus Neumann, 1908 and R. turanicus Pomerantsev, 1936. Systematic Parasitology **10**: 3–26.

• Pesapane R, Foley J, Thomas R, Castro LR. 2019. Molecular detection and characterization of *Anaplasma platys* and *Ehrlichia canis* in dogs from northern Colombia. Veterinary Microbiology **223**: 184-189.

• Schuit DM. 2010. *Rhipicephalus sanguineus*, preventie en tekenbestrijding in het kader van *Ehrlichia canis* op Aruba [Research report]. Utrecht Centre of Tick-borne Diseases, Utrecht.

• Silva CBd, Santos HA, Navarrete MG, Uzedo Ribeiro CCD, Gonzalez BC, Zaldivar MF, Pires MS, Peckle M, Costa RLd, Vivas Vitari GL, Massard CL. Molecular detection and characterization of *Anaplasma platys* in dogs and ticks in Cuba. Ticks and Tick-borne Diseases **7** (5): 938-944.

• Sonenshine DE, Roe RM. 2014. Biology of Ticks. Oxford University Press, New York.

• Spickler, AR. 2009. *Rhipicephalus appendiculatus*. CFSHP. Available from http://www.cfsph.iastate.edu/DiseaseInfo/ factsheets.php (accessed in March 2022).

• Spickler, AR. 2021. Lyme Disease. CFSPH. Available from cfsph.iastate.edu/diseaseinfo/factsheets/ (accessed in January 2021).

• Spruit L. 2012. Onderzoek naar het voorkomen van de teek *Rhipicephalus sanguineus*, de tekenbestrijding bij de hond en het voorkomen van teken bij bijzondere dieren op het eiland Curaçao [Ph.D. Thesis]. Universiteit Utrecht, Utrecht.

• Starkey LA, Newton K, Brunker J, Crowdis K, Edourad EJP, Meneus P, Little SE. 2016. Prevalence of vector-borne pathogens in dogs from Haiti. Veterinary Parasitology **224**: 7-12.

• Straten van der G. 2008. A survey of ticks, *Ehrlichia canis* and current control methods on dogs on the island of Aruba [Research report]. Utrecht Centre of Tick-borne Diseases, Utrecht.

• Sykes JE, Foley JE. 2014. Chapter 29 – Anaplasmosis. Pages 290-299 in Sykes JE, editor. Canine and Feline Infectious Diseases. Saunders, Missouri.

• Toledo Vieira Fd, Lima Acosta IC, Fernandes Martins T, Moraes Filho J, Silva Krawczak F da, Mar Barbieri AR, Egert L, Fernandes DR, Ribeiro Braga F, Bahia Labruna M, Dietze R. 2018. Tick-borne infections in dogs and horses in the state of Espírito Santo, Southeast Brazil. Veterinary Parasitology **249**: 43–48.

• Vugteveen V. 2010. *Ehrlichia canis* infections on the island of Aruba [Research report]. Utrecht Centre of Tick-borne Diseases, Utrecht.

• Walker AR, Bouattour A, Camicas JL, Estrada-Peña A, Horak IG, Latif AA, Pegram RG, Preston PM. 2003. Ticks of Domestic Animals in Africa: a Guide to Identification of Species. Bioscience Reports, Edinburgh.

• Westra NAL. 2012. *Ehrlichia canis* infectie bij de hond op Curaçao. Een overzicht van het klinisch beeld, de diagnostiek, de effectiviteit van de therapie en de predisponerende factoren bij honden [Research report]. Utrecht Centre of Tick-borne Diseases, Utrecht.

• Wills MK, Kirby AM, Lloyd VK. 2018. Detecting the Lyme Disease Spirochete, *Borrelia Burgdorferi*, in Ticks Using Nested PCR. Journal of Visualized Experiments 132 (e56471) DOI: 10.3791/56471.

• Yabsley MJ, McKibben J, Macpherson CN, Cattan PF, Cherry NA, Hegarty BC, Breitschwerdt EB, O'Connor T, Chandrashekar R, Paterson T, Perea ML, Ball G, Friesen S, Goedde J, Henderson B, Sylvester W. 2008. Prevalence of *Ehrlichia canis, Anaplasma platys, Babesia canis vogeli, Hepatozoon canis, Bartonella vinsonii berkhoffii*, and *Rickettsia* spp. in dogs from Grenada. Veterinary Parasitology **151**: 279-285.

Appendices

List of the Appendices:

Appendix 1: Questionnaire

SAMPLE ID																	
DATE																	
CLINIC																	
DOG'S NAME																	
SEX																	
BREED																	
AGE																	
STATUS (pet/feral)																	
TOWN																	
SYMPTOMS																	
HEALTH HISTORY haematocrit/serology																	
N° of TICKS collected																	
TOTAL TICKS on dog																	
Feeding/**C**rawling																	
LOCATION OF TICK body part of the dog																	
TICK-CONTROL ✓/✘																	
Which tick control **PRODUCT**																	
LAST DOSE																	

Appendix 2: Geneaid manual for DNA extraction

Genomic DNA Mini Kit (Tissue)

For research use only

Sample: up to 30 mg of tissue (tailsnips, liver, kidney, brain, adipose tissue, earpunches, insects etc.)
Yield: 10-20 µg (0.5 cm of mouse tail, 20 mg of mouse liver), 20-50 µg (20 mg of mouse kidney)
Format: spin column
Time: within 30 minutes
Elution volume: 30-200 µl
Storage: dry at room temperature (15-25ºC)

Geneaid
CERTIFICATE NO. QAIC/TW/50077
ISO 9001:2008 QMS

Introduction

The Genomic DNA Mini Kit (Tissue) was designed specifically for purifying total DNA (including genomic, mitochondrial and viral DNA) from a variety of tissue and insect samples. The provided micropestle can efficiently homogenize tissue samples to shorten the time in the Lysis Step. Proteinase K and chaotropic salt are used to lyse cells and degrade protein, allowing DNA to be easily bound by the glass fiber matrix of the spin column. Once any contaminants have been removed, using a Wash Buffer (containing ethanol), the purified DNA is eluted by a low salt Elution Buffer, TE or water. The entire procedure can be completed without phenol/chloroform extraction or alcohol precipitation. The purified DNA (approximately 20-30 kb) is suitable for use in PCR or other enzymatic reactions.

Quality Control

The quality of the Genomic DNA Mini Kit (Tissue) is tested on a lot-to-lot basis by isolating genomic DNA from a 20 mg mouse liver sample. The purified DNA (more than 10 µg with an A260/A280 ratio of 1.8-2.0) is quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Contents

Component	GT004	GT050	GT100	GT300
GT Buffer	3 ml	30 ml	30 ml	75 ml
GBT Buffer	4 ml	40 ml	40 ml	75 ml
W1 Buffer	2 ml	45 ml	45 ml	130 ml
Wash Buffer* (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	25 ml (100 ml)	50 ml (200 ml)
Proteinase K** (Add ddH_2O)	1 mg (0.1 ml)	11 mg (1.1 ml)	11 mg x 2 (1.1 ml x 2)	65 mg (6.5 ml)
Elution Buffer	1 ml	30 ml	30 ml	75 ml
GS Columns	4	50	100	300
2 ml Collection Tubes	8	100	200	600
Micropestle	4	50	100	300

Order Information

Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	GDM010/25
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300
gSYNC™ DNA Extraction Kit	50/100/300 preps	GS050/100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Genomic DNA Maxi Kit (Plant)	10/25 preps	GPM010/25
GENEzol™ DNA Reagent Plant	100/200 rxns	GR100/200
Presto™ Mini gDNA Yeast Kit	100/300 preps	GBY100/300
Presto™ Mini gDNA Bacteria Kit	100/300 preps	GBB100/101/300/301
Geneius™ Micro DNA Extraction Kit	100/300 preps	GMB100/300
Presto™ Buccal Swab gDNA Extraction Kit	100/300 preps	GSK100/300
Presto™ 96 Well Blood gDNA Extraction Kit	4/10 x 96 preps	96GBP04/10
Presto™ 96 Well Plant gDNA Extraction Kit	4/10 x 96 preps	96GPP04/10

*Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
**Add ddH_2O (see the bottle label for volume) to prepare Proteinase K (vortex to dissolve and spin down) and store at 4ºC

Caution

GBT Buffer contains guanidine hydrochloride. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Genomic DNA Mini Kit (Tissue) Functional Test Data

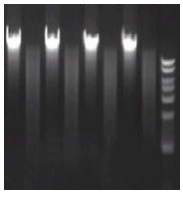

Figure 1. Genomic DNA from a variety of tissue samples was extracted using the Genomic DNA Mini Kit (Tissue). The purified genomic DNA (30-40 kb) was *Eco*RI digested and analyzed by electrophoresis on a 1% agarose gel.
1 = Mouse Liver
2 = Mouse Tail
3 = Fish Muscle
4 = Fruit Fly (Drosophila)
M = Geneaid 1 Kb DNA Ladder

Genomic DNA Mini Kit (Tissue) Protocol

IMPORTANT BEFORE USE

• Add ddH_2O (see the bottle label for volume) to prepare Proteinase K (vortex to dissolve and spin down) and store at 4ºC
• Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
• Additional requirements: microcentrifuge tubes, absolute ethanol, (optional) RNase A (10 mg/ml), ddH_2O

Tissue Dissociation	• Cut up to **30 mg of animal tissue (or 0.5 cm of mouse tail)** then transfer it to a 1.5 ml microcentrifuge tube. NOTE: If tissue has a higher number of cells (e.g. spleen or liver), reduce the starting material to 10 mg. • Use the provided **Micropestle** to grind the tissue to a pulp. • Add **200 µl of GT Buffer** to the tube and homogenize the sample tissue by grinding. • Add **20 µl of Proteinase K** to the sample mixture then shake vigorously and incubate at 60ºC for 30 minutes. NOTE: During incubation, invert the tube every 5 minutes.
Step 1 Lysis	• Add **200 µl of GBT Buffer** then shake vigorously for 5 seconds. • Incubate at 60ºC for at least 20 minutes to ensure the lysate is clear. NOTE: During incubation, invert the tube every 5 minutes. If insoluble material is present following incubation, centrifuge for 2 minutes at 14-16,000 x g then transfer the supernatant to a new 1.5 ml microcentrifuge tube. At this time, preheat the required **Elution Buffer** (200 µl per sample) to 60ºC (for Step 4 DNA Elution). **Optional Step: RNA Degradation** (If RNA free gDNA is required, perform this optional step) • Following 60ºC incubation, add 4 µl of RNase A (10 mg/ml) to the sample lysate then shake vigorously. • Incubate at room temperature for 5 minutes.
Step 2 DNA Binding	• Add **200 µl of absolute ethanol** to the lysate then immediately shake vigorously for 10 seconds. NOTE: If precipitate appears, break it up as much as possible with a pipette. • Place a **GS Column** in a **2 ml Collection Tube**. • Transfer the mixture (including any precipitate) to the **GS Column** then centrifuge at 14-16,000 x g for 2 minutes. • Discard the **2 ml Collection Tube** then transfer the **GS Column** to a new **2 ml Collection Tube**.
Step 3 Wash	• Add **400 µl of W1 Buffer** to the **GS Column** then centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through then place the **GS Column** back in the **2 ml Collection Tube**. • Add **600 µl of Wash Buffer** (make sure ethanol was added) to the **GS Column**. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through then place the **GS Column** back in the **2 ml Collection Tube**. • Centrifuge for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 4 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approx. 200 µl. • Transfer the dried **GS Column** to a clean 1.5 ml microcentrifuge tube. • Add **100 µl of pre-heated Elution Buffer** or TE to the CENTER of the column matrix. • Let stand for at least 5 minutes to ensure the **Elution Buffer** or TE is completely absorbed. • Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	**Too much tissue was used** • If using more than 30 mg of tissue, separate into multiple tubes. **Sample tissue was not lysed completely** • Add additional Proteinase K and extend the incubation time in the Lysis Step. • Following the Lysis Step, centrifuge for 2 minutes at 14-16,000 x g to remove sample debris. Transfer the supernatant to a new microcentrifuge tube and proceed with the DNA Binding Step. **Precipitate was formed at DNA Binding step** • Reduce the sample material. • Following ethanol addition, break up any precipitate as much as possible prior to loading GS Column.
Low Yield	**Sample tissue was not lysed completely** • Add additional Proteinase K and extend the incubation time in the Lysis Step. **Column was clogged at DNA Binding step** • Following the Lysis Step, remove the insoluble debris by centrifugation. • Prior to loading the column, break up the precipitate in the ethanol-added lysate. **Incorrect DNA Elution Step** • Ensure that the Elution Buffer or TE is added to the center of the GS Column matrix and is absorbed completely. **Incomplete DNA elution** • Elute twice to increase the DNA recovery.
Eluted DNA does not perform well in downstream applications	**Residual ethanol contamination** • Following the Wash Step, dry the GS Column by centrifuge at 14-16,000 x g or incubate at 60ºC for 5 minutes. **RNA/Protein contamination** • Perform optional RNA Degradation step/reduce the sample amount. **Genomic DNA was degraded** • Use fresh samples or freeze fresh samples in liquid nitrogen immediately and store at -80ºC.