Diagnostic update

SNAP Leish 4Dx Test and improved SNAP *Leishmania* Test: improved utility, same great performance

Vectors and the diseases they transmit have become increasingly prevalent throughout Europe. A recent publication summarizing over 224,000 SNAP* 4Dx* Plus Test results and 211,000 SNAP* *Leishmania* Test results from 2016–2020 in Europe found that dogs are commonly exposed to vector-borne pathogens, such as *Leishmania* spp. transmitted by sand flies and the tick-borne disease agents *Anaplasma* spp. and *Ehrlichia* spp.[1] The geographic distribution of these vectors and the pathogens they transmit continues to expand. Selected countries (>n= 1000 for Anaplasma/Ehrlichia and >n=100 for Leishmania respectively) are projected on a map with the proportion positive results for *Anaplasma* spp. and *Ehrlichia* spp. (figure 1) and *Leishmania* spp. (figure 2).

The SNAP Leish 4Dx Test from IDEXX can be used to detect the antigen of *Dirofilaria immitis* and antibodies against *Anaplasma phagocytophilum, Anaplasma platys, Ehrlichia canis, Ehrlichia ewingii, Ehrlichia chaffeensis* and *Leishmania infantum* in a single whole blood, plasma or serum sample.[2–4] To help veterinarians routinely test for and more accurately diagnose these vector-borne diseases, the SNAP Leish 4Dx Test incorporates species-specific recombinant proteins for *Leishmania infantum* in addition to highly specific peptides currently used on the SNAP 4Dx Plus Test, including *A. phagocytophilum*, *A. platys, E. canis* and *E. ewingii* as well as an optimized antibody pair for detection of adult *D. immitis*. These reagents have been previously evaluated in canine experimental infections as well as patient samples.[5,6]

This SNAP Leish 4Dx Test is intended to better meet the needs of practicing veterinarians and their patients, enabling them to confidently test for evidence of these tick-borne pathogens, *D. immitis* and *Leishmania*. Equally important, the SNAP Leish 4Dx Test helps uncover evidence that dogs have been exposed to multiple infectious organisms either through bites from multiple vectors or coinfections carried by the same tick vector. This helps in diagnosis, treatment and awareness of leishmaniosis, dirofilariosis and various tick-borne diseases.

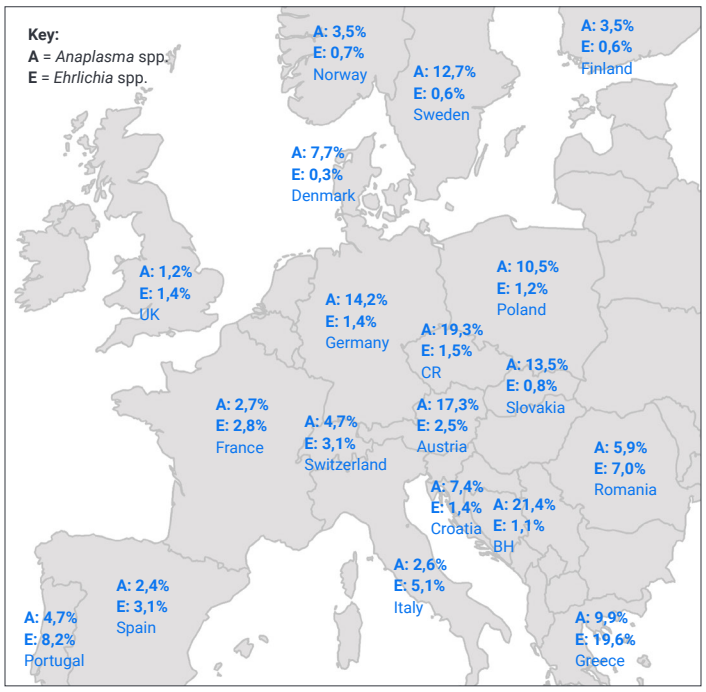


Figure 1: *Anaplasma* spp. and *Ehrlichia* spp. seropositivity (%) by country.[1] A: antibodies to *Anaplasma* spp., E: antibodies to *Ehrlichia* spp.; total numbers of tested dogs: Austria (n = 4.572), BH/Bosnia and Herzegovina (n = 3.671), CR/Czech Republic (n = 6.238), Croatia (n = 2.417), Denmark (n = 7.784), Finland (n = 6.084), Germany (n = 20.582), Greece (n = 6.488), France (n = 18.070), Italy (n = 64.879), Norway (n = 3.051), Poland (n = 3.812), Romania (n = 13.995), Slovakia (n = 1.584), Switzerland (n = 1.006), Spain (n = 39.526), Sweden (n = 10.047), Portugal (n = 1.285), UK (n = 2.631).

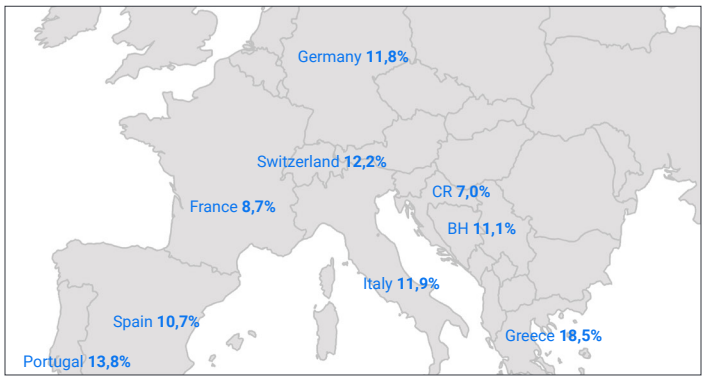


Figure 2: *Leishmania* seropositivity by country[1]

Total number dogs tested by country: BH/Bosnia Herzegovina (n = 172), CR/Croatia (n = 1.761), France (n = 5.307), Germany (n = 686), Greece (n = 9.568), Italy (n = 90,532), Portugal (n = 1.329), Spain (n = 98.737), Switzerland (n = 221)

Same great performance with the addition of *Leishmania* detection

The SNAP Leish 4Dx* Test continues to exhibit sensitivity and specificity consistent with the performance shown in numerous, peer-reviewed publications.[2–4]

Analyte	Reference method	SNAP Leish 4Dx Test result		Total	Sensitivity (95% CL)
		+	–		Specificity (95% CL)
Dirofilaria immitis[a]	+	48	1	49	98,0% (89,1%–99,9%)
	–	0	461	5	100,0% (99,2%–100%)
***Anaplasma* spp.**[b]	+	80	5	85	94,1% (86,8%–98,1%)
	–	7	418	425	98,4% (96,6%–99,3%)
***Ehrlichia* spp.**[c]	+	99	7	106	93,4% (86,9%–97,3%)
	–	13	391	404	96,8% (94,6%–98,3%)
Leishmania infantum[d]	+	82	3	85	98,8% (93,5%–100%)
	–	1	272	273	98,9% (96,8%–99,8%)

Table 1: SNAP Leish 4Dx Test versus reference methods.[3]

Reference methods

a. Necropsy or PetChek* Heartworm ELISA positive and PetChek* Heartworm ELISA negative[4]

b. *A. phagocytophilum* IFA and *Anaplasma* spp. ELISA[4]

c. *E. canis* IFA and *E. ewingii* ELISA[4]

d. IFA[7]

The sensitivity and specificity for antibodies to *Leishmania* allows veterinarians to confidently screen dogs for evidence of this important pathogen while also getting trusted results for heartworm, *Anaplasma* spp. and *Ehrlichia* spp., all with a single sample and test device.

In addition to accurately detecting *Leishmania* antibodies, the sensitivity of the SNAP Leish 4Dx Test for detection of antibodies to common tick-borne pathogens, *Anaplasma* spp. and *Ehrlichia* spp., allows veterinarians to uncover dogs that may have vague or no clinical signs at the time of testing, giving them the opportunity to further evaluate for evidence of anaplasmosis and ehrlichiosis. With some tick-borne pathogens, acute disease might occur soon after tick attachment. For example, in most dogs, the clinical signs of canine anaplasmosis are nonspecific and confined to the acute phase of the infection.[8]

Thrombocytopenia was evident in dogs experimentally infected with *A. platys* or *A. phagocytophilum* within 10 days post-infection.[9,10] Thus, anaplasmosis poses a diagnostic challenge, and early detection is particularly relevant. The highly specific peptides used in the SNAP Leish 4Dx Test allow for detection in many of these acute-phase infections, closely aligned with when PCR would be positive.[11]

As with all vector-borne diseases, accurate diagnosis enables timely treatment in clinically affected dogs and helps support discussions with pet owners on control measures and preventative recommendations.

Leishmania: different threats and risks

Canine *Leishmania* infections are predominantly due to *Leishmania infantum*. *L. infantum* infection is typically transmitted by sand flies, which represent the main risk of transmission. *L. infantum* may infect a wide range of mammal species, including cats and humans. Other modes of transmission without vectors have also been demonstrated including sexual, vertical and via blood transfusion.

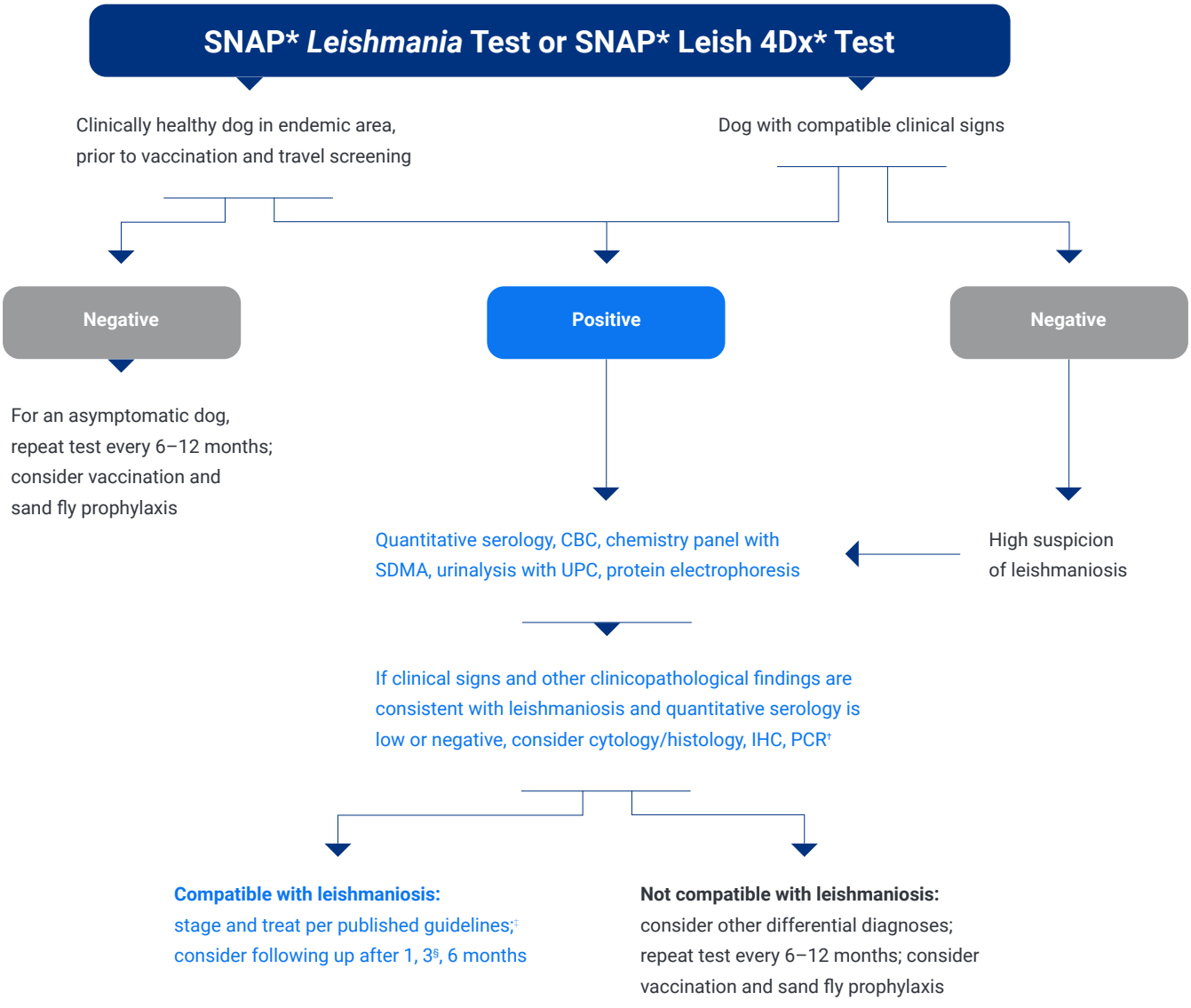
Screening is recommended for apparently healthy dogs living in or travelling to or from endemic areas including the following:

+ Annually in endemic areas as part of annual *Leishmania* health check and prior to *Leishmania* vaccination
+ Imported dogs
+ Blood donors
+ Breeding dogs

Diagnosis is based on clinical signs and/or clinicopathological findings consistent with disease combined with confirmation of *L. infantum* infection, predominantly using serological and/or molecular diagnostics. Clinical signs may include weight loss, lymphadenopathy, changes in appetite, lethargy, splenomegaly, fever, vomiting, diarrhea, mucous membrane pallor, polyuria, polydipsia, dermatitis (exfoliative, papular, nodular or pustular), ocular lesions, epistaxis, lameness (joint diseases), and neurological disorders.

Diagnosis of canine leishmaniosis is supported by detection of *Leishmania infantum* antibodies. Infection with *Leishmania* spp. does not indicate disease. Subclinical infections are common and usually have low antibody levels. Clinically sick dogs typically present with high antibody titers (>1:400) that are readily detectable.[12] Point-of-care test accuracy aids in diagnosis of dogs with subclinical infection, clinically sick dogs, and differentiation of infected rather than vaccinated animals (DIVA). Dogs that have previously tested positive for *Leishmania* should be monitored routinely with quantitative testing (ELISA or IFAT), a complete minimum database including urine analysis with UPC and qPCR if applicable for changes in disease status.

Diagnostic algorithm for diagnosis and staging of leishmaniosis



†Recommended sample types: bone marrow, lymph node, spleen, skin or conjunctival swabs.

‡For more information on the clinical staging, treatment and prognosis of leishmaniosis, visit the LeishVet website: leishvet.org/fact-sheet/clinical-staging.

§Quantitative serology recommended 3 months after initial therapy and then every 6–12 months.

References

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