Diagnose one, get one free': co-infections in imported dogs, consequences and complications

Ian Wright, chair of ESCCAP

IDEXX

DISCLOSURES

+ I am chair of the European Scientific counsel for Companion Animal Parasites (ESCCAP) which is a non for-profit organisation sponsored by pharmaceutical and diagnostic companies

+ A full list of ESCCAP sponsors can be found at www.esccap.org

+ I have personally received honoraria for lectures and articles presented for IDEXX in the last 12 months including this one

+ *The information contained herein is intended to provide general guidance only. As with any diagnosis or treatment, you should use clinical discretion with each patient based on a complete evaluation of the patient, including history, physical presentation, and complete laboratory data. With respect to any drug therapy or monitoring program, you should refer to product inserts for a complete description of dosages, indications, interactions, and cautions. Diagnosis and treatment decisions are the ultimate responsibility of the primary care veterinarian.*

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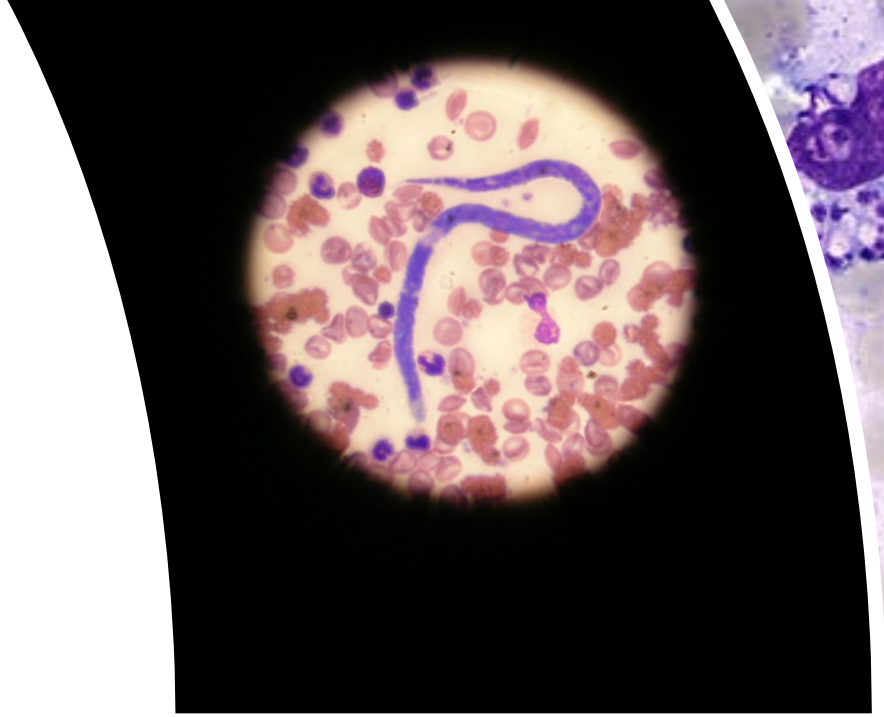
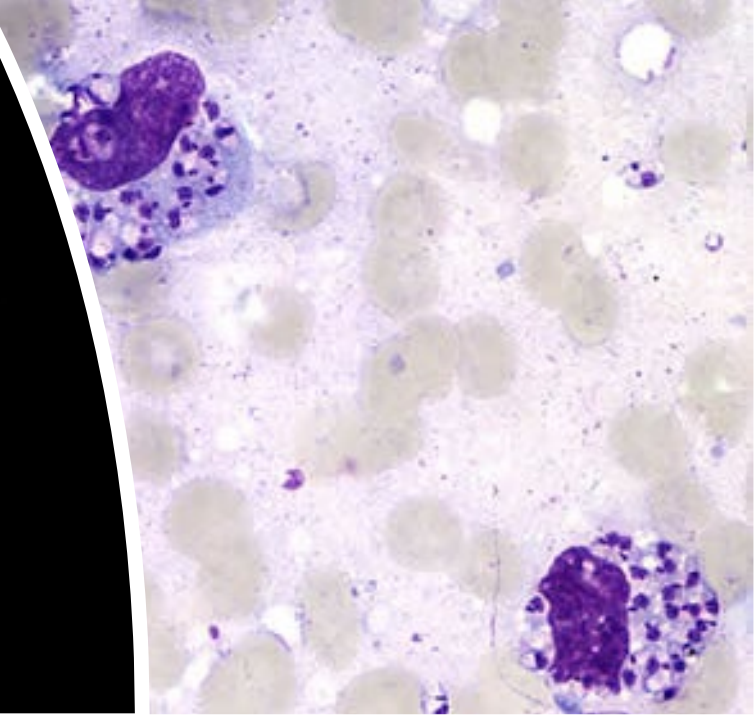
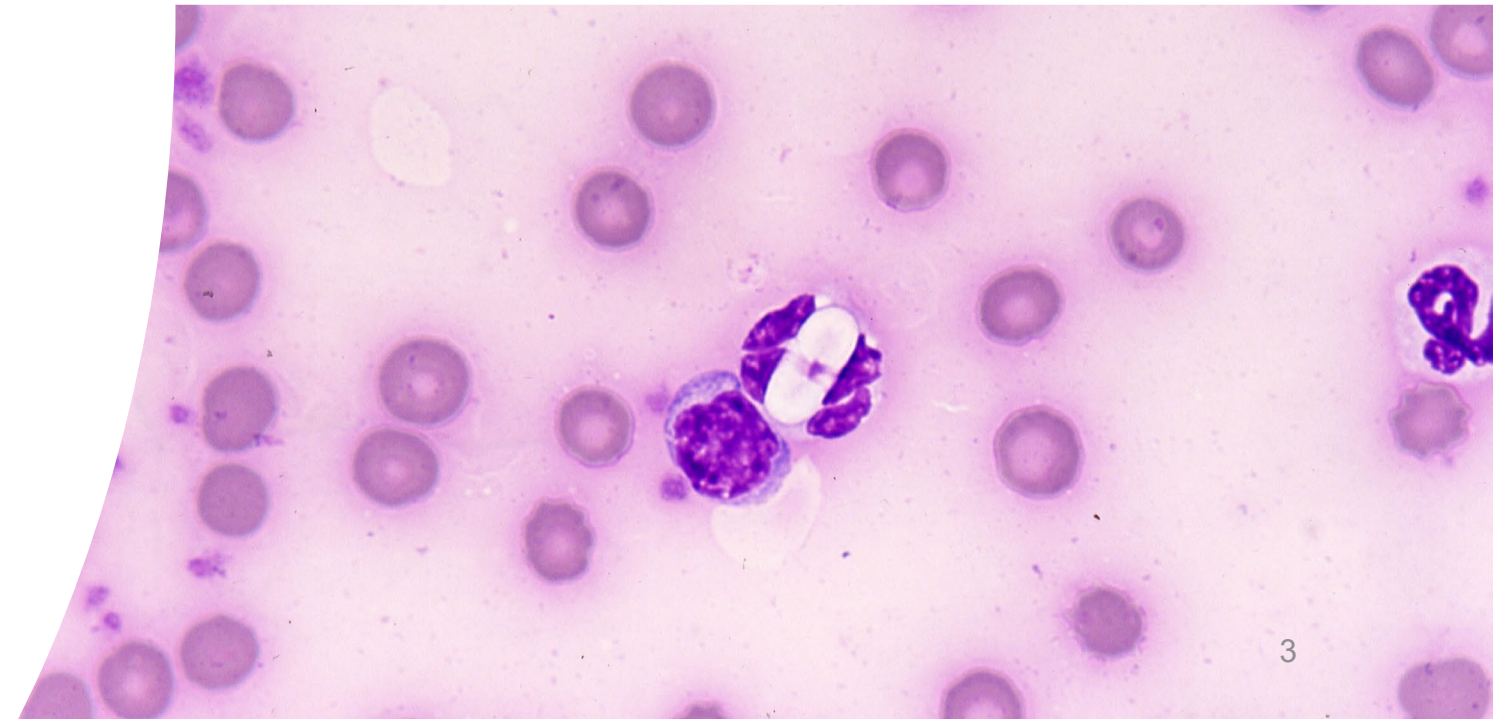
Parasites in imported dogs

- Tick- borne pathogens
  - *Ehrlichia canis*
  - *Hepatozoon canis*
  - *Anaplasma* spp
  - *Babesia* spp
- *Leishmania infantum*
- Fly–borne nematodes
  - *Dirofilaria immitis*
  - *Dirofilaria repens*
  - *Thelazia callipaeda*
- *Linguatula serrata*





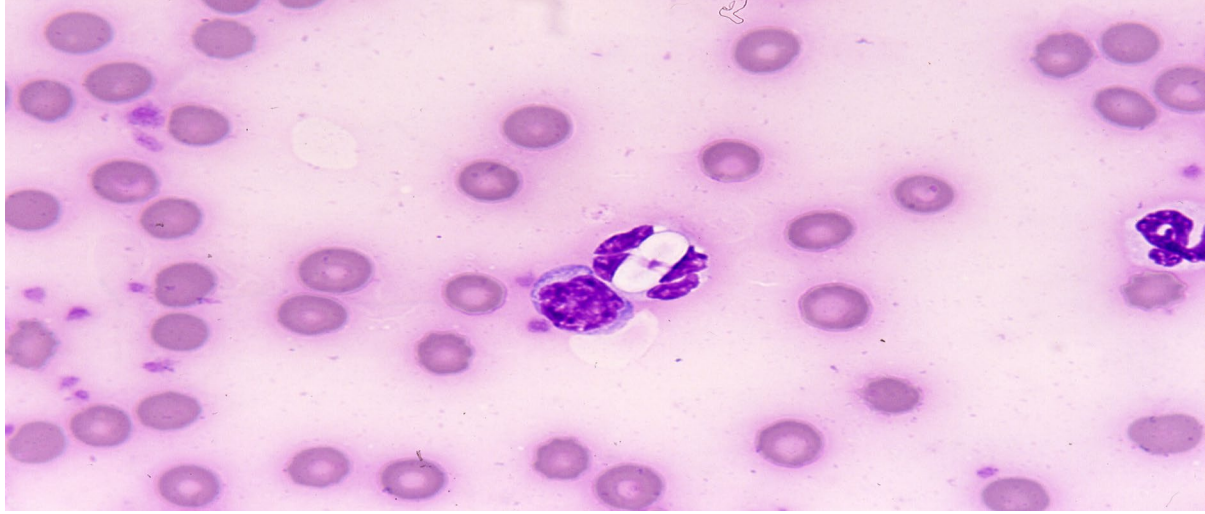


Co-infections

Common for a variety of reasons

- Common vectors
  - Rhipicephalus sanguineus vector for *Ehrlichia canis*, *Hepatozoon canis*, *Anaplasma platys*
  - Mosquito vectors transmit *Dirofilaria immitis* and *Dirofilaria repens*
- Parasites making hosts susceptible to further infections
  - *Leishmania* predisposing to range of other parasitic infections?
  - *Ehrlichia canis* predisposing hosts to *Leishmania* infection?
- Geographic overlap
  - Many parasites are present in the same geographic region
  - *Babesia canis* often present with *Rhipicephalus*-borne tick infections

Tick-borne coinfections



- Ticks on dog or in owner's house
- Travel history
- Relevant clinical signs?
  - Anaemia and thrombocytopaenia – *Babesia, Ehrlichia canis, Anaplasma platys*
  - Lymphadenopathy and pyrexia
  - Neurological signs – TBEV, *Ehrlichia canis*
- Blood smear – especially useful for *Hepatozoon canis* and may also pick up other coinfections
- Serology – *Ehrlichia* (quantitative, 2 weeks apart), *Anaplasma*
  - Available as combination test (SNAP 4Dx Plus Test)
- PCR – *Ehrlichia*, *Babesia* (allows speciation), *Anaplasma*, *Hepatozoon*
  - In house analysers or external lab packages

Leishmania co-infections

- Prevalence studies showing *Leishmania* positive dogs more likely to have coinfections
- Possible immune modulatory effects
- Where *Leishmania* is identified consider possibility of other coinfections
- Many clinical signs shared with other vector-borne infections

TABLE 2. POSITIVITY TO OTHER VECTOR-BORNE PATHOGEN IN *LEISHMANIA-POSITIVE (G1)* AND *LEISHMANIA*-NEGATIVE (G2) DOGS. *,**

PATHOGENS	GROUP 1- % (N)	GROUP 2 - % (N)
***Ehrlichia* spp.**	58.6 (34) aA	48.3 (28) aA
Dirofilaria immitis	3.4 (2) cA	0 bA
***Anaplasma* spp.**	0 cA	0 bA
Ehrlichia* spp. + *D. immitis	17.2(10) bA	0 bB
***Ehrlichia* spp. + *Anaplasma* spp.**	5.2 (3) bA	1.7 (1) bA
***Ehrlichia* spp. + D. *immitis* + *Anaplasma* spp.**	5.2 (3) bA	0 bA

* Lowercase letters in the same column indicate statistical difference (P<0.05).

** Capital letters in the same line indicate statistical difference (P<0.05).

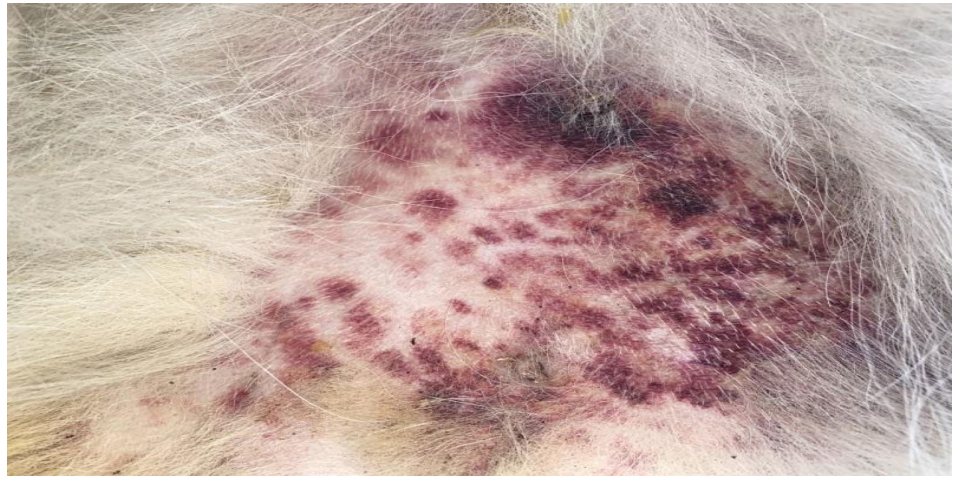
Ramos RAN, Giannelli A, Ubirajara-Filho CRC, et al. **Vector-borne pathogens in dogs from areas where leishmaniosis is endemic.** *Vet Parasitol Reg Stud Reports.* 2022;32:100746. doi:10.1016/j.vprsr.2022.100746

Leishmania and *Ehrlichia canis*

+ Some longitudinal studies suggest that *Ehrlichia* may predispose dogs to *Leishmania* infection over time

+ Relevant as *Leishmania* has a long incubation period

+ Fever, lymphadenopathy and coagulopathies may be present in both leishmaniosis and ehrlichiosis



10.1111/j.1469-0691.2008.02150.x

Ehrlichia canis and _Leishmania infantum_ co-infection: a 3-year longitudinal study in naturally exposed dogs

Y. Mekuzas[1], L. Gradoni[2], G. Oliva[3], V. Foglia Manzillo[3] and G. Baneth[1]

[1]School of Veterinary Medicine, Hebrew University, Rehovot, Israel, [2]MIPI Department, Istituto Superiore di Sanità, Rome and [3]Department of Veterinary Clinical Sciences, University Federico II, Naples, Italy

Leishmania Diagnosis

- History – Signs can develop months or years after travel
- Qualitative serology – useful initial screen if can be combined cheaply with other serology (*Ehrlichia*, *Anaplasma*) and/or heartworm (e.g., SNAP 4Dx Plus Test)
- Quantitative serology – high confirms leishmaniosis with clinical signs
- FNA – Giemsa staining for Amastigotes. Low sensitivity.
- PCR – EDTA, conjunctival swabs, lymph node, bone marrow aspirates/biopsies
- Biopsy – skin, lymph node and bone marrow

Dirofilaria immitis

- Mosquito transmitted
- Large geographic overlap with *D.repens* infection
- Likely dogs will be exposed to both parasites during mosquito feeding
- Geographic overlap with tick-borne pathogens and *Leishmania*
- Always test if any of these pathogens are present
- Coagulopathy from *Leishmania*, *Ehrlichia* or *Anaplasma platys* may affect treatment risk of thromboembolism
- Prognosis depends on severity and chronicity of other infections e.g., chronic ehrlichiosis

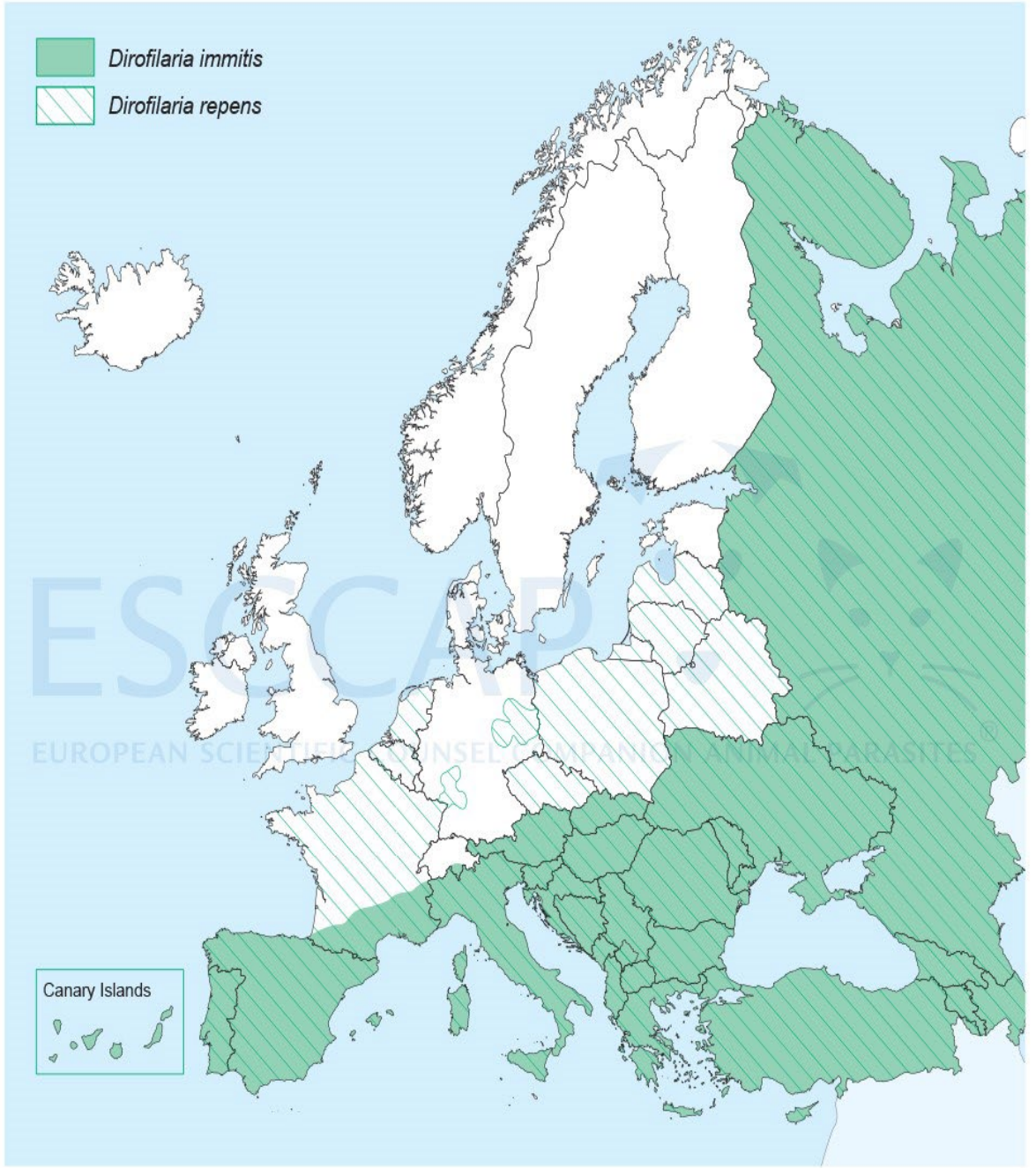
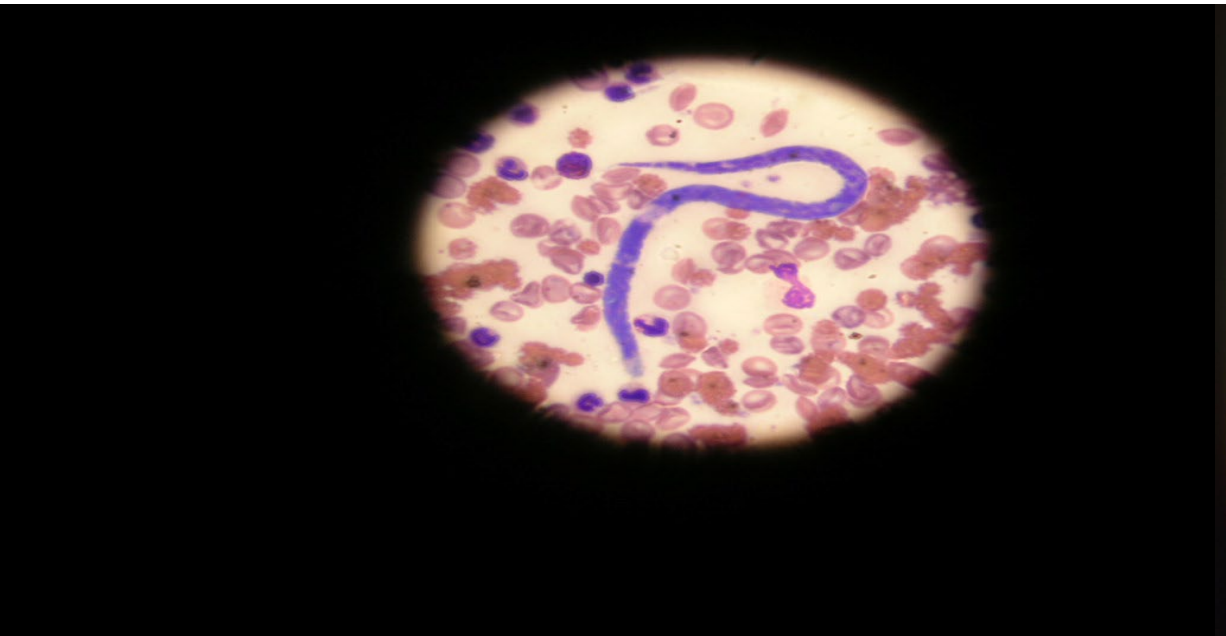
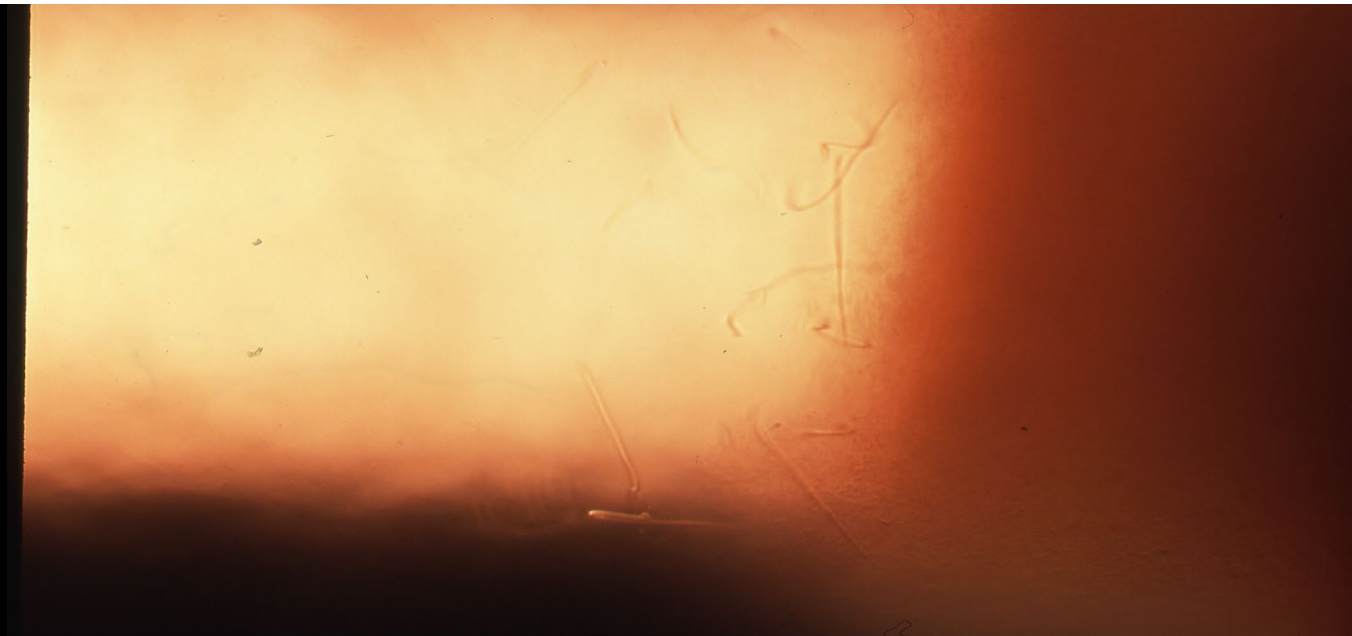


Figure 6: Approximate distribution of *Dirofilaria immitis* and *Dirofilaria repens* in Europe (© ESCCAP)

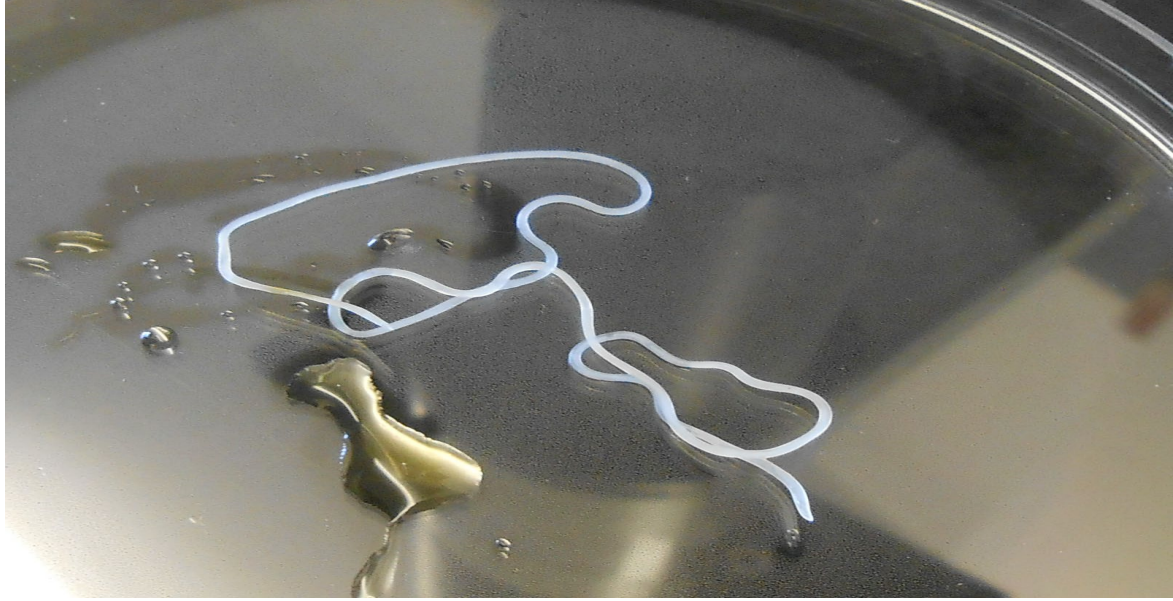
Diagnosis – blood examination for microfilariae

- Direct smear – low sensitivity but microfilaria sometimes detected
- Knott's test – 1ml citrated blood centrifuged with 9mls formalin
  - Also allows screening for *D.repens*
- Micro haematocrit centrifugation – microfilaria visible in buffy coat





Dirofilaria repens adults



- Adults in skin or skin nodules
- Sometimes found during surgery
- Sometimes found in nodules around the eye

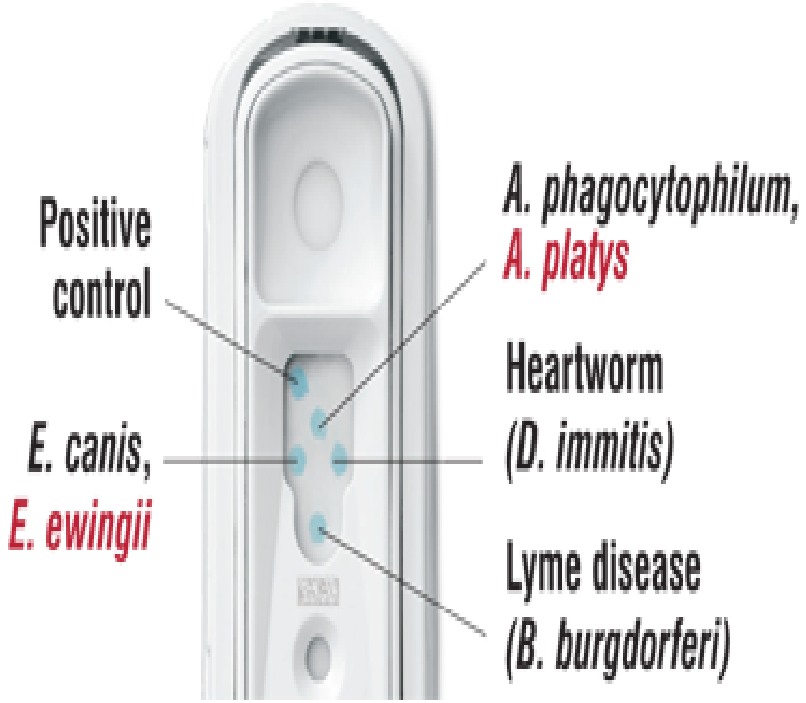
+ APHA in collaboration with ESCCAP UK & Ireland is offering free of charge morphological identification of suspected cases of *T.callipaeda*, *D.repens* and *L.serrata* seen in veterinary practices in England and Wales

+ Sample submissions must be accompanied by full clinical history to qualify for free testing. Further, information on how to submit them can be found here http://apha.defra.gov.uk/vet-gateway/surveillance/experts/parasitology.htm.

- Dogs positive for *D.repens* should also be tested for *D.immitis*

Diagnosis – antigen serology

+ Antigen in uterine secretions
+ Gold standard test in dogs, at least 93% sensitivity
+ Specificity close to 100%
+ Heat treating serum increases sensitivity but decreases specificity
+ SNAP 4Dx Plus Test also includes *E.canis* and *Anaplasma* spp testing



Case example “Mr Boo”

11 year old Labrador retriever

Imported from Singapore 7 years previously

Presented with

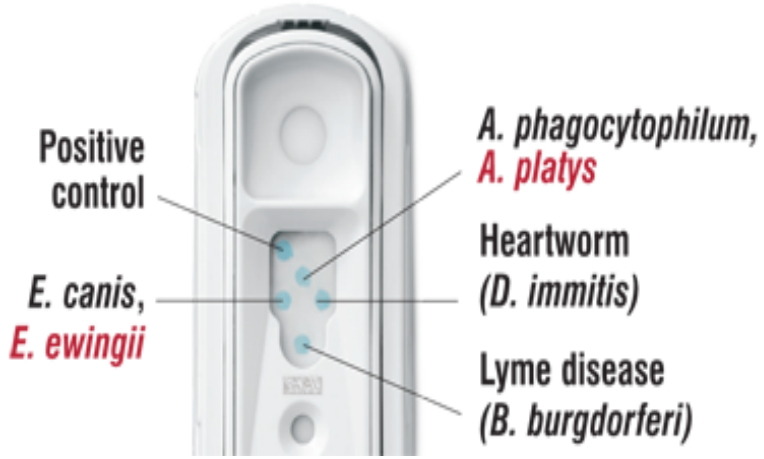
- Lethargy
- Intermittent vomiting and diarrhoea
- Intermittent cough past 3-4 months

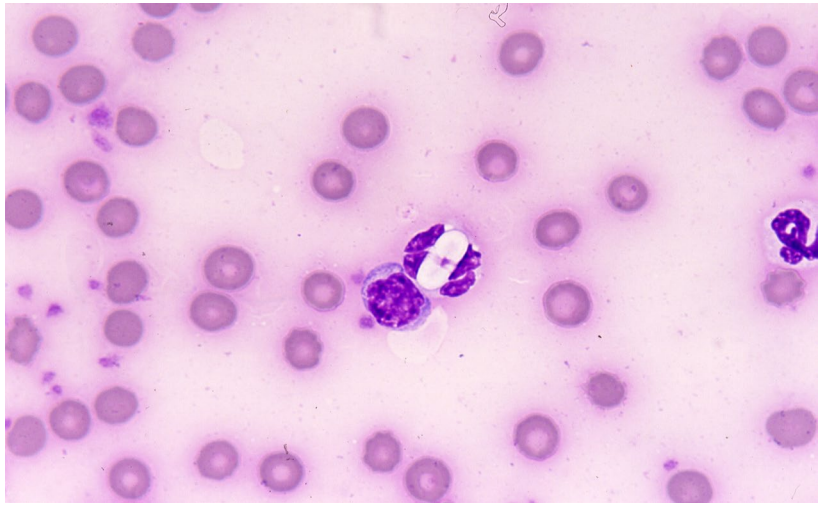
Clinical exam unremarkable other than

- Palpable splenomegaly
- Mild increase in tracheal reflex

Biochemistry unremarkable other than

- *Slight raise in liver enzyme (ALT 165 U/L)*
- *Mild hypoalbuminemia and hyperglobulinemia*





If you can, test for everything!

+ *Leishmania* – quantitative serology, PCR
+ Heartworm – antigen blood test, Knott's test
+ *Ehrlichia canis* and *Anaplasma* – serology, PCR
+ *Hepatozoon canis* – blood smear, PCR
+ *Babesia* - PCR
+ *Brucella canis* – consult external labs

Further investigation

- Thrombocytopenia and lymphopenia
- No significant changes on thoracic radiographs
- Ultrasound guided FNA of spleen revealed
  - Extramedullary haematopoiesis
  - Increased plasma cells and neutrophils
  - Microfilariae
- *Ehrlichia canis* antibody and Heartworm antigen positive
- Option to start treatment with doxycycline and moxidectin but given very guarded prognosis treatment was not pursued
- *Leishmania* status?

Test	Result	Units	Reference range
RBC	4.86	10^{12}/l	5.39–8.70
Haemoglobin	11.4	g/dl	13.4–20.7
HCT	0.36	l/l	0.38–0.56
MCV	74.1	Fl	59.0–76.0
MCH	23.5	Pg	21.9–26.1
MCHC	31.7	g/dl	32.6–39.2
Absolute reticulocyte count	47.6	10^{9}/l	≤110.0
Platelets	39 (no platelet clumping seen)	10^{9}/l	143–448
WBC	3.9	10^{9}/l	4.9–17.6
Neutrophils (absolute)	3.16	10^{9}/l	2.94–12.67
Lymphocytes (absolute)	0.2	10^{9}/l	1.06–4.95
Monocytes (absolute)	0.31	10^{9}/l	0.13–1.15
Eosinophils (absolute)	0.23	10^{9}/l	0.07–1.49
Basophils (absolute)	–	10^{9}/l	–

Overview Sample Slide

- Blood smears and direct blood work.
- Combine tests
- Test in house
- Take advantage of lab testing packages and bundles

Testimonial Slide

EDIT KEY TAKEAWAY

All five (of my) dogs were put down. They were the innocent party in this

– DR. JENNIFER SMITH, contracted *Brucella canis* from an imported dog

ESCCAP UK & Ireland

National Head

Professor Hany Elsheikha

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