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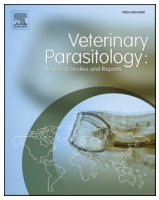

Original Article

Molecular screening of selected vector-borne pathogens circulating in owned dogs in the Caribbean archipelago of Guadeloupe (France)

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ABSTRACT

Vector-borne diseases represent a major health challenge, both because of the complexity of their control, their common zoonotic nature, or the pathology they can cause in the individual. In tropical areas, surveillance of these diseases is even more important, since the activity of vectors is usually continuous throughout the year. To develop effective prophylaxis and surveillance programs, it is important to know the identity and prevalence of these pathogens as well as their distribution in a given territory. In Guadeloupe, a French archipelago located in the Lesser Antilles of the Caribbean, no information exists about vector-borne diseases in companion animals. With this aim, blood samples were obtained from 46 owned dogs with outdoor access from five different veterinary clinics located in the two mainland islands, and the presence of DNA of the main canine vector-borne pathogens (CVBP) was investigated through diverse PCR protocols. At least one pathogen was detected in 30.4 % of the dogs. The most frequently detected CVBP was *Coxiella burnetii* (17.4 %), followed by *Dirofilaria immitis* (8.7 %), and *Candidatus* Mycoplasma haematoparvum, *Hepatozoon canis* and *Rickettsia* spp. (2.2 % in all cases). One dog was coinfected with *Candidatus* M. haematoparvum and *D. immitis*. All samples were negative for *Anaplasma* spp., *Ehrlichia* spp., *Bartonella* spp., *Borrelia burgdorferi* sensu lato, piroplasmids, and *Leishmania* spp. No significant differences in pathogen occurrence were observed between the two main islands or according to the dog's sex and age groups. This study contributes to filling a relevant gap in the knowledge of vector-borne diseases in the Caribbean.

1. Introduction

Canine vector-borne diseases (CVBDs) comprise a relevant and globally distributed group of disease agents (i.e., viruses, bacteria, protozoa, and helminths) affecting wild and domestic canids, transmitted by hematophagous arthropods such as ticks, fleas, lice, triatomines, mosquitoes, and sand flies (Otranto et al., 2009). The risk of canine vector-borne pathogen (CVBP) infection depends on several intrinsic and extrinsic factors, such as the breed, the animal's genetics, the presence of concomitant infections, its diet, travel between areas with different disease risks, the animal's environment and management (indoor/outdoor lifestyle, application of antiparasitic treatments…), the vector abundance, and the environmental conditions (Cevidanes et al., 2023; ESCCAP, 2012; Otranto et al., 2009). Because of their climatic characteristics and geographical attributes, tropical regions remain the most affected areas by CVBDs and account for more than two-thirds of the world's biodiversity, including insects and ticks, as well as a diversity of hosts involved in the circulation of CVBPs (Otranto et al., 2024). In addition, climatic change has caused a net geographic expansion of CVBDs in recent years, and warmer temperatures have indeed been shown to affect the behavior, physiological characteristics, and life cycle of vectors and pathogens (Thomson and Stanberry, 2022). The Caribbean is also the crossroad of intercontinental exchanges between the Americas, Europe, and Africa, which particularly encourages the

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introduction and spread of vectors and VBDs, especially through the movement of animals (through legal or illegal trade and bird migration) (Díaz-Sánchez et al., 2018; Molia et al., 2008). Despite the high prevalence of animal and zoonotic infectious diseases in tropical areas, limited epidemiological knowledge of the diversity of vectors and vector-borne pathogens, together with the lack of experience in the management of VBDs, limit the development of effective control strategies in the Caribbean (Gondard et al., 2020).

Information about the epidemiological situation of CVBDs in the Caribbean area is scarce and limited to a handful of islands. Studies carried out in Haiti, Grenada, Trinidad, St. Kitts, Cuba, and the Dominican Republic showed in dogs high prevalences of *Anaplasma* (mainly *A. platys*), *Babesia* (*B. vogeli* and *B. gibsoni*), *Hepatozoon canis* and *Dirofilaria immitis*; while other vector-borne pathogens have been found at a lower prevalence, such as *Bartonella henselae, Bartonella vinsonii* and hemoplasmas (Georges et al., 2008; Roblejo-Arias et al., 2022; Yabsley et al., 2008). A case of leishmaniosis due to *Leishmania martiniquensis* was detected in a human patient in Martinique (Liautaud et al., 2014). *Leishmania* sp. was also found in *Rhipicephalus microplus* ticks collected on cattle from Martinique (Gondard et al., 2020). By contrast, *Borrelia burgdorferi* sensu lato has never been reported in the Caribbean (Maggi and Krämer, 2019; Starkey et al., 2016).

The archipelago of Guadeloupe is a French territory located in the Caribbean with many connections, both touristic and commercial, with neighboring countries and territories, North America and Europe. In the past decades, the archipelago has suffered several outbreaks of emerging infectious diseases in both humans and animals (West Nile virus, Chikungunya, dengue, leptospirosis, etc.) (Gruel et al., 2021). Knowledge about VBP in Guadeloupe is restricted to large animals (Camus and Barre, 1995; Gondard et al., 2017), however, no information is available on the CVBPs circulating in Guadeloupe. Veterinarians diagnosed certain CVBDs in dogs, such as ehrlichiosis and dirofilariosis, based on clinical signs and/or commercial diagnostic tests (Oliver Ibene, pers. comm., 2023), but no molecular surveys have been performed to date to confirm those observations and to identify the etiological agents. Knowing the exact etiology of the CVBDs in a given area is essential to carry out accurate differential diagnosis, and choosing the correct diagnostic tools and treatments, and to establish appropriate control measures. In consequence, this work aims to provide molecular and epidemiological data on the CVBPs circulating in domestic dogs in the French archipelago of Guadeloupe.

2. Materials and methods

The Guadeloupean archipelago, in the Caribbean region (16°17′ N, 61°29′ W), consists of more than 700 islands, islets, reefs, and cays. The archipelago can be divided into a "continental" part, composed of the two major islands, Grande-Terre (GT) and Basse-Terre (BT), separated by a narrow arm of the sea, and in a group of annexed islands. Guadeloupe average annual temperature and relative humidity of about 27 °C and 80 %, respectively. Fluctuations in temperature and humidity vary especially between the dry season, from January to June, and the rainy season, from July to December. According to Gompper (2014), the domestic dog population rounded 115,800 individuals in Guadeloupe in 2011.Veterinary clinicians of Guadeloupe were approached and requested to take a sample during their practice. Clinics were chosen to cover the entire territory of the continental part of the archipelago. Five clinics agreed to participate in the study: three from the GT island and two from the BT island (Fig. 1). Due to strict time limitations to perform the survey, dogs were selected based on convenience sampling. Informed consent was signed by every dog owner. Between the 15th and 30th of June 2023, 46 dogs were sampled. Of these, 23 were males and 23 females. Eleven dogs were younger than one year, and 35 were adults (Supplementary File 1). Blood was collected from the jugular or the cephalic veins and a drop (about 100 uL) was applied to an FTA™ Nucleic Acid Collection Cards (Whatman, Maidstone, Kent, UK), air-dried, and placed into Eppendorf tubes. These filter papers ensure long-term storage, stabilization, and protection of nucleic acids at room temperature. For each animal, the following epidemiological information was registered: age, sex, place of residence, parasitic treatments,

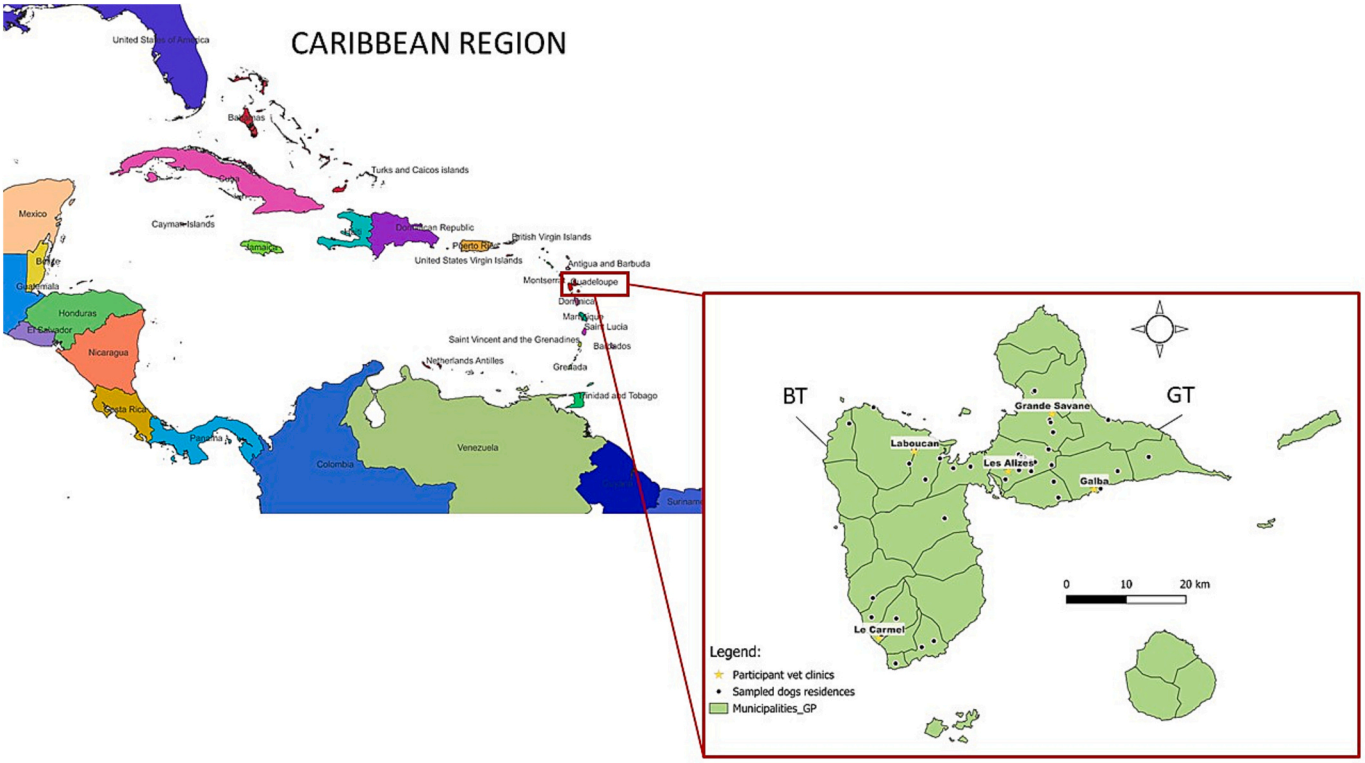

Fig. 1. The Guadeloupe archipelago with the origin of the sampled dogs and name of the participant veterinary clinics. GT: Grande Terre; BT: Basse Terre.

vaccines, previous clinical history, and presence of ectoparasites.

A circular punch of 1 mm in diameter of the blood spot was placed in a microtube for the DNA extraction process. A first cleaning step was done by incubating the punch in 180 µl of Tris-EDTA (10 mM/0,1 mM, pH 8) for 15 min at room temperature to remove preservative substances and possible PCR inhibitors from the sample. After a short spin and 15 min of incubation at room temperature, all the Tris-EDTA was removed and replaced by 180 µl of extraction medium. The extraction medium consisted of an aqueous solution of Chelex© sigma resin (5 % *w/v*). Tubes were then quickly vortexed and placed in a thermoblock for 10 min at 100 °C, and after that, the tubes were vortexed again and submitted to a second incubation at 100 °C for 25 min. In the end, the total volume of the DNA extraction medium was 180 µl for each sample. To verify the suitable DNA extraction of each sample, a verification PCR were carried out by amplifying a fragment of the gene MC1R (coding for the melanocortin1 receptor), present in all canine genomes (Table 1). The kit BIAN®-Taq Polymerase (IBIAN Technologies, Zaragoza, Spain) was used, and the reaction volume was 10 µl, including 5 µl of DNA, 1 µl of each primer (2 µM), 1 µl of 10× buffer (without Mg), 0.3 µl of Mg (50 mM), 0.8 µl of dNTPs (2.5 mM each), 0.06 µl of Taq Polymerase from IBIAN (5 U/µl), and 0.84 µl of distilled water.

The presence of DNA of *Rickettsia* sp., Anaplasmataceae, *Coxiella burnetii*, *Bartonella* sp., *Borrelia burgdorferi* sensu lato, hemotropic *Mycoplasma*, *Hepatozoon* sp., piroplasmids, *Leishmania* sp., and filarial nematodes was investigated by diverse protocols (Table 1). All conventional and nested PCR assays were run in a total reaction volume of 10 µl, using the 2XPCR Master Mix (Promega©), 0.5 µl of each primer at 10 µM, and 4 µl of DNA. PCR products were revealed by electrophoresis on a 1.5 % agar gel. For the detection of *Coxiella burnetii*, DNA samples were screened by Real-Time PCR using a TaqMan real-time PCR using the SsoAdvanced Universal Probes Supermix (Bio-Rad) and the conditions described in Tilburg et al. (2010).

Amplicons obtained from positive PCR protocols were sent for sequencing to MacroGen (Madrid, Spain). The primary genomic sequences obtained were visualized with Chromas©, and then compared with those available in GenBank® by BLAST analyses (http://www.ncbi.nlm.nih.gov/blast). Sequence alignments were performed using the ClustalW tool implemented in UGENE software (http://ugene.net) and Maximum Likelihood phylogenetic trees were generated using MEGA X software (Kumar et al., 2018). Based on the lowest Bayesian information criterion (BIC), the selected trees were constructed for each gene fragment by bootstrap analysis with 1000 replicates.

The occurrence was calculated as the ratio of the number of positive animals to the total number of dogs tested. For each CVBP, a sample was considered positive if amplification was observed in at least one PCR assay and was then confirmed by sequencing. The maximum possible prevalence for the undetected pathogens was calculated using WinEpi (WinEpi Program, n.d.). Differences between islands, sexes, and age groups in the overall occurrence of pathogens as well as for specific pathogens with occurrences higher than 10 % were tested using Fisher's exact test.

New sequences were uploaded to GenBank© with accession numbers PP965251-PP965254, PP980958, PP970336 and PP965183.

3. Results

All samples were positive for the MC1R gene. At least one CVBP was detected in 30.4 % of the dogs (Table 2, Supplementary File 1). Specifically, eight were found positive for *Coxiella burnetii* (17.4 %), four for Filariidae (8.7 %), and one for *Rickettsia* sp., *Hepatozoon* sp., and *Mycoplasma* sp. (2.2 % each). One dog was coinfected with *Candidatus* M. haematoparvum (CMhp) and *D. immitis*.

Molecular characterization of the COI gene of the four Filariidae-positive samples revealed sequences with 100 % identity with *D. immitis* sequences from a dog from Thailand (GenBank accession number: MK250756). Sequencing of the positive case of *Hepatozoon*

Table 1
PCR primers and protocols used to detect vector-borne pathogens in dogs, Guadeloupe.' = minutes, "= seconds, F = Forward, R = Reverse.

Target gene	PCR type	Primers F/R	Annealing	Fragment length (bp)
MC1R		ChocF/ ChocR	60,2 °C, 30"	450
Anaplasma spp. (16S rRNA)	Conventional PCR	16SF/16SR	42 °C, 30"	421
Anaplasma spp. (rpoB)	Conventional PCR	RpoBF/ RpoBR	55 °C, 45"	577
Anaplasma spp. (gltA)	Conventional PCR	F1b/ HG1085R	45 °C, 45"	459
Anaplasmatacae (16S rRNA)	Conventional PCR	16sR/16sD	62 °C, 30"	345
Rickettsia spp. (16S rRNA)	Conventional PCR	fd1/Rc16	54 °C, 30"	416
Rickettsia spp. (ompA)	Conventional PCR	OmpAF/ OmpAR	54 °C, 30"	650
Rickettsia spp. (ompB)	Conventional PCR	OmpBF/ OmpBR	53 °C, 30"	618
Rickettsia spp. (gltA)	Conventional PCR	GltAF/GltaR	45 °C, 45"	360
Mycoplasma spp. (16S rRNA)	Conventional PCR	Mycop16S rRNA-F/ Mycop16S rRNA-R	60 °C, 30"	384
Mycoplasma haemocanis (16S rRNA)	Conventional PCR	Mhf-OH-OK1/ Mycop-00CB-r1	58,4 °C, 45"	175
Candidatus Mycoplasma haematoparvum (16S rRNA)	Conventional PCR	M sp./C Mhp	56,5 °C, 30"	175
Bartonella spp. (ITS-1)	Conventional PCR	ITS1F/ITS1R	59 °C, 45"	675
Bartonella spp. (gltA)	Conventional PCR	BhCS871.p/ BhCS1137.n	51 °C, 45"	321
Bartonella spp. (rpoB)	Conventional PCR	rpoB 1400F Barto/rpoB 2300R Barto	53 °C, 45"	379
Borrelia burgdorferi group (23S—5S rRNA intergenic spacer regions)	Nested PCR	23SN1/ 23SC1 23SN2/ 5SCB	52 °C, 30″ 52 °C, 20"	380 225
Coxiella burnetii (IS1111)	Real-time PCR	QKF3/QKR3	60 °C, 1'	
Piroplasmida (18S rRNA)	Conventional PCR	PiroA/PiroB	58 °C, 30	360
Piroplasmida and *Hepatozoon* sp. (18S rRNA)	Conventional PCR	PiroF/PiroR	"64 °C, 45"	408
Hepatozoon spp. (18S rRNA)	Conventional PCR	HepaF/ HepaR	50 °C, 30"	574
Leishmania spp. (ITS-1)	Conventional PCR	ITSR/L58S	52 °C, 45"	300–350
Filariidae (ITS-2)	Conventional PCR	DIDRF/ DIDRR	60 °C, 30"	430–664
Filariidae (COI)	Conventional PCR	COIfilF/ COIfilR	52 °C, 45"	631

Table 2
Observed occurrences of vector-borne pathogens in dogs, Guadeloupe. Overall: proportion of dogs infected by at least one pathogen.

Detected pathogen	Observed occurrence (%)	95 % CI
Coxiella burnetii	17.39	[6.45; 22.34]
Dirofilaria immitis	8.70	[0.56; 16.83]
Candidatus M. haematoparvum	2,17	[0.00; 6.39]
Hepatozoon canis	2.17	[0.00; 6.39]
Rickettsia sp.	2.17	[0.00; 6.39]
Overall	30.43	[17.14; 43.73]

yielded a sequence with an identity of 99.81 % with published sequences of *H. canis* from dogs in Brazil (MT081050). Sequencing of the *Mycoplasma* amplicon showed 99.69 % homology with *Candidatus* Mycoplasma haematoparvum (CMhp) from a dog in Chile (KY117661). Phylogenetics confirmed the identity of these two pathogens (Supplementary File 2). Sequencing of the *Rickettsia* positive sample showed an identity of 96.76 %, with uncultured *Rickettsia* sp. from a sheep in Spain (KT733038) and with several fragments of *Rickettsia aeschlimannii* (KY233291, KY233290, MF098413.1, MW398879, MW398877). Unfortunately, none of the additional protocols for *Rickettsia* turned out positive, preventing us from characterizing this sample. No dog was positive for Anaplasmataceae, *Bartonella* sp., *B. burgdorferi* s.l., piroplasmids or *Leishmania* sp. For all these undetected pathogens, the maximum possible prevalence was 6.3 %.

No significant differences between islands, sexes, and age groups were found either for overall pathogen occurrence or for *C. burnetii* (in all cases, Fisher's $p > 0.05$). Out of the four dogs positive for *D. immitis*, one was already diagnosed with heartworm disease by a commercial antigenic test, and the co-infected dog was noticed with hematuria and coughs at the time of sampling. None of those was regularly treated against parasites. No relevant clinical history was reported for the two remaining positive dogs or the dogs positive for the other pathogens.

4. Discussion

The present work represents the first molecular study of CVBP in Guadeloupe, a Caribbean island highly connected with the rest of the Caribbean region, Europe, and the American continent. Identifying the CVBPs present in the archipelago is key to understanding the dynamic of such pathogens in the Caribbean, controlling them, and preventing outbreaks.

The present survey suffers from a small sample size, that might have affected the detection of some of the studied pathogens. Also, the selection of owned dogs may also have repercussion in the results, as those dogs usually receive prophylactic treatments and veterinary care, being less exposed to parasites and pathogens than feral dogs. Nevertheless, the dogs investigated were allowed to roam free, being more exposed to ticks, fleas and mosquitoes than those with permanent confinement. Finally, the use of conventional PCR protocols instead of real-time PCR protocols for the majority of the pathogens may also have affected the sensitivity of our survey.

Notwithstanding this, we observed a low pathogen variability among the sampled dogs and only one case of co-infection, which is generally observed in the Lesser Antilles but differs with larger islands, or close to the continent, and continental territories (Georges et al., 2008; Kelly et al., 2013; Maggi and Krämer, 2019; Starkey et al., 2016; Yabsley et al., 2008). This phenomenon can be associated with the negative diversity-isolation of the “island effect”, whereby continental territories and nearby islands are sources of biodiversity for the little islands, decreasing as we move away from the continent (Wang et al., 2022).

In the Caribbean, *C. burnetii* is mostly found in cattle, sheep, and goats. Nevertheless, the bacteria has been also identified in ticks collected on horses in Cuba (Noda et al., 2016), and one seropositive cat was found in St Kitts (Johnson et al., 2020), but no report of infected dogs has been done until now. Moreover, a study carried out among military dogs in Martinique, the Guadeloupe sister island, showed no evidence of *Coxiella* infection, while dogs from other French territories studied showed positivity, especially in French Guyana (Boni et al., 1998; Epelboin et al., 2023). Therefore, the present work is the first report of *C. burnetii* infection in dogs in the Caribbean. Although *C. burnetii* infection in dogs is generally asymptomatic, the zoonotic nature of this CVBP represents a public health concern. Surveys should be carried out all over the archipelago on domestic animals, especially ruminants and milk tanks, to prevent possible spillovers to humans.

All the Filariidae-positive samples were identified as *D. immitis*. In the Caribbean, *D. immitis* is very frequent in dogs (Maggi and Krämer, 2019; Starkey et al., 2016). Although the local veterinarians were aware of the presence of filarial parasites in the archipelago, the etiological agent was not identified until the present study. *Hepatozoon canis* is widely spread in the Caribbean islands and other tropical areas such as Brazil and Costa Rica (Kelly et al., 2013; Maggi and Krämer, 2019; Starkey et al., 2016; Yabsley et al., 2008). Guadeloupean veterinarians generally affirm that tick-borne diseases, such as hepatozoonosis, are generally less diagnosed in the past 20 years. Thus, this may explain why the observed occurrence in this work is lower than the mean prevalence reported in the rest of the Caribbean (around 9 %) (Kelly et al., 2013; Starkey et al., 2016; Yabsley et al., 2008), although this can be caused by the island effect previously mentioned or to the low sample size.

Regarding *Rickettsia* sp., only one positive sample was found, but it was not possible to carry out a full molecular characterization. The potentially identified species, *Rickettsia aeschlimannii*, which belongs to the Rickettsia spotted-fever group (SFG), has been reported in infected humans but never in other mammals, although it has been found in ticks collected on small ruminants, domestic dogs, and cattle in Pakistan (Majid et al., 2023). *Rickettsia aeschlimannii* has never been reported in the Caribbean, although other species from the SFG, such as *Rickettsia africae* and *Rickettsia conorii* has been found in dogs (Kelly et al., 2013; Maggi and Krämer, 2019). In Guadeloupe, *Rickettsia africae* and *Rickettsia conorii* has been reported in humans, cattle, and goats, but no other *Rickettsia* species has ever been reported (Gondard et al., 2020; Parola et al., 1999).

In the Caribbean, CMhp was reported in dogs from Cuba (Roblejo-Arias et al., 2022), and in a woman from Grenada (Maggi et al., 2013). The other canine hemoplasma, *M. haemocanis*, was not found in our sampling. This can be the result of true absence due to the island effect abovementioned, or the consequence of failing to reach the required sample size. Transmission routes of hemotropic Mycoplasmas are not well known. Although originally these were considered to be transmitted by ticks, other ways of transmission, such as via predation, fights, or vertically, cannot be excluded (Cevidanes et al., 2023; Maggi et al., 2013; Tasker, 2022). This incognita represents an obstacle to the sustainable development of effective control strategies. In Guadeloupe, the tick species suspected to be able to transmit hemoplasmas, such as *Rhipicephalus sanguineus* sensu lato, are present (Gondard et al., 2020; Tasker, 2022). Combined with the presence of feral dogs all over the territory, it should represent a great concern for the transmission of the pathogen in the archipelago, keeping in mind its zoonotic potential (Maggi et al., 2013). *Ehrlichia canis* was not found during this survey. This agrees with local veterinarians' observations about a marked decrease in the incidence of this pathogen, as mentioned above for *H. canis*.

In conclusion, this study is a first step to encourage wider studies on the CVBP circulating in Guadeloupe. Although the provided data are based on limited sampling, we were able to confirm several observations made by local veterinarians and identify by molecular means, for the first time, the presence of relevant CVBP on the Guadeloupean territory, some of them with zoonotic potential. All these pathogens were previously confirmed in dogs in other Caribbean islands, except for *C. burnetii*, which is reported for the first time in this study. Further studies should be directed to determine the actual prevalence of the detected CVBP and their vectors, to implement surveillance campaigns and efficient prevention measures in Guadeloupe.

CRediT authorship contribution statement

Mélody Imbert: Writing – original draft, Visualization, Methodology, Investigation, Data curation. **Clara Muñoz-Hernández:** Writing – review & editing, Methodology, Investigation. **Marta Sánchez-Sánchez:** Writing – review & editing, Investigation. **Luis V. Monteagudo:** Writing – review & editing, Supervision, Investigation. **Isabel G. Fernández de Mera:** Writing – review & editing, Resources, Methodology, Investigation. **Javier Millán:** Writing – review & editing,

Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2024.101132.

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