WSAVA CONGRESS 2022 | 29-31 October Lima, Peru

Canine Babesiosis: a challenge for veterinarians

Gaetano Oliva

University of Naples Federico II, Italy

Dipartimento Medicina Veterinaria Produzioni Animali

WSAVA One Health Committee

Some materials of this lecture have been kindly provided by prof. Gad Baneth, Hebrew University, Israel, due to our long-time friendship and scientific collaboration

Once again, thanks !

Who we are

- *Babesia* are apicomplexan hemoparasites transmitted by ticks to mammals or bird

- They belong to the order Piroplasmida, because of their particular shape during the intraerythrocytic parasite stage, the piroplasm (Greek: pera = pear)

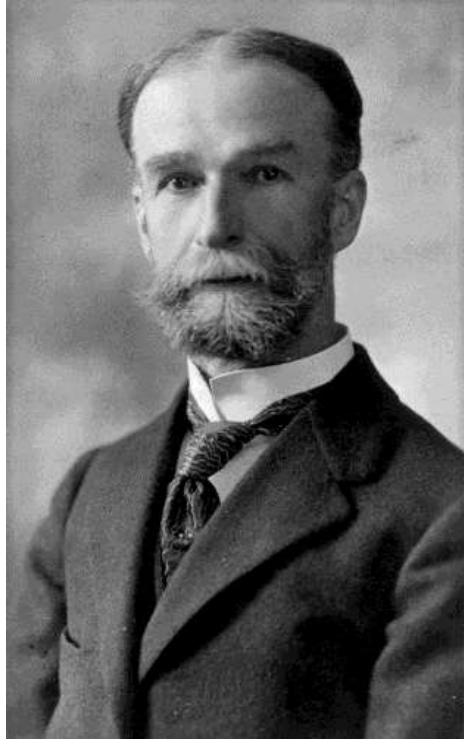
History

- Victor Babeş 1854-1926
- Romanian pathologist
- Described the causative agent of febrile hemoglobinuria in cattle (1888)
- Canine babesiosis reported in Europe for the 1st time from Italy in 1895

Victor Babeş 1854-1926

Theobald Smith

- Theobald Smith 1854-1926
- American MD that worked for the USDA
- Described the life cycle of *Babesia bigemina* (initially *Piroplasma bigeminum*), the cause of the cattle disease Texas Fever in 1893, with Frederic Kilborne.
- This was the first time that an arthropod was definitively linked with the transmission of any infectious disease

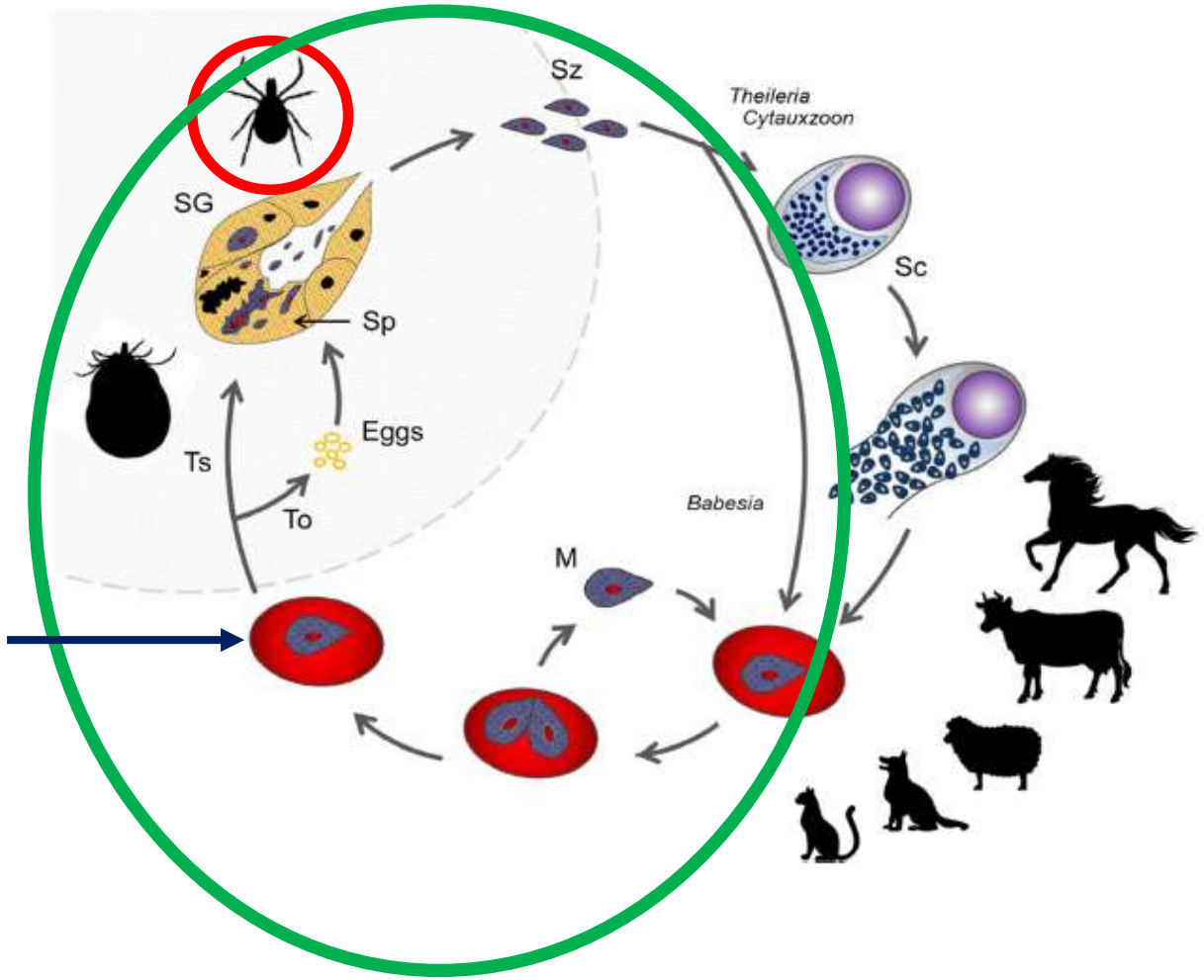


Theobald Smith 1859-1934

REMEMBER THAT:
Babesiosis may affect humans

- First well documented case of human babesiosis described in 1957 in Croatia, (former Yugoslavia) Skrabalo Z, Deanovic Z: Piroplasmosis in man: report of a case. Dot Med Georgr Trop 1957; 9: 11-16.
- A 33 y. old splenectomized farmer
- Incrimined pathogen: *Babesia bovis* (perhaps *Babesia divergens*)
- Fatal infection

Life cycle



Green circle: *Babesia spp*.

Red circle: Infected tick

SG: Salivary glands

Sz: Sporozoites

M: Merozoites

Blue arrow: gametocyte
(late Merozoite, no further division)

When another tick feeds on an infected vertebrate, gametocyte-infected erythrocytes are ingested and gametocytes egress and develop in *Babesia* into extracellular, ray-shaped isogametes

Schnittger et al., 2022

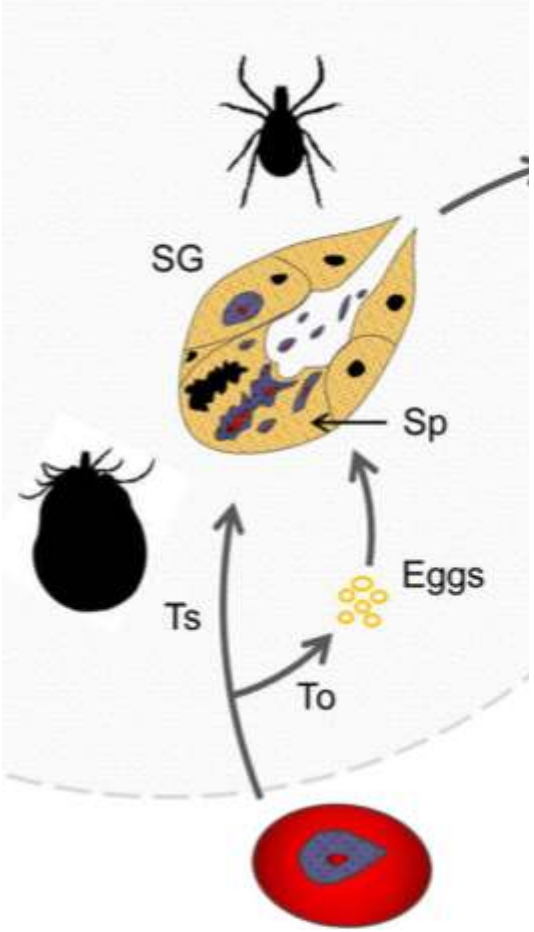
WHAT HAPPENS IN THE TICK

- Gametes fuse in the tick midgut and turn into the diploid zygote, or ookinete stage, which invades the gut epithelium

- Subsequent intracellular asexual replication results in the formation of primary kinetes that, in the case of *Babesia*, invade other tick tissues

- Secondary kinetes are generated and invade salivary glands. In salivary glands, metamorphosis of kinetes into sporozoites occurs, which are injected into a vertebrate during a blood meal, closing the cycle

In *Babesia* sensu stricto……



- Kinetes can invade the tick ovaries and then eggs, passing into the next tick generation (transovarial transmission)
- Depending on the species, kinetes will develop in sporozoites in the larvae or in the nymph salivary glands

Babesia affecting domestic dogs

Schnittger et al., 2022

Species	Candidate or confirmed tick hosts	Distribution	Pathogenicity	Merozoite size[b]	Clade[c]	Reference
B. vogeli	[a]*Rhipicephalus sanguineus* s.l.	Worldwide	Subclinical or mild in adults; severe in pups	L	*Babesia* s.s. (Clade VI)	Uilenberg et al. 1989; Zahler et al. 1998; Carret et al. 1999; Schnittger et al. 2012
B. canis	[a]*Dermacentor reticulatus*	Europe	Mild to severe depending on the individual	L		
B. rossi	[a]*Haemaphysalis elliptica*, *Haemaphysalis leachi*	Southern Africa, Nigeria, Sudan	Severe	L		
R. vitalii	[a]*Amblyomma aureolatum*	South America	Severe	S/M		Loretti and Barros 2005; Soares et al. 2011
Babesia sp. Coco	nk	USA	Severe in immunosuppressed dogs	L		Birkenheuer et al. 2004; Sikorski et al. 2010
B. gibsoni	[a]*H. longicornis*, *H. bispinosa*, *R. sanguineus* s.l.	Southeast Asia, USA, Australia, Europe	Mild to severe depending on the individual	S		Patton 1910
Babesia sp. Akita610	*Ixodes ovata*	Japan	Asymptomatic	nk		Inokuma et al. 2003
B. negevi	*Ornithodoros tholozani* ✦	Israel	Severe	S/M	Western group (Clade II), *Babesia* s.l.	Baneth et al. 2020
B. conradae	nk	USA, China	Severe	S		Kjemtrup et al. 2006
B. vulpes	*D. reticulatus*, *Ixodes hexagonus*, *I. ricinus*, *I. canisuga*, *R. sanguineus* s.l.	Europe, North America	Severe	S	*Babesia vulpes*-group (Clade Ib), *Babesia* s.l.	Baneth et al. 2015, 2019

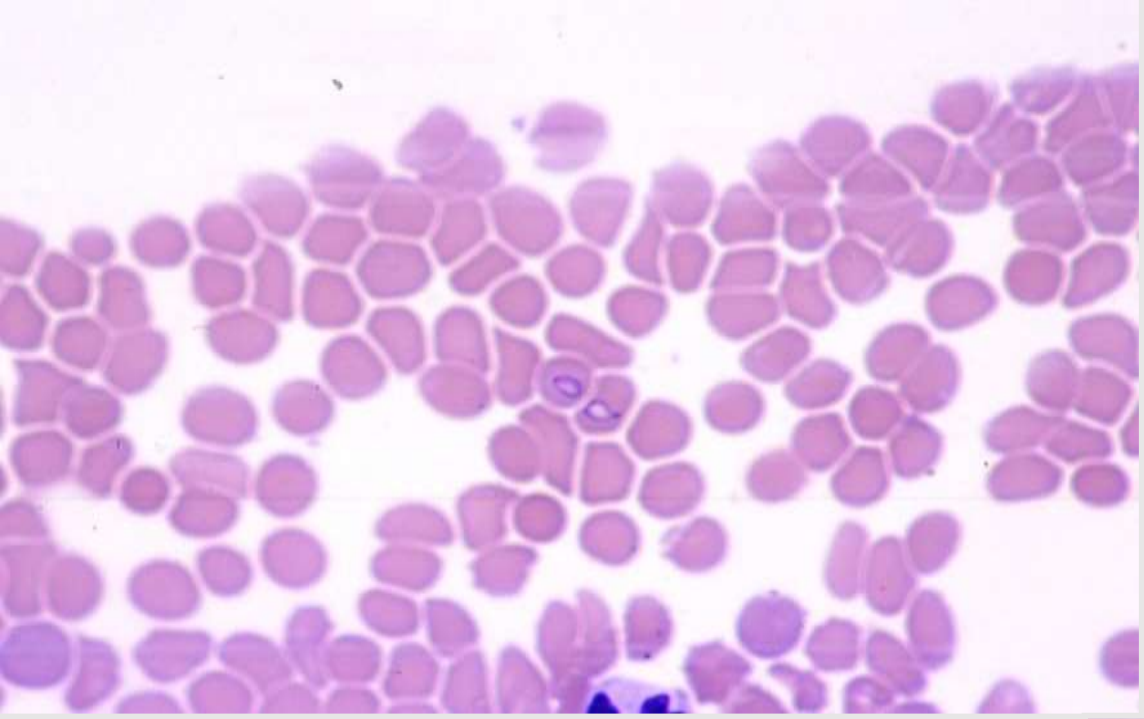
✦ *First record of Babesia in Argaside tick*

Microscopy identification of *Babesia spp*. in dogs

Large form *Babesia*	Small form *Babesia*
2.5-5.0 μm	1-2.5 μm

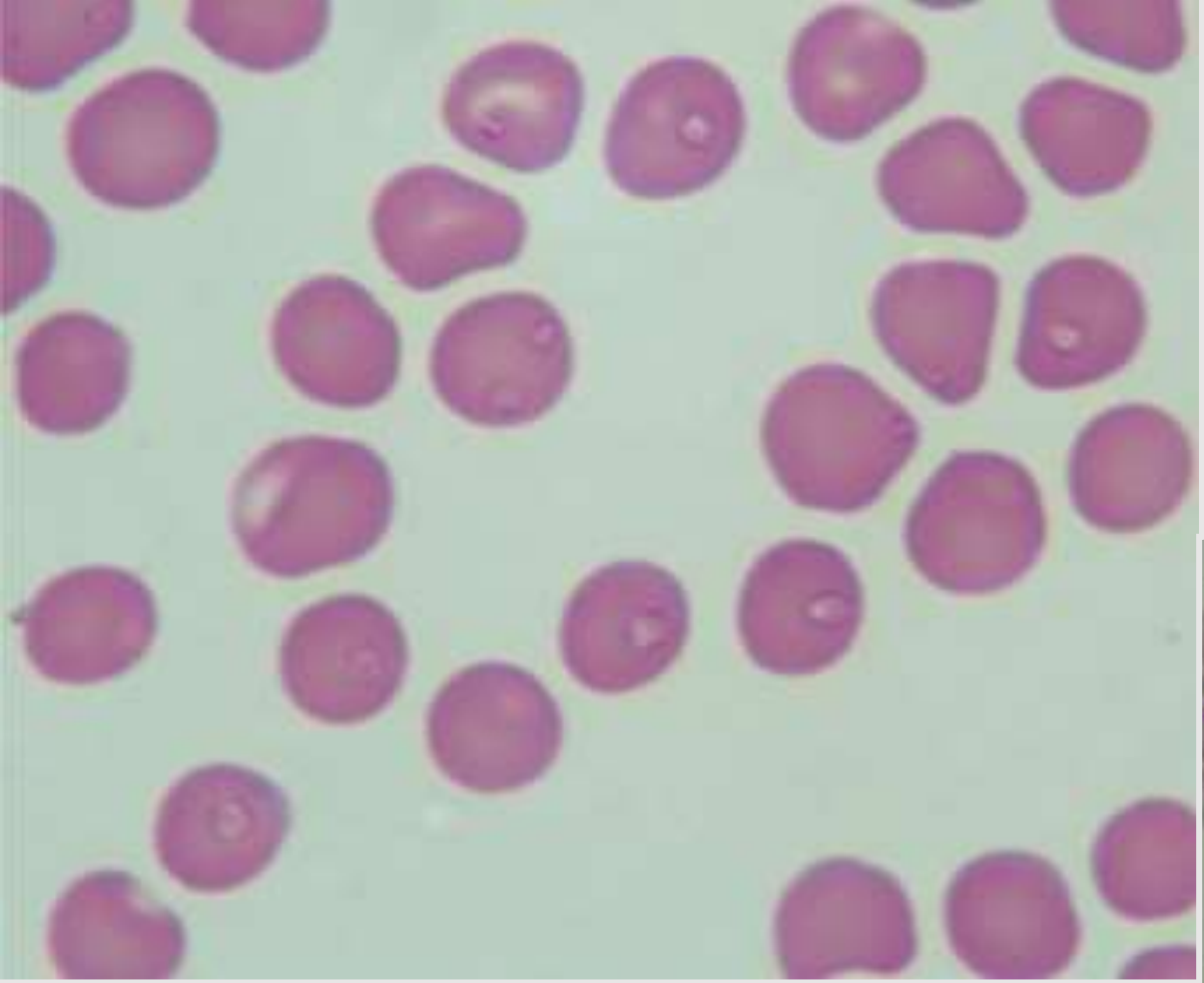
Babesia canis

Babesia gibsoni



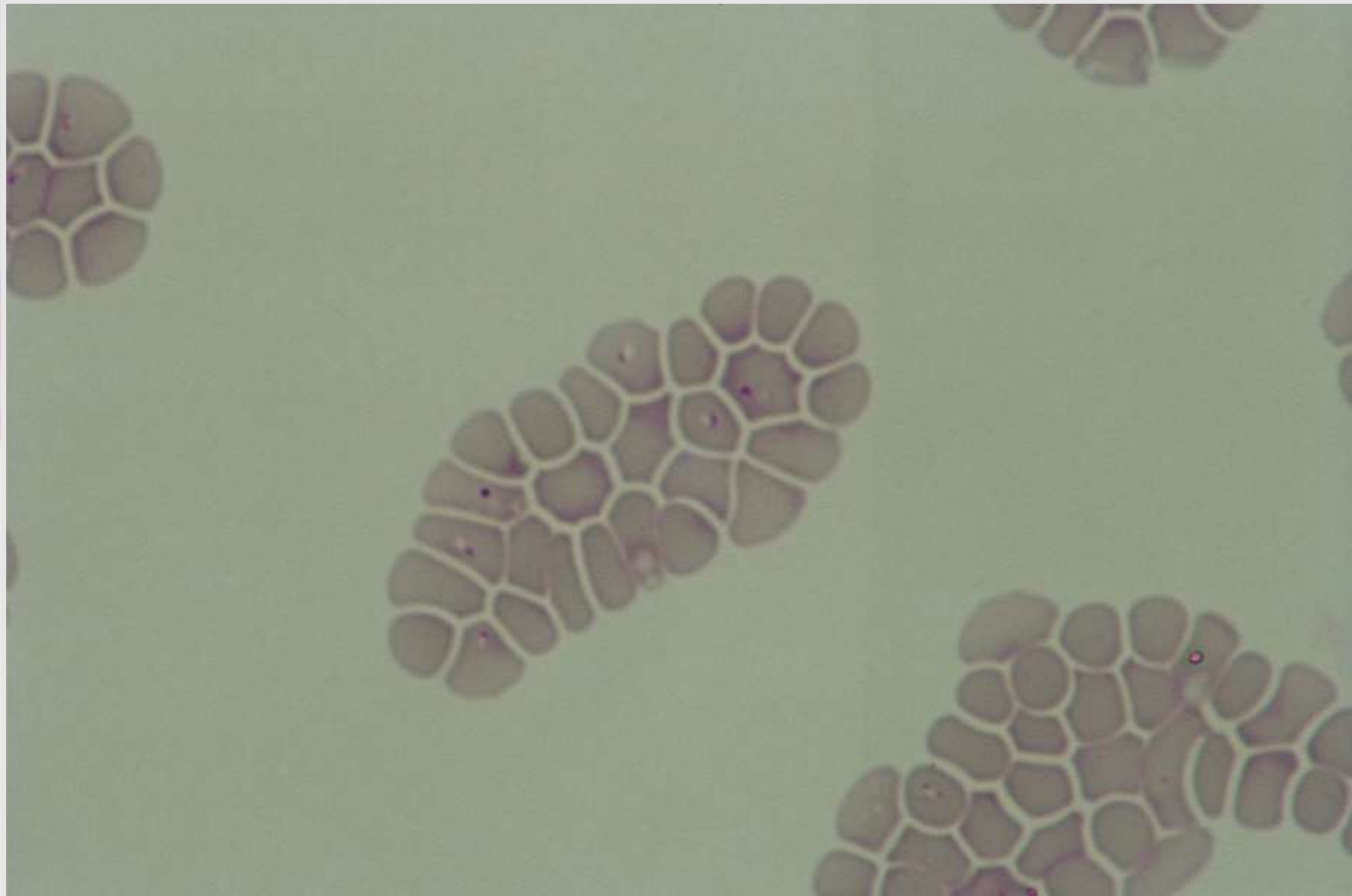
Large *Babesia*
(*Babesia vogeli*)

Courtesy: Prof G. Baneth

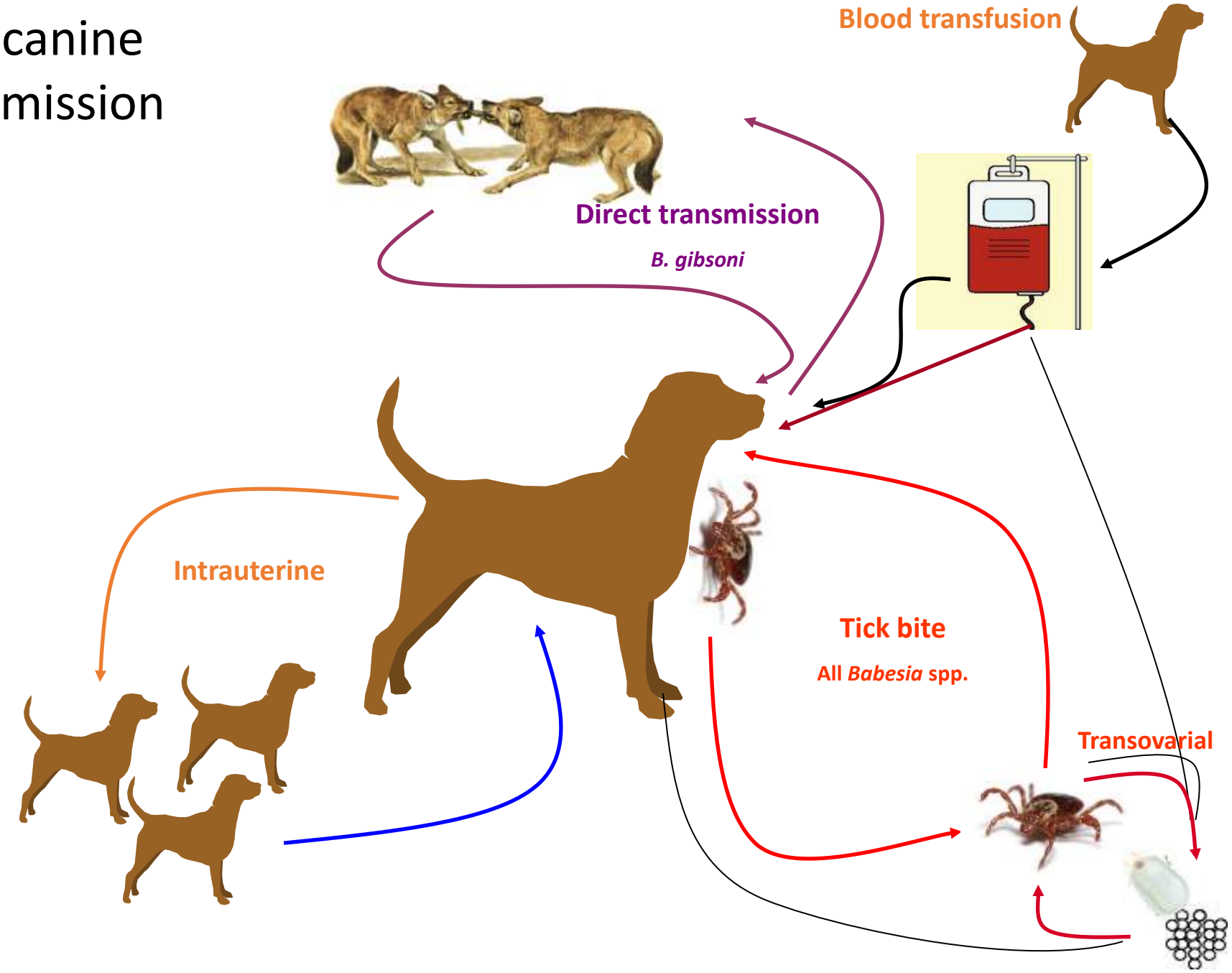


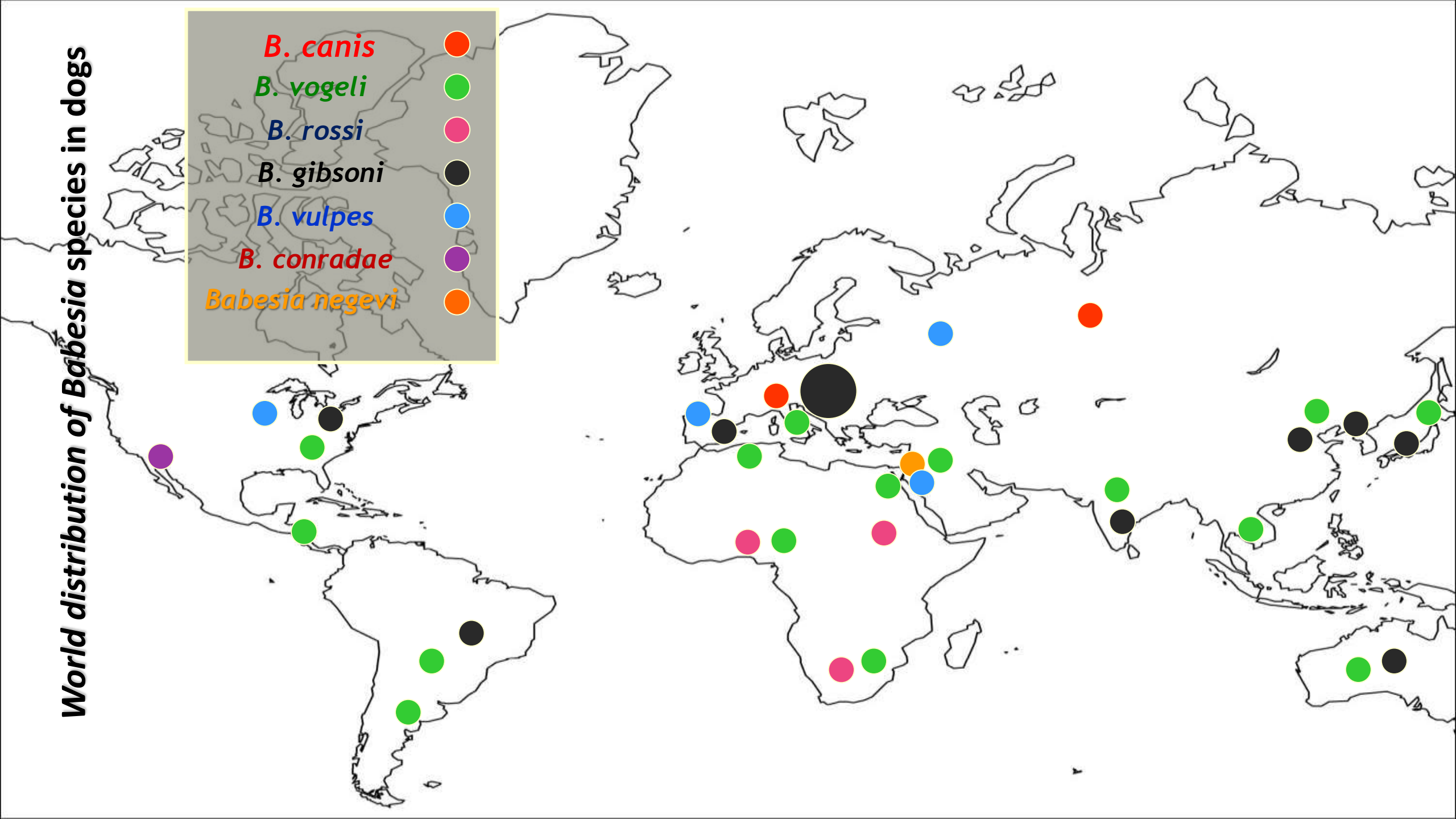
Small *Babesia*
(*Babesia gibsoni*)

Courtesy: Prof G. Baneth



Pathways of canine *Babesia* transmission




World distribution of Babesia species in dogs
B. canis
B. vogeli
B. rossi
B. gibsoni
B. vulpes
B. conradae
Babesia negevi

Map picture: Karasova et al., 2022

The «strange case» of *B. gibsoni*

- *Babesia gibsoni* first occurring in Asia, is spreading alla round the world, probably due to its particular way of transmission
- A Spanish study of the vector-borne disease's prevalence in 153 dogs from Barcelona confirmed the presence of *B. gibsoni* in 2% of the dogs examined (Tablar et al., 2009)
- A more recent italian study on 1311 randomly selected healthy dogs confirmed the presence of the parasite (positive: 0.2%) (Veneziano et al., 2018)
- Frequent detection of clinical cases in Balcanic and Central Europe Countries
- **<u>The most frequest infected dogs belong to dog fighting breeds</u>**

Figure 1. Occurrence of *Babesia gibsoni* in Europe.

The disease

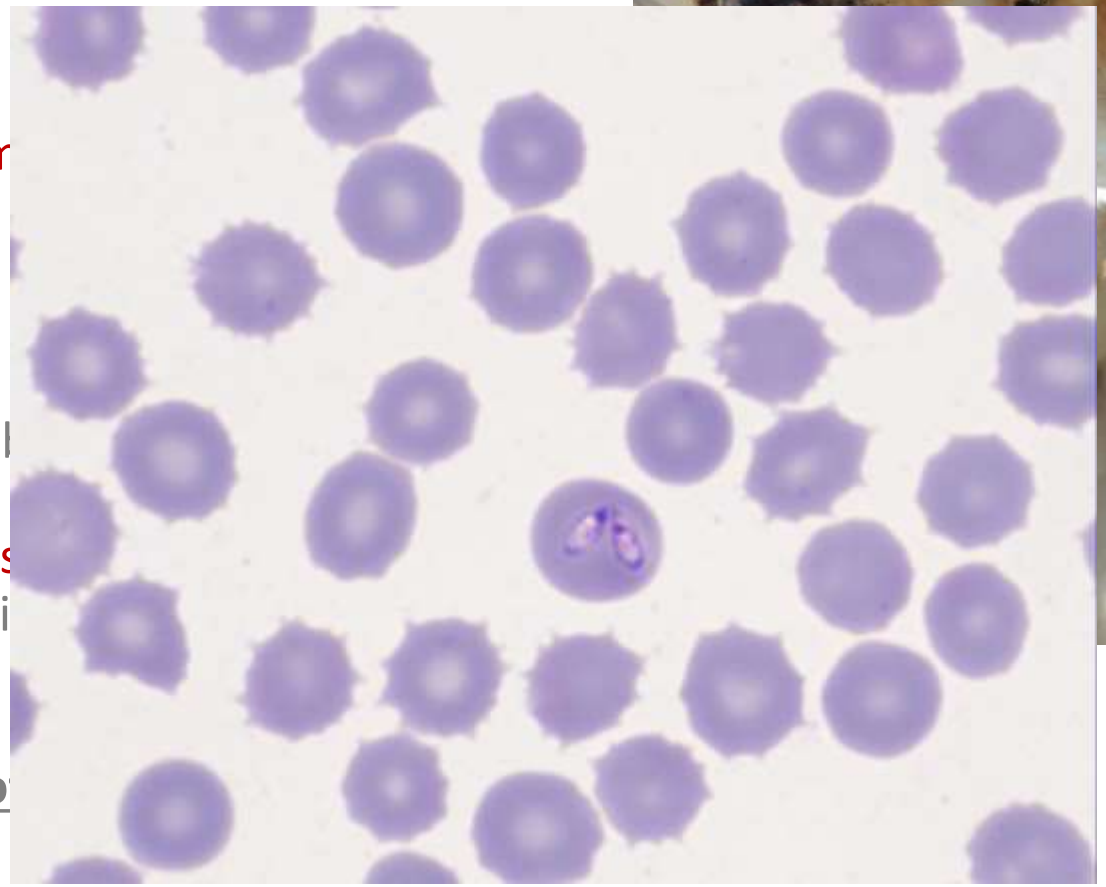
- Infection of dogs by *Babesia* spp. results in very varying clinical presentations, depending on the species as well as the age, immune status, and concomitant infections of the affected animal

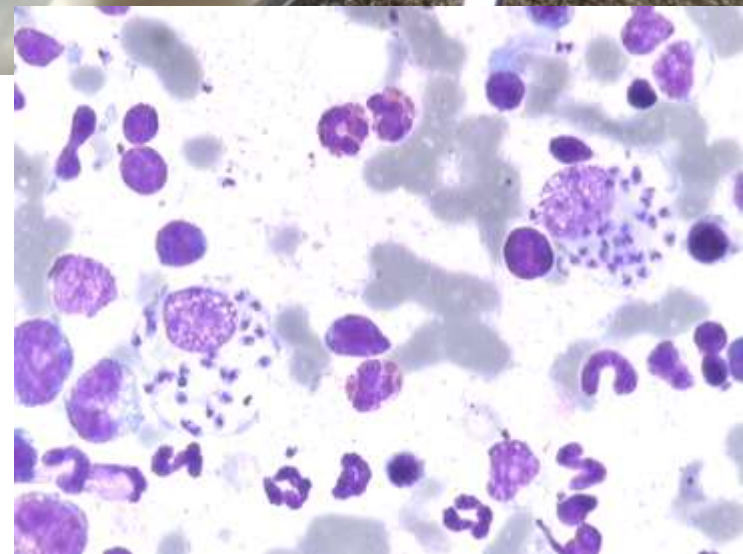
- Among large *Babesia*, *B. rossi* appears the most pathogenetic, while *B. vogeli* results in subclinical or mild disease

- Small *Babesia* pathogeneticy is currently under investigation, however most of them (*B. vogeli* example) may give severe disease

Factors that determine the severity of babesiosis in dogs

- Species of piroplasm infection
- Age
- Dog breed susceptil
- Host immune status suppression/neoplasi disease)
- **Co-infection** with o



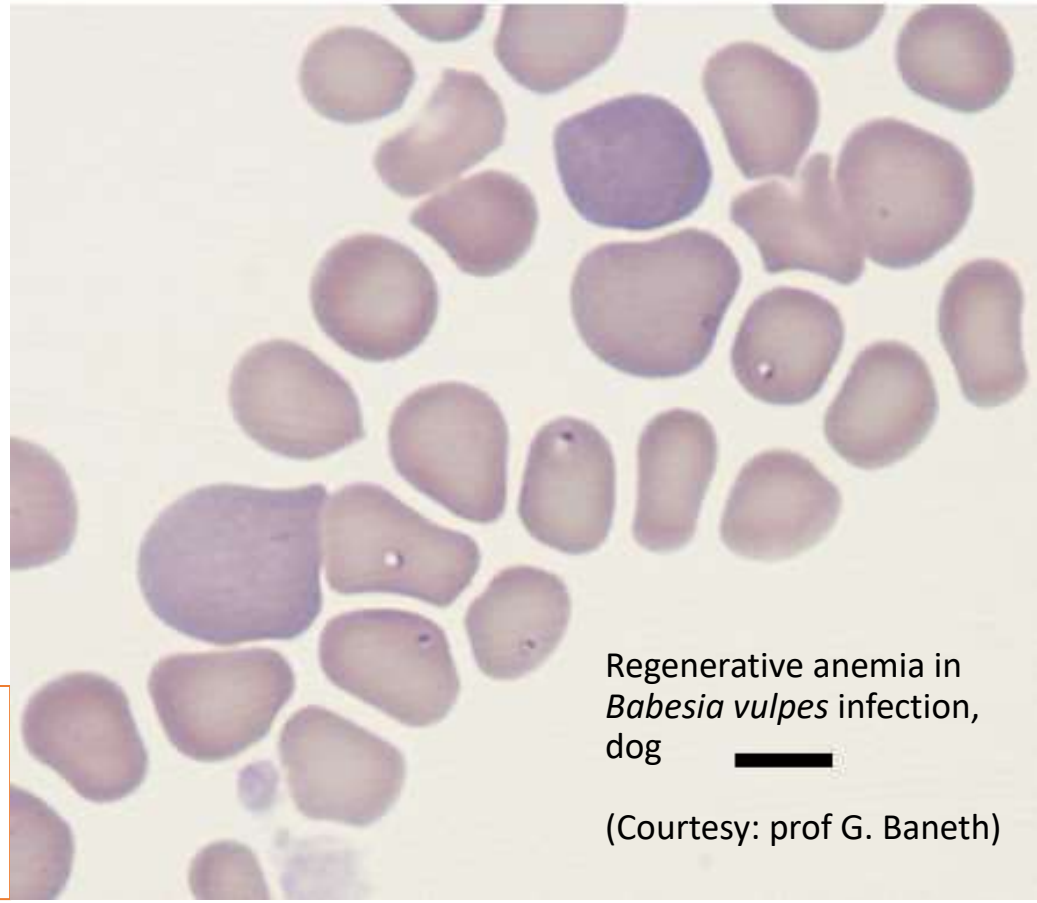


General Manifestations of *Babesia* infection

- Anorexia/lethargy/fever
- Lymphadenomegaly
- Splenomegaly
- Icterus
- Pigmenturia
- Weight loss
- Weakness

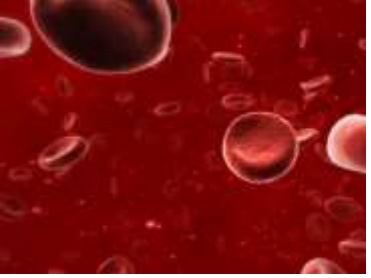
- Hemolytic anemia
- Thrombocytopenia

- Tissue hypoxia
- Cerebral babesiosis
- Acute renal failure, hepatopathy, pulmonary edema



Regenerative anemia in *Babesia vulpes* infection, dog

(Courtesy: prof G. Baneth)



Pathogenesis of babesiosis

- More mechanisms for hemolytic anemia other
- than direct parasite destruction of RBC:
  - Discharge of hemolytic toxins
  - Oxidative injury to RBC
  - Extravascular hemolysis
  - Intravascular hemolysis

Bullet points

- After invasion, *Babesia* and *Theileria* parasites reside, in contrast to *Plasmodium*, without a parasitophorous vacuole, directly within the cytoplasm of the erythrocyte
- *Babesia* evolved highly intricate means to escape the host immune defense by antigenic variation
- Babesia causes the appearance of adhesive protuberances on the surface of infected erythrocytes that provoke their sequestration in capillaries, avoiding destruction in the spleen

Babesia: different degrees and severity of anemia (different mechanism when compared to *Leishmania*), however consider subclinical/chronic cases

Piastrinopenia could be the main haematological alteration, together with mild non regenerative anemia)

(Furlanello et al., 2005)

Parameter	N. dogs ↓	N. dogs ↑	N. dogs N	Abnormal value	Min/max	Reference ranges ↓	Reference ranges ↑
Seg neu	17	0	6	2684 ± 797	806-3807	3900	8000
PLT	23	0	0	38.3 ± 19.8	11-79	143	400
MPV	0	22	1	20.7 ± 3.5	15-28	7	11
PCT	18	0	5	0.06 ± 0.02	0.03- 0.1	0.11	0.28
PDW	0	22	1	69.8 ± 7.5	54-83	37	51
aPTT	0	17	6	13.8 ± 0.9	12.9-16.3	10.6	12.8
PT	0	2	21	8.8 ± 0.9	8.3-9.9	6.7	8.2
Fibrinogen	0	23	0	758 ± 152	5.07-898	150	400
FDPs	0	5	18	-	-	<2.5	
D- Dimer	0	4	19	0.7 ± 0.26	0.4-1.03	0.01	0.33
AT	11	0	12	91 ± 6.3	81-99	100	148
CK	0	17	6	692 ± 1669	121-7105	45	100
AST	0	20	3	151 ± 161	45-760	15	37
ALT	0	15	8	168 ± 229	52-924	15	50
ALP	0	19	4	249 ± 196	107-954	20	150
Tot Bilirubin	0	16	7	2 ± 3.3	0.27-13.6	0.15	0.26
Tot Protein	12	0	11	5.6 ± 1.1	3.9-5.6	5.7	7.7
Fe	12	0	11	55 ± 18	22-89	100	220
TIBC	12	0	11	267 ± 19	232-295	300	410
Lactic acid	0	12	2	34 ± 22	15-88	0	9

Zoonotic Risk of Babesiosis

- All known *Babesia* species hav
- The main species of
  - *B. di*

- Complications develop in approximately half of the patients hospitalized with babesiosis
  - Acute respiratory distress syndrome (ARDS)
  - Disseminated intravascular coagulopathy (DIC)
  - Congestive heart failure
  - Coma
  - Splenic rupture
  - **Fatality rates: 6-9%**

- **No ca as been implicated as zoonotic to date**
- **Some c *gibsoni* have been reported. Source of infection under investiga on**

BIBA

a Vet’s dog…

Biba: (end of April 2022)

- Neutered female
- Mongrel
- 3 Ys
- 23kg
- Adopted from a public kennel when she was 1 year old

History:

- After the adoption, she was diagnosed for hypoadrenocorticism
- At the age of two years, she resulted serologically positive to *Babesia spp. Leishmania spp*. and *Rickettsia spp*. during the routine clinical and biochemical follow up
- Mild Anemia and Thrombocytopenia, no other clinical signs
- Treatment for Leishmaniasis (MIL + ALL) and Rickettsiosis (Doxi)
- Normalization of both parameters after one month of treatment, relapses after 3 months
- Referred to OVUD (April 2022) for lameness and generalized painful joints, again mild anemia and thrombocytopenia.
- IFAT Leishmania: 1:320; lymph node n-PCR +
- 18S rRNA gene PCR + (*Babesia spp*), then sequencing: *B. gibsoni*

Treatment

- Actually:

- Allopurinol (10 mg/kg, orally, every 12 h),
- Desoxycortone pivalate (Addison)

- Previously:
- Atovaquone (13,3 mg/kg, orally, every 8 h) + Azithromycin (10 mg/kg SID)

- Clindamycine - Metronidazole - Doxicicline

April 2022

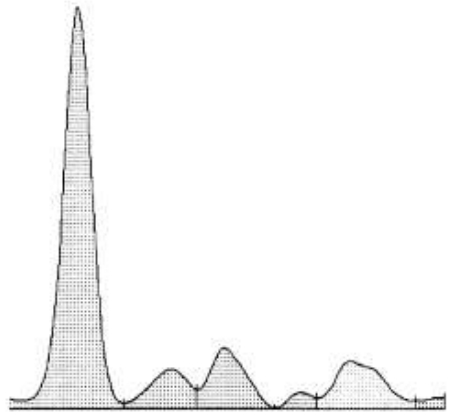
Haemogram

Parametro	Risultato	Range
RBC	4,80	5.65 – 8.87 (M/uL)
HCT	30,0	37.3 – 61.7 (%)
HB	10,6	13.1 – 20.5 (g/dL)
MCV	62,5	61.6 – 73.5 (fL)
MCH	22,1	21.2 – 25.9 (pg)
RET	5,3	10,0 – 110,0 (K/uL)
WBC	9,82	5.05 – 16.76 (K/uL)
NEUT	5,09	2.95 – 11.64 (K/uL)
LINFO	2,89	1.05 – 5.10 (K/uL)
MONO	0,79	0.16 – 1.12 (K/uL)
EOSIN	1,03	0.06 – 1.23 (K/uL)
BASOF	0,02	0.00 – 0.10 (K/uL)
PLT	117	148 – 484 (K/uL)
MPV	12,0	8.7 – 13.2 (fL)

Biochemistry

Parametro	Risultato	Range
AZO	42	25 – 50 (mg/dL)
CREA	1,32	<1.8 (mg/dL)
TRIGL	20,7	20 – 60 (mg/dL)
CHOL	Nd	135 – 270 (mg/dL)
PT	5,58	6 – 7.8 (g/dL)
GLU	91	60 – 110 (mg/dL)
ALT	15	10 – 47 (IU/L)
BIL TOT	0,41	0.10 – 0.50 (mg/dL)
ALP	123	<180 (IU/dL)

ELETTROFORESI DELLE SIEROPROTEINE



SPECIE : CANE

Frazioni	Val. %	Riferimenti %	Val. g/dL	Riferimenti g/dL
Albumina	64.6	51.0 - 63.0	3.55	3.06 - 4.72
Alfa1	8.2	4.2 - 4.7	0.45	0.25 - 0.35
Alfa2	11.6	5.3 - 8.5	0.64	0.32 - 0.64
Beta1	2.0	3.2 - 8.3	0.11	0.19 - 0.62
Beta2	12.4	5.0 - 13.8	0.68	0.30 - 1.03
Gamma	1.2	12.0 - 13.0	0.07	0.72 - 0.98

Proteine Totali : 5.50 g/dL [6.00 - 7.50]
Rapporto A/G : 1.82 [0.50 - 1.30]

Commenti

Urinary profile

Parameter	Risultato	Range
Color	Vogel II	-
Glucose	Ass	Ass
Bilirubin	Ass	Ass
SG	1015	>1030
Blood	Pres+	Neg
pH	6,0	
Protein	Ass	Ass
Urobilinogen	Norm	Norm
Nitrites	Neg	Neg
Chetons	Ass	Ass
Leucocytes	Ass	Ass
UPC	0,13	-
Sediment	Eritrociti	Neg

Diagnostics

- Examination of the dog's history
- Epidemiological data in the area
- Complete clinical examination/clinicopathological
- Concurrent diseases
- Splenectomy/blood transfusion
- Bites by another dog

Identification of the species responsible for infection is imperative

THEN…

- B… …pillary vein)
- Se… - IFAT/ELISA useful to detect chronic infected dogs with low parasitemia)
- Mole… …ar approach (18S rRNA gene as target, then specific PCR/sequencing)

The use of PCR specific for *Babesia spp*. in regions where both *B. canis* and *B. gibsoni* are common, a negative test will exclude Babesia disease

When results are positive a further Investigation should be done by using specific PCR for determining of the species and thereafter the appropriate treatment

Treatment

- To date, no treatment of *Babesia* infection that would be able to completely eliminate parasites from the body has been described
- The owner of the dog treated for *Babesia* infection should be understand the risk of recurrence of the disease. In addition, treated dogs may become a source of infection and potential reservoir
- In the case of small *Babesia* spp., frequent recurrences of the disease occur after treatment, even though the dog appears clinically healthy
- Anti-Babesia drugs are able to improve the clinical condition
- Treated dogs may have very low parasitemia that makes PCR not sensible to detect it

<u>THESE CONCEPTS MAY BE APPLIED TO MANY CVBDs</u>

Drugs

Imidocarb Proprionate - aromatic diamidine

- Blocks the uptake of inositol into the infected erythr… …ion)
- Damage to parasite DNA

Therapeutic dosage:

- 6.6 mg/kg of body we… …s at 14-day intervals

Side eff…

P… …osis) and

…ic symptoms (Atropine premedication – 0.05 mg/kg)

Imidocarb proprionate is not efficacy against Small *Babesia*, particularly against *B. gibsoni*. Good efficacy against *B. canis*

Drugs

Diminazene Aceturate - aromatic diamidine:

- Mechanism of action not fully elucidated
- Disruption of parasite DNA synthesis and aerobic glycolysi

Therapeutic dosage:

- 3-5 mg/kg of body weight, IM or s ervals
- alternative protocol: 2 mg/kg of utaneous applications every 48 h + Clindamycin 25 mg/kg of body weight, or

SEVERE sid

- Neurol , ataxia, convulsions, paralysis, and death, especially in dogs
- P recurrences
- nany Countries

Diminazene Aceturate has a proven efficacy against Large and Small *Babesia*, strongly limited from its toxicity

Drugs

Atovaquone – synthetic hydroxinaphthoqu

- inhibition of pyrimidine and adenosine triphosph
- Anti-Malaria drug, largerly used in humans

ydrocloride)

Therapeutic dosage:

- 13,3 mg/kg of body weig
- + Azithromycin 10 m

days

Pitf

cloride in many Countries

Atovaquone-Azithromycin is considered the treatment of choice for *B. gibsoni* infection; treatment does not allow definitive clearence, relapses may occur. Many other anthibiotic combination, by using Doxycyclin, Enrofloxacin, Clyndamicin have been used with no clear advantage

Supportive care

Depending on clinical condition:

- oxygen therapy
- Blood transfusion
- Fluid therapy to correct acid-base imbalance and electrolyte abnormalities

- Immune-suppressive corticosteroid therapy is controversial

  Like in the other CVBDs treatment

Prevention

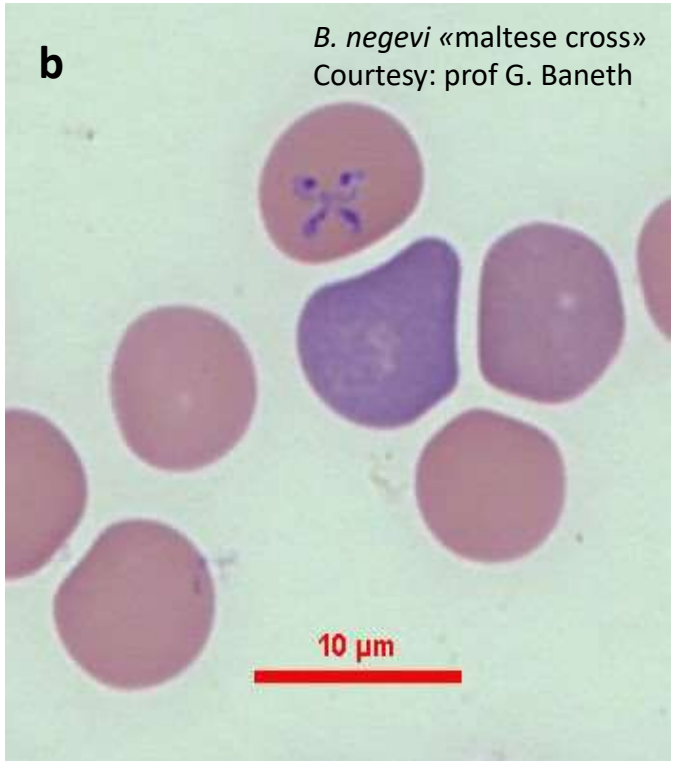
- Tick avoiding-bite drugs (Pyrethroids)
- Insecticides (Isoxazolines)
- Tick removing/killing

- Avoid blood transfusion from infected dogs
- Check blood product (PCR) before transfusion in endemic area
- Avoid mating with infected dogs (vertical transmission)

- Vaccine (Pirodog®) developed for *B. canis and rossi*, does not prevent the infection

Summary and conclusions

- Canine babesiosis is caused by different and divergent pathogens that induce diseases with a high degree of similarity
- The genus *Babesia* will probably be split into more than one genera in the future
- Identification of the species responsible for infection is imperative



The University of Naples was founded in 1224, at the behest of King Federico II

The Veterinary School was founded in 1795, at the behest of King Ferdinand IV

Dipartimento
Medicina Veterinaria
Produzioni Animali