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How Covid-19 Isolate Afected to the Epidemiology (Prevalence) of Main Mediterraneum Vector-Borne Diseases (VBD) in Dogs

Sandra López Fernández[1], Carmen Chicharro[2], Javier Nieto[2], Fernando Fariñas Guerrero[3], Eduardo Martínez Manzanares[1,4] and Encarnación Clavijo Frutos[1,4]

[1]*Department Medical Microbiology, Faculty of Medicine, University of Malaga, Calle Louis Pasteur 32, 29010 Malaga, Spain*

[2]*Leishmaniasis and Chagas Disease Unit, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain*

[3]*Institute of Clinical Immunology and Infectious Diseases, Grupo YNMUN Biomedicina, 29018 Malaga, Spain*

[4]*FIMABIS, Andalusian Public Foundation for Research in Biomedicine and Health of Malaga, Spain*

***Corresponding author:** Sandra López Fernández, Department Medical Microbiology, Faculty of Medicine, University of Malaga, Calle Louis Pasteur 32, 29010 Malaga, Spain

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ABSTRACT

Diptera spp. and Ixodidae spp. can transmit several diseases through their bite, a lot of them are vector-borne zoonoses that can affect humans who live near dogs. It is knowledge that isolation of reservoirs of vector-borne diseases helps to presser the transmission and down the prevalence on the diseases. During the epidemy of SARS-COVID-19 confinement in Spain, owners and dogs were isolated into their homes, reducing the Potential reservoirs for VBD and giving an awesome opportunity to check how this hypothesis is true for VBD. Sample of 3135 dogs were tested along 7 years with Quantitative Immunoassay (IFA, ELISA) and PCR test to detect antibodies and pathogen of main Mediterraneum VBD *(Leishmania infantum, Anaplasma phagocytophilum, Dirofilaria immitis, Babesia canis and Ehrlichia canis)*, health check, blood test (hemogram, biochemical, proteinogram).

Results were separated into 2 populations, pre-pandemic tested vs pandemic tested and compared both groups. We found that the levels of positive dog's pre-pandemic group were much higher than pandemic levels.

This study shows that isolation of positive dogs far to healthy dogs and vectors help to low risk of contagious for other dogs and, most importantly, Humans. This information can help to preserve Public Health and avoid contagion.

Keywords: Leishmaniasis; Dirofilariasis; Anaplasmosis; Babesiosis; Piroplasm; Ehrlichiosis; Vector Borne Diseases; Mediterraneum Vectorial Zoonosis; Sars-Covid-19 Confinemen

Background

Five Vector-Borne Diseases (VBD) zoonosis agent are the most common in Mediterraneum [1] dogs population, two bacteria (*Ehrlichia canis and Anaplasma phagocytophilum*), one nematode (*Dirofilaria immitis*) and two protozoans (*Babesia canis, Leishm ania infantum*). *E. canis* is transmitted transstadially and intrastadially only by ticks known "Brown Dog tick" [2,3] *Rhipicephalus sanguineus* complex. A. *phagocytophilum* has only one vector, *Ixodes Ricinus* [4,5] but *Ehrlichia* spp. Has been found in *I. persulcatus* [6], but not in Spain by now. Both bacteria infect the tick through the feeding [7] and can infect new subjects when ticks molt and bite again a new host or pass to a new tick generation by trans-ovarian transmission. *Dirofilaria immitis* is one nematode transmitted by several species [8] of mosquitoes [9] from genera Culex, Aedes, Anopheles and Ochlerotatus. All filaria have filiform elongated body.

Females produce embryos, the microfilaria, who swim in vascular system and go to the Diptera mosquitoes bite the vertebrate carrier and act like secondary host, developing the third stage of the larvae.

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1 or 2 weeks after the bite, the new bite transmits the larvae to a new host, human or animal. Under the name *Babesia canis*[10,11] there are three related subspecies of this Genus, *B. canis canis*[12], *B. canis rossi, and B. canis vogeli*. They are grouped known as "Large Piroplasmids". Neither can be identified under microscope observation, the three subspecies share similar external antigens, so immunoassays do not distinguish from each other, being necessary PCR test to identify each one. Truly, Clinical difference is not important for treatment, but it is a main factor by epidemiologist studies. All of them are transmitted by the "Hard-Ticks" Dermacentor reticulatus [13,14]. The female tick bites one carrier host and can transmit the protozoa to a new host after the molting or directly pass the new tick generation though trans-ovary transmission, in this way, new immature tick can carry the parasites since first stage.

At least, *Leishmania infantum* is a flagellated protozoa transmitted by sandflies in Spain, it has high incidence [15] in Mediterraneum coast and is a neglected [16,17] zoonosis includes in the EDO [18] list (obligatory declaration list) in Spain. A lot of dogs are infected by this parasite displaying symptoms or not [19]. One sandfly female [20] feeds on a carrier vertebrate, eating amastigote form of *L. infantum*. The parasite changes to a promastigote form, stretching the flagella, and goes to cranial intestine where begging to multiplicate between intestinal microvilli. New leishmanias migrate to the head where finish their maturation, and then, flagellum Metacyclic Promastigote can be inoculated in a new host though sandfly bite 2 weeks after infection. At the beginning of SARS-COVID-19, Spanish government declared the state of health alert [21] prohibiting the exit of people from their homes except in exceptional cases. Because of this, dogs were isolated at home with their owners providing an exceptional opportunity to study how dogs' isolation affects vector-borne diseases transmission.

Methods

This was a retrospective observational study in 1320 dogs that were selected from the database (5000 initial subjects) of a previous epidemiological survey which sought to explore the distribution of canine vector-borne zoonoses in South of Spain. from 3135 dogs checked in Shelters, Pounds, and clinics from Malaga we selected dog from homes left in pounds or shelters and dogs from veterinary clinics and all tests were done under sedation (Table 2).

Different variables were collected from each subject (Table1) samples selected were:

1) Blood: Three sets were separated, one set for Málaga University (UMA), one set for Health Institute Carlos III (ISCIII) in Madrid and other set for reference laboratory.

The blood was separated into three tubes for each set, one Heparin-Li, one EDTA and one silicone gel. As well one drop was affixed onto tree slides to check microfilaria larvae and other parasite forms. From the UMA copy gel tube, another 4 more drops were collected to make Lateral-flow test (quick rapid antibodies test [22]). Serum and blood were sent to reference laboratory to issue for the performance of different tests (Table 1).

Table 1: DIAGNOSTICAL TEST: blood and tissues where collected and tested in the same day or processed and freeze at -20ºC (not blood).

DISGNOSIS TECHNICS	Anaplasma phagocytophilum	Leishmania infantum	Babesia canis	Ehrlichia canis	Dirofilaria immitis
SERUM	IFA (100%)		ELISA(100%)	IFA(100%)	ELISA(100%)
	LATERAL FLOW (10%)		-	LATERAL FLOW (10%)	
BLOOD	SMEAR (25%)				
SPLEEN and	FINE NEEDLE ASPIRATED (FNA)				
LYNPHONODES	FOR PCR (5+5%) AND MICROSCOPY (100%) DIAGNOSIS				
SKIN and	SCRAPING FOR PCR (5+5%)				
MUCOSES	AND MICROSCOPY (100%) DIAGNOSIS				
BLOOD FRACTION	BLOOD ANALYSIS				
HAEMOGRAM	Leukocytes, Basophils, Eosinophils, Segment/Nucleated Neutrophils, Monocytes, Immature Neutrophils, Hematocrit, Erythrocytes, Hemoglobin, Reticulocytes, Platelets, Microscopy Disturbances, Mean Cell Hemoglobin, Mean Corpuscular Volume, Red cell Distribution Width, Mean Corpuscular Hemoglobin Concentration, Mean Platelet Volume, Reticulocytes Hemoglobin Concentration				
PROTEINOGRAMME	Serum Proteins, Total Proteins, Total Globulins, Albumin, Albumin/Globulins, Globulins (Alpha 1-Glob, Alpha 2-Glob, Betta-Glob, Gamma-Glob)				
BIOCHEMISTRY	Urea, Creatinine, SDMA, Glucose, Alkaline Phosphatase, GOT (AST), GPT (ALT), Bilirubin, Cholesterol, Triglycerides, Creatin-Kinase, Phosphorus, Cl, k, Calcium, Na, Na/k				
Clinical parameters	Lymph-adenomegaly, Digestive sings like Diarrhea or Vomits, Appetite disorders, Polyuria/Polydipsia, Lethargy, Fever, low Weigh, Mucosal Pallor with or not Ulcer, Exfoliative Dermatitis with or not Ulcer, Nodules, Onychogryphosis, Blepharitis/Conjunctivitis, Uveitis, Breathing Difficulty, Exercise Intolerance, Poor Stamina				

Table 2: Sedative drugs used to collect samples. Chemical Drug name are add with the empirical doses and were injected intra-muscular way.

SEDATION IM	DRUG	DOSE	VIA
BENZODIAEPINES	A) DIZEPAM	0,5 mg/kg	INTRAVENOUS
	OR		
	B) MIDAZOLAM	0,25 mg/kg	INTRAVENOUS
PHENOTIAZINES	ACEPROMAZINE	0.005-0.04 mg/kg	INTRAMUSCULAR
ALFA-2-ADRENERGIC	A) XYLACIN	0.2-1 mg/kg	INTRAMUSCULAR
	OR		
	B) MEDETOMIDINE	5 a 10 µg/kg	INTRAVENOUS
DISSOCIATIVES	KETAMINE	25 mg/Kg	INTRAVENOUS, 20% WITH NACL ISOTONIC
OPIATES	A) METHADONE	0,3 a 08 mg	INTRAMUSCULAR
	OR		
	B) BUPRENORFPHINE	10-20 mg/kg	INTRAMUSCULAR
	OR		
	C) MORPHINE	0.02-0,04 mg/kg	INTRAMUSCULAR

The blood was separated into three tubes for each set, one Heparin-Li, one EDTA and one silicone gel. As well one drop was affixed onto tree slides to check microfilaria larvae and other parasite forms. From the UMA copy gel tube, another 4 more drops were collected to make Lateral-flow test (quick rapid antibodies test [22]). Serum and blood were sent to reference laboratory to issue for the performance of different tests (Table 1).

2) Tissues: Three sets were collected as well, for UMA, ISCIII and reference Laboratory. The tissues selected were:

a) Bone marrow form right Rib-Cartilage edge, by FNA technique (Table 1) with 21G needle.

b) Lymph-node from popliteus gland, by FNA technique (Table 1) with 21G needle.

c) Mucous Membrane from lips, by Scraping technique (Table 1), with nº 20 Surgical Blade.

d) Skin from internal side of ears, by Scraping technique (Table 1), with nº 20 Surgical Blade.

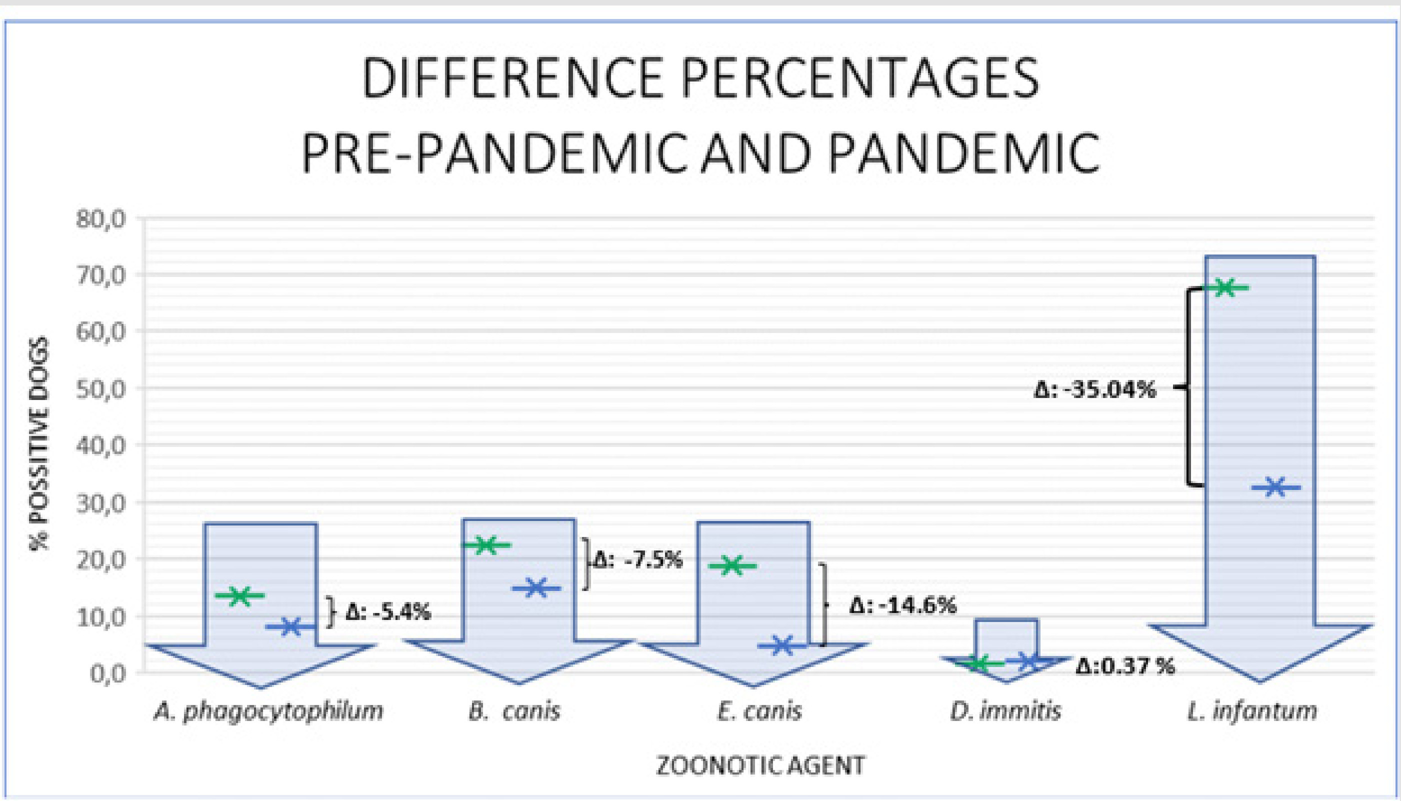
Samples were entered in a tube with Tris-EDTA buffer solution [23] until arrival at the university laboratory, where were homogenized and DNA purified with commercial kit [24] and freeze at -20ºC before to send to ISCIII and the other laboratories to keep at -80ºC. DNA sets were tested with PCR for *L. infantum* [25] in ISCIII and *B. canis* [26] in Reference laboratory [27]. 5% of the Spleen, lymph-nodes, mucosa, and skin sampled collected, and mucosa for microscope observation (Commercial [28] Diff-Quick stain). *D. immitis* [29] was checked from blood samples, all with ELISA and Lateral flow techniques and 25% was checked using simple microscopy technique, one drop of blood adds with one drop of sterile NaCl 9% physiological [30] serum and observed directly. An additional 5% were checked with MSD parasite diagnoses. The data obtained were listed in the statistical Table and analyzed with Fisher test and also submitted to the analyses of Correlation of Pearson.

Results

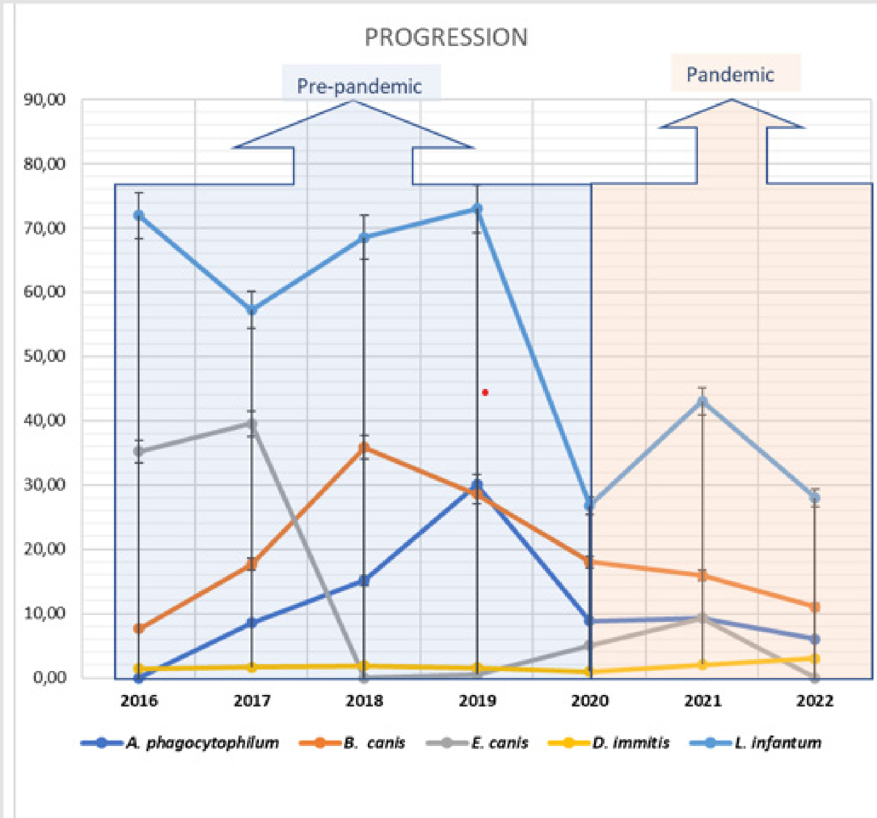
A. phagocytophilum did not get carriers in 2016, as well *E. canis* in 2018 and 2022. The parasite most common was *L. infantum* all the years and *D. immitis* was the least represented. *A. phagocytophilum* and *E. canis* obtained similar results (Table 3). Tick borne bacteria *A. phagocytophilum* showed a decrease of -5.4% from Pre-pandemic (Graph 1) to Pandemic years while it in the other bacteria, *E. canis,* was -14.6% of decrease. The rate of *A. phagocytophilum* is smaller than *E. canis* based on *E. canis* of average prevalence is 18,8% in Pre-pandemic and decreases to 4.8% while *A. phagocytophilum* begins with 13,4% and decreases only to 8,0%. A similar process occurs if we compare *B. canis* front of *A. phagocytophilum* (Graph 1), being *B. canis* rate -7.5% of decreasing, 22,5% Pre-pandemic to 15,0% of Pandemic years. Mosquitoes borne diseases, *D. immitis* virtually has remained essentially unchanged, being the only parasite who shows a slight increase (Graph 1), 0.37%. It begins from 1,6% Pre-pandemic and climbs to just to 2,0% in Pandemic. However, *L. infantum* persists over time high prevalence levels (Graph 1), starting with 67,7% Pre-pandemic and plunging -35.04% of incidence until the 32,6% in Pandemic. Excluding *D. immitis*, the remaining diseases showed a significant reduction since Pandemic Isolated period as we can see in (Graph 2). Faced with more than one diagnosis technique, based on an image (Figure 1), laboratorial alterations (Figure 2), Clinical signs (Figure 3) and Serology (Figure 4). On an additional basis, Blood and tissues were tested by PCR technique to verification the laboratory results (Figure 5).

Table 3: Aggregate results of all years in %.

YEAR	Nº SUBJECTS	% SUBJECTS SICK WITH:				
		A. phagocytophilum	*B. canis*	*E. canis*	*D. immitis*	*L. infantum*
2016	210	0	7,62	35,2	1,43	71,9
2017	187	8,56	17,6	39,6	1,6	57,2
2018	159	15,1	35,8	0	1,89	68,6
2019	196	30,1	28,6	0,51	1,53	73
2020	317	8,83	18	5,05	0,95	26,8
2021	151	9,27	15,9	9,27	1,99	43
2022	100	6	11	0	3	28
AD:7	1320	11,1	19,2	13,6	0,23	52,1



Graph 1: Comparison of prevalence levels before (green) and after (blue) Pandemic Isolation and precent of variation (Δ) in each species.



Graph 2: Growth Index pre-pandemic and Pandemic for each disease.

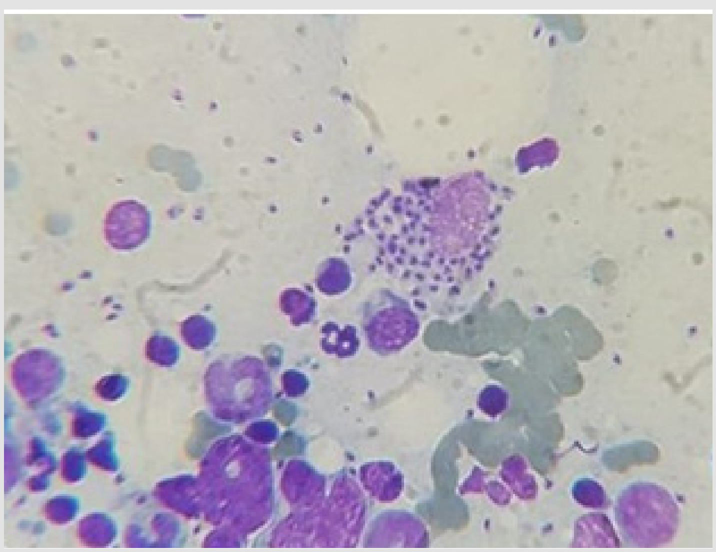


Figure 1: Popliteal lymph-node with amastigote form of L. infantum in dog.

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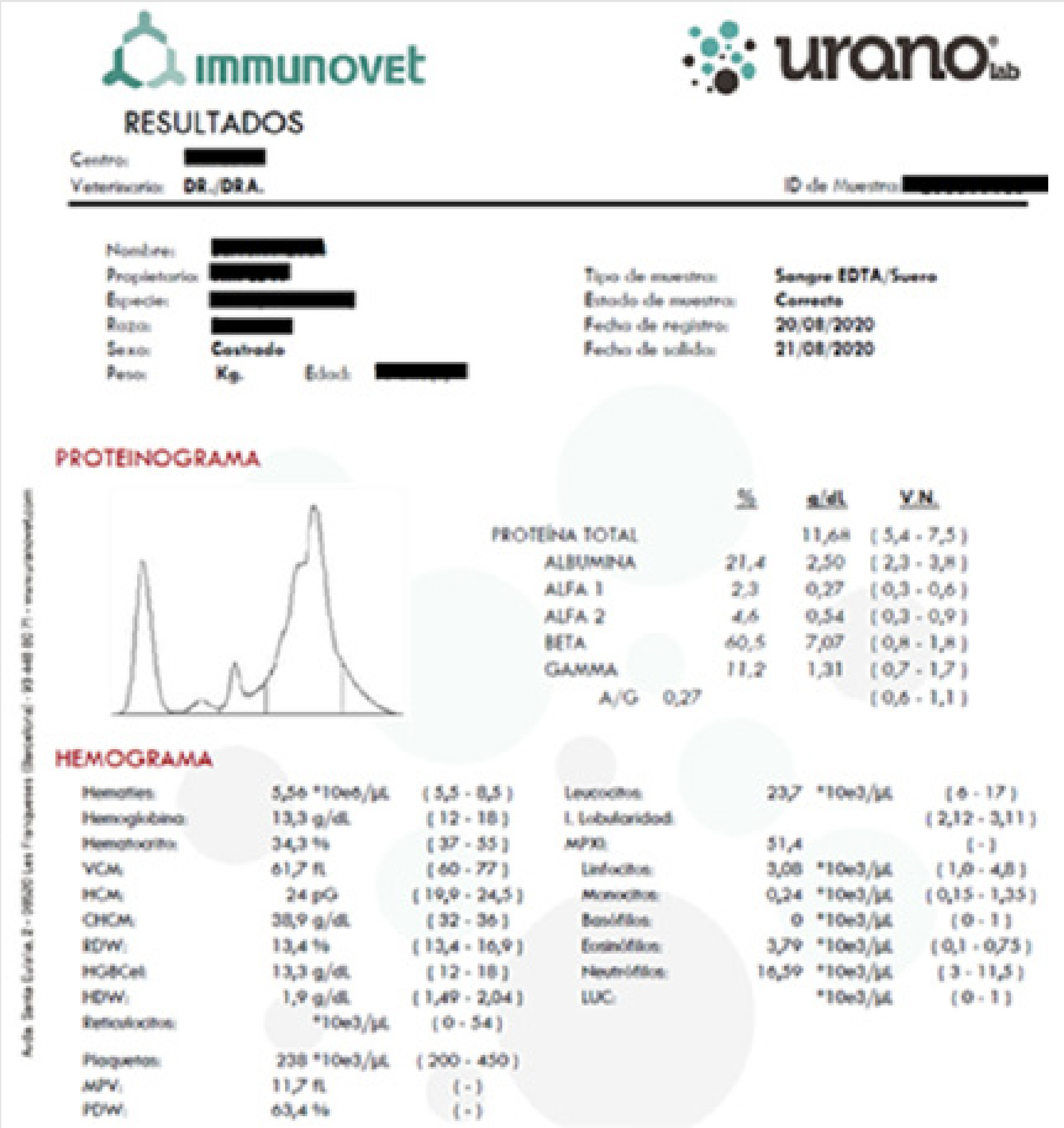

immunovet

urano lab

RESULTADOS

Centro: [redacted]
Veterinario: DR./DRA.
ID de Muestra: [redacted]

Nombre:	[redacted]		
Propietario:	[redacted]	Tipo de muestra:	Sangre EDTA/Suero
Especie:	[redacted]	Estado de muestra:	Correcto
Raza:	[redacted]	Fecha de registro:	20/08/2020
Sexo:	Castrado	Fecha de salida:	21/08/2020
Peso:	Kg. Edad: [redacted]		

PROTEINOGRAMA

	%	g/dL	V.N.
PROTEÍNA TOTAL		11,68	(5,4 - 7,5)
ALBUMINA	21,4	2,50	(2,3 - 3,8)
ALFA 1	2,3	0,27	(0,3 - 0,6)
ALFA 2	4,6	0,54	(0,3 - 0,9)
BETA	60,5	7,07	(0,8 - 1,8)
GAMMA	11,2	1,31	(0,7 - 1,7)
A/G	0,27		(0,6 - 1,1)

HEMOGRAMA

Hematíes:	5,56 *10e6/µL	(5,5 - 8,5)	Leucocitos:	23,7	*10e3/µL	(6 - 17)
Hemoglobina:	13,3 g/dL	(12 - 18)	I. Lobularidad:			(2,12 - 3,11)
Hematocrito:	34,3 %	(37 - 55)	MPXI:	51,4		(-)
VCM:	61,7 fL	(60 - 77)	Linfocitos:	3,08	*10e3/µL	(1,0 - 4,8)
HCM:	24 pG	(19,9 - 24,5)	Monocitos:	0,24	*10e3/µL	(0,15 - 1,35)
CHCM:	38,9 g/dL	(32 - 36)	Basófilos:	0	*10e3/µL	(0 - 1)
RDW:	13,4 %	(13,4 - 16,9)	Eosinófilos:	3,79	*10e3/µL	(0,1 - 0,75)
HGBCel:	13,3 g/dL	(12 - 18)	Neutrófilos:	16,59	*10e3/µL	(3 - 11,5)
HDW:	1,9 g/dL	(1,49 - 2,04)	LUC:		*10e3/µL	(0 - 1)
Reticulocitos:	*10e3/µL	(0 - 54)				
Plaquetas:	238 *10e3/µL	(200 - 450)				
MPV:	11,7 fL	(-)				
PDW:	63,4 %	(-)				

Figure 2: Reference laboratory results example, proteinogram and hemogram from a subject tested with Ehrlichiosis. Data and reference have been deleted for privacy. Language in Spanish.

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Figure 3: Leishmaniasis in dogs;

A. Lip-mucosa inflammation (Spanish waterdog),

B. Skin nodules in abdomen (mixed 4 months dog, negative serology but positive microscopy identification) and

C. Skin Ulcera (Malinoise with mucocutaneous leishmaniasis).

IDEXX LABORATORIES

	Resultado	Valores de Referencia
SEROLOGIA CANINA		
RATIO LEISHMANIA ELISA	0.09	<= 0.55
Ac anti-LEISHMANIA Equivalencia en IFI	Negativo	.
Ac anti-EHRLICHIA CANIS IFI	NEGATIVO	<0.9

Valores hasta 1/40 pueden ser producidos por la enfermedad o ser el resultado de reacciones inespecificas.
Valores superiores a 1/40 se consideran positivos.

Ag DIROFILARIA IMMITIS CANINO ELISA	NEGATIVO	NEGATIVO
Ac anti- BABESIA CANIS ELISA	<0.1	.

TU < 14 : NEGATIVO

TU 14.0 - 19.0: DUDOSO

TU > 19: POSITIVO

Nuevos valores de referencia por cambio de técnica.

Figure 4: Serology results form reference laboratory. Language is Spanish and all owner and dog details have been deleted for privacy.

Canine Babesia or Theileria sp. by PCR (DNA detection)

Result: Negative

Normal reference ranges:

This test has a sensitivity of 99.9% and a specificity of 100%. Detect any kind of babesia or theileria. "Theileria annae" is a small species first detected in dogs in 2000 and is usually diagnosed in smears as "Babesia gibsoni"

Less than 100 parasites / ul: carrier, possibly asymptomatic.
100 to 1,000 parasites / ul: Mild infection.
1,000 to 100,000 parasites / ul: Mild or chronic infection, although anemia is common.

Figure 5: Babesia PCR test from blood sample in a dog. Data was deleted for privacy.

Discussion

There are several possible conclusions to be drawn from the results obtained. First of all is the transmission of vector-borne diseases can be controlled protecting dogs from the vector, whether isolating (thus not feasible) or keeping out dogs from vector and this can be achieved using repellent substances for ticks and mosquitoes using collar or spot-on for example. If the vector cannot access the dog, the transmission is severely disrupted. The geographical area of South of Spain shows an important presence of vectors, mosquitoes, sandflies and ticks infected with infectious agents, so humans should be protected from vector. In the case of mosquitoes and sandflies, using Chemical Repellents (at evening outs), mosquito-nets on doors and windows, Electrical Devices Repelling at home (that provide extra-protection to the dogs). About tick, using high-top shoes and protective clothing in trips to the country, gardens, or parks, sprayed onto clothes repellent substances.

Adults must comprehensively review the body of kids and elderly persons after walks in the countryside. property garden should be often sprayed to kill adults and eggs of vector. The parents should be careful and teach their kids not to touch animals foreign. Relevant authorities are obliged to ensure the Public Health so, based on these results, they should take the necessary steps to protect population with actions like clean rivers, punctual fumigations, and follow the measures proposed by WHO [31] and OIE [32,33].

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