Vectorial capacity of *Haemaphysalis hystricis* and *Rhipicephalus sanguineus* ticks for *Babesia gibsoni* in dogs in Taiwan

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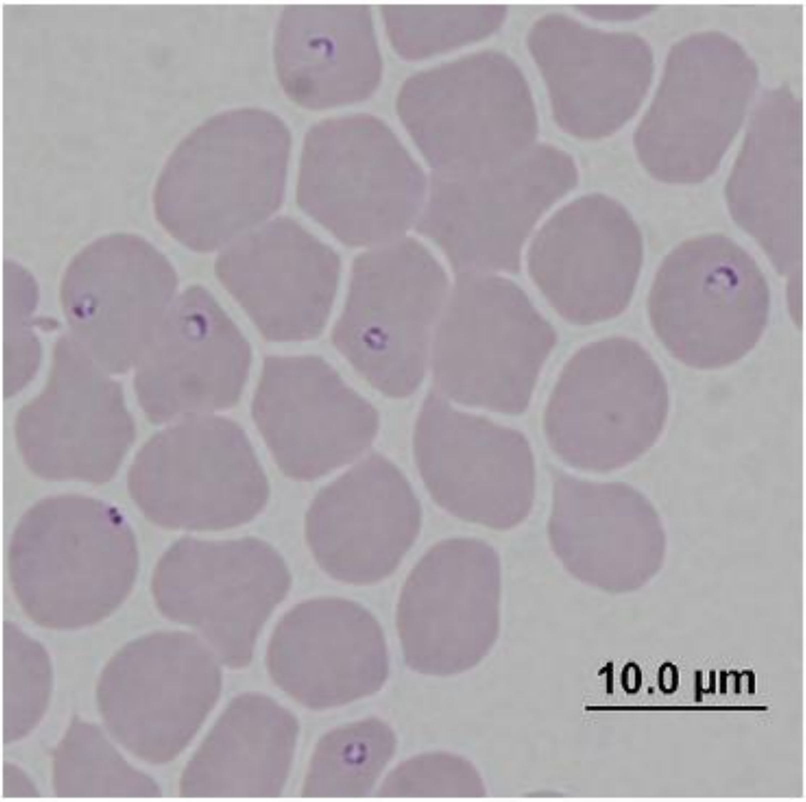

B. gibsoni in erythrocytes (Irwin 2010)

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1 Abstract

BACKGROUND: *Babesia gibsoni* is a tick-borne blood protozoan parasite, which is responsible for causing babesiosis in dogs. It has been reported that this parasite is mainly transmitted by the ticks *Haemaphysalis longicornis* and *Rhipicephalus sanguineus* in Asian countries. The disease relationship of *Haemaphysalis hystricis* has not been studied, therefore it is not known if this species may also be a vector for *B. gibsoni*.

METHODS: *H. hystricis* and *R. sanguineus* ticks were collected from dogs in Taiwan and were sent to the Utrecht Centre for Tick-borne Diseases (UCTD). At the UCTD a Polymerase Chain Reaction (PCR) and Reverse Line Blot hybridization (RLB) were done. Moreover, DNA samples from dogs in Taiwan were also tested at the UCTD by PCR and RLB. A second generation *H. hystricis* nymphs was obtained from one collection first generation *H. hystricis* adults, which were also tested at the UCTD.

RESULTS: A total of 185 ticks were used for testing of which 104 were *H. hystricis* adults, 80 were *R. sanguineus* adults and 1 was a *H. hystricis* nymph. *B. gibsoni* infection was detected in 17,3% (18/104) of *H. hystricis* ticks, whereas none of the *R. sanguineus* ticks (0/80) were infected. Furthermore, *B. gibsoni* infection was not found in the second generation of *H. hystricis* nymphs. Also, 122 DNA samples were tested at the UCTD, which revealed that 20,5% (25/122) was positive for *B. gibsoni*. Finally, *Babesia vogeli* (n=4) was also found in *R. sanguineus* adults.

CONCLUSIONS: This study provides evidence to support the hypothesis that *H. hystricis* may be the vector of *B. gibsoni* and not *R. sanguineus*. The vector competence of both ticks need to be further studied.

Keywords *Haemaphysalis hystricis* · *Rhipicephalus sanguineus* · Tick · *Babesia gibsoni* · Taiwan

2 Introduction

2.1 *Babesia gibsoni*

Babesiosis is an infection often seen in animals worldwide, which is caused by many different *Babesia* species. It is gaining increasing interest due to its emerging zoonotic aspects in humans (Yao et al. 2014; Imre et al. 2013). *Babesia gibsoni* is a tick-borne blood protozoan parasite in the group of small *Babesia* species, which is responsible for causing babesiosis in dogs. It is the pathogen responsible for canine babesiosis in Taiwan. There is no evidence that *B. gibsoni* can infect humans. However, dogs and cats can serve as a source of infected ticks for humans due to the close companionship (Imre et al. 2013; Mandal et al. 2015; Otranto et al. 2009; Lin et al. 2012; Yamauchi et al. 2009; Solano-Gallego & Baneth 2011).

Also, *Babesia vogeli* is found in Taiwan*.* It is a parasite in the group of large *Babesia* species beside *Babesia canis* and *Babesia rossi.* All large *Babesia* subspecies show morphological similarities, but all have different vectors and pathogenicity. *B. vogeli* is found worldwide in tropical and subtropical climates including Asia. Moreover, this parasite is transmitted via the vector tick *R. sanguineus* (Irwin 2010; Adaszek & Winiarczyk 2008; Cacciò et al. 2002).

2.1.1 Different types of small *Babesia* species

Three different small *Babesia* species have been found. Small *Babesia* infections are now attributed to the Asian isolate (*B. gibsoni*), the Californian isolate and *B. microti*-like organism in Europe (Boozer & Macintire 2003). A synonym used for the microti-like organism is *Theileria annae*, which is not a *Theileria* but a parasite most closely related to *Babesia microti*. It is said that this parasite differs from both *Theileria* and *Babesia,* therefore a new genus may be required. *Babesia vulpes* is another name used for *B. microti* (Baneth et al. 2015; Uilenberg 2006).

The Asian isolate is identical to the strain identified in most of the United States, excluding California. The Californian strain has been reported in many breeds of dogs, while *B. gibsoni* almost exclusively affects pit bulls and American Staffordshire terriers in the United States. Other breeds of dogs can be affected by *B. gibsoni*, mostly due to an attack by pit bulls (Boozer & Macintire 2003).

2.1.2 Geographical distribution of *B. gibsoni*

B. gibsoni is endemic in Southeast Asia. However, *B. gibsoni* is also found in the United States, South America, Australia and Europe (in particular Spain, Germany and Italy). Figure 1 shows the distribution of several *Babesia* species including *B. gibsoni* in Europe (Solano-Gallego & Baneth 2011)[1].

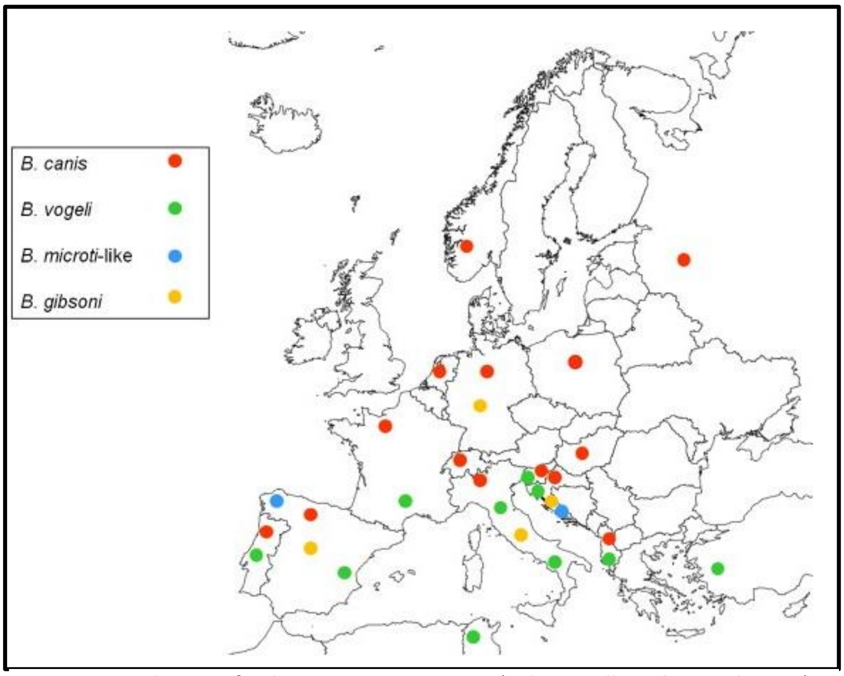


Figure 1: Distribution of Babesia species in Europe (Solano-Gallego & Baneth 2011)

2.1.3 Transmission

B. gibsoni can be transmitted by vector ticks, as discussed on page 8-9. Moreover, the transmission of *B. gibsoni* during aggressive interactions between fighting dogs is recognized as the major route of infection in countries outside Asia. Certain breeds as the American pit bull and Staffordshire terriers are used for illegal fighting, which can cause transmission of pathogens including *B. gibsoni*. This is the reason for the geographical distribution and clonal expansion (Irwin 2010).

Also, *B. gibsoni* can also be transmitted via blood transfusion or iatrogenic inoculation with contaminated needles or surgical instruments. So, recipient dogs can develop life-threatening immune-mediated hemolytic anemia without exposure to the classical tick vector (Otranto et al. 2009; Chen et al. 2015). Finally, *B. gibsoni* can be transmitted vertically via the uterine route (Fukumoto et al. 2005).

[1] *B. microti*-like refers to *B. vulpes* (Baneth et al. 2015)

2.1.4 Mechanism of action and clinical signs

B. gibsoni replicates in erythrocytes and initiates a mechanism of antibody-mediated cytotoxic destruction of circulating red blood cells which can cause clinical signs (Chen et al. 2015). The acute form in canines is associated with the presence of remittent fever, anorexia, progressive anemia, lethargy, thrombocytopenia, haemoglobinuria, splenomegaly and hepatomegaly. The anemia increases over a period of two to four weeks and corresponds with an increasing parasitemia. At the end of this period death or recovery occurs (Mandal et al. 2015; Groves & Dennis 1972).

2.1.5 Diagnosis

Diagnosis of *B. gibsoni* is made by identifying organisms on blood smears and the parasite can be readily distinguished from larger *Babesia* species as *B. vogeli,* which is shown in table 1. It can be complicated to make this diagnosis because low levels of parasitemia are common even with patent infections. To increase parasite detection, buffy coat smears or blood smears made from capillary blood can be examined. Even though the organisms may be low in number in chronic or subclinical infections, which makes it difficult to find the organisms, an infection is easy to find in clinical cases due to the clinical signs (Boozer & Macintire 2003; Mandal et al. 2015; Groves & Dennis 1972).

Characteristic	Large *Babesia* species (*B. canis, B. rossi* and *B. vogeli*)	*B. gibsoni*
Morphology	Typically pear-shaped with a heavy ring of cytoplasm and small vacuole. Marked tendency for ameboid activity resulting in bizarre shapes	Small delicate rings of bluish cytoplasm surrounding a vacuole with one to two chromatin dots located at the periphery of the cytoplasm. Oval, elongate, and band-like parasites seen rarely
Size	Typical parasites 3.0 – 5.0 μm in length, occupy more than 1/5 the area of the red blood cell (RBC).	Rings forms 1.5 – 2.5 μm in diameter, occupy less than 1/5 the area of the RBC
Multiply parasitized RBCs	Frequent	Only in heavy infections and then rarely more than two parasites/RBC

Table 1: The difference between B. gibsoni and large Babesia species on blood smears (Groves & Dennis 1972; Adaszek & Winiarczyk 2008)

<u>2.1.6 Therapy for *B. gibsoni* infections in canines</u>

No single drug is sufficient for the treatment of *B. gibsoni*. Therefore, drug combinations appear to be a better choice for treating this parasite. It has been reported that atovaquone with azythromycin is an effective therapeutic strategy for suppressing parasitemia. However, after this treatment *B. gibsoni* could still be detected by PCR. Doxycycline with diminazene aceturate is another treatment strategy, but persistent parasitemia can still be detected in blood after this treatment. Clindamycin has also been evaluated. This drug is one of the lincomycin antibiotics, which has been shown to be effective against human babesiosis. However, treatment with clindamycin alone it not enough to eliminate the clinical symptoms and babesia in the blood. A combination of clindamycin, doxycycline and metramidazole can be a successful therapy. Nevertheless, some dogs showed persistent parasitemia after treatment with this combination. No current therapeutic strategy has been found to completely eliminate *B. gibsoni* infections (Lin et al. 2012; Suzuki et al. 2007). Finally, imidocarb dipropionate is not effective as therapeutic strategy for *B. gibsoni* (Chen et al. 2015).

It is important to remember that treating babesiosis reduces parasitemia and supports resolution of clinical signs. However, the infection itself may not be eliminated after treatment.

Therefore, dogs diagnosed with *Babesia* should be considered as permanent carriers of the infection (Chen et al. 2015).

Ticks cannot transmit an infection immediately upon first attachment to a host. They require a period of 24 to 48 hours of initial feeding before organisms are able to pass through the salivary glands into the vertebrate host. Therefore, tick removal should not be delayed (Chen et al. 2015).

2.1.7 Prevention

To prevent an infection with *Babesia* species various measures can be taken. First, topical and environmental acaricidal treatments aim at reducing the exposure to vector ticks and pathogen transmission to dogs. Collars, spot on formulations and sprays (which include permethrin, amitraz, fipronil, imidacloprid and other chemicals) are most effective in controlling tick infestations on individual animals (Solano-Gallego & Baneth 2011).

Also, screening canine blood donors is recommended as *Babesia* species are transmitted by blood product transfusions. Moreover, aggressive interactions between dogs should be reduced (Solano-Gallego & Baneth 2011).

2.2 Vector ticks for *B. gibsoni*

B. gibsoni is mainly transmitted by the ticks *Haemaphysalis longicornis* and *Rhipicephalus sanguineus* in Asian countries (Víchová et al. 2016; Irwin 2010). *H. longicornis* is the predominant tick in dogs and cats and is thought to be the main vector for *B. gibsoni* in East Asian countries (Iwakami et al. 2014; Maeda et al. 2015; Taylor et al. 2007).

R. sanguineus is known as the kennel tick or brown dog tick, which is found on dogs in (sub)tropical regions of the world. *R. sanguineus* is also a dominant tick species on wild and domestic canines in Asia. The tick can survive in indoor and outdoor environments and it is a vector of a wide range of pathogens including *Rickettsiae conorii*, *R. rickettsii*, *E. canis*, *H. canis,* and *B. vogeli*, besides *B. gibsoni* (Imre et al. 2013; Otranto et al. 2009; Shimada et al. 2003).

Furthermore, *H. hystricis* is a tick found in subtropical and temperate zones. The disease relationship of this species is not recorded yet. Therefore, it is not known if this tick species could be a vector for *B. gibsoni*. Further research is required (Shimada et al. 2003; Geevarghese & Mishra 2011).

2.2.1 Geographical distribution of the vector ticks

H. longicornis is widely distributed in the Far East and Australasia. In addition, this species shows a geographical distribution from northern to southern Japan. *R. sanguineus* is believed to have originated in Africa, but is now considered to be the most widely distributed tick species in the world (Shimada et al. 2003; Taylor et al. 2007).

H. hystricis is found in the Oriental Region. The distribution of *H. hystricis* is restricted to a subtropical and temperate belt reaching out from the eastern Himalayas of India to the coasts of Vietnam and Fukien Province in China, and to Taiwan, Ryukyu islands and Okinawa islands. Furthermore, *H. hystricis* is found in Thailand, Malaysia, East Indies (Southeast Asian islands), Sri Lanka, northern Burma and Laos as shown in figure 2. However, the distribution of *H. hystricis* may be wider than reported before (Shimada et al. 2003; Hoogstraal et al. 1965; Geevarghese & Mishra 2011).

Also, the seasonal occurrence of *R. sanguineus* and *H. longicornis* has been reported. *H. longicornis* is mainly found in September, followed by June and May. Fewer *H. longicornis* is collected in October and November than in other months. *R. sanguineus* is mainly active in September, followed by October and June. No *R. sanguineus* ticks were found in November (Shimada et al. 2003).

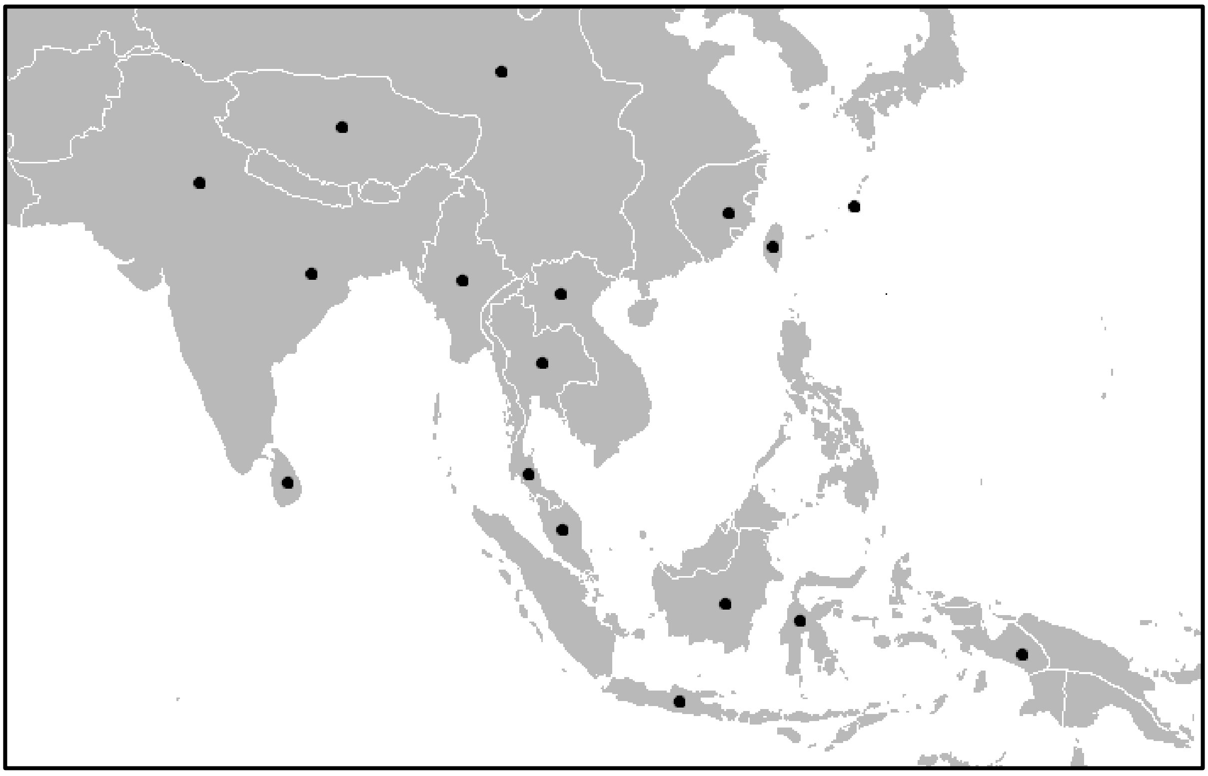


Figure 2: Distribution of H. hystricis ticks in the Oriental Region

3 Materials and methods

3.1 Tick collection

Both adult *R. sanguineus* and *H. hystricis* ticks were collected from dogs in Taiwan, either confirmed carrying *B. gibsoni* by PCR or were negative by PCR. These ticks were received and tested at the Utrecht Centre for Tick-borne Diseases (UCTD).

3.2 Identification & feeding

When received, the ticks were fed on laboratory animals at the University. Thereafter they were identified in the acaridarium using the books "Ticks of Domestic Animals in Africa" and "Ticks of Domestic animals in the Mediterranean Region". Also, an article was used for the identification of especially *H. hystricis* adults (Estrada-Peña et al. 2004; Walker et al. 2003; Hoogstraal et al. 1965).

3.3 Storage

Living ticks were stored in stove 1 (28°C) to oviposit and for the eggs to hatch, death ticks were stored in the International Tick Collection 2015. Some living ticks were moved to stove 3 (12°C) for storage. After hatching, the larvae were further incubated to allow their maturation to complete.

3.4 Number of ticks used

A total of 185 ticks were used for testing of which 104 were *H. hystricis* adults, 80 were *R. sanguineus* adults and 1 was a *H. hystricis* nymph of the first generation (see figure 3). The 104 *H. hystricis* adults consist of 75 female ticks and 29 male ticks. 62 female adults and 18 male adults were used to examine the *R. sanguineus* species. Larvae were not used in this survey. Sample informaton has been attached in Appendix D.

Furthermore, 40 engorged nymphs of the second generation of the *H. hystricis* species of collection V were used for PCR and RLB.

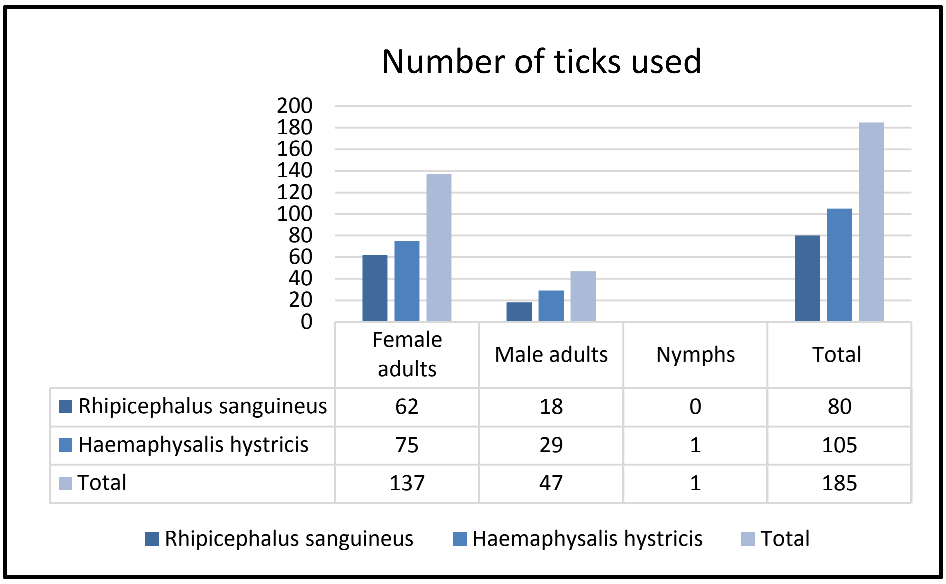


Figure 3: An overview of the total number of ticks used in this research

3.5 Number of DNA samples from dogs used

A total of 122 DNA samples from dogs in Taiwan were sent to the UCTD for PCR and RLB. These dogs carried the ticks which were examined first at the UCTD.

3.6 DNA extraction

DNA was extracted from all dead ticks. Ticks were put in lysis buffer, after fifteen minutes at -80°C a 7 mm or 5 mm metal bead was added to the adults and the nymph. Then, the TissueLyser LT causes disruption of the samples. The test protocol has been attached in Appendix A.

3.7 Polymerase Chain Reaction (PCR)

After the DNA extraction, a PCR was used. This test amplifies specific DNA fragments. A master mix containing H2O, 5x phire reaction buffer, dNTPs, forward primer, reverse primer and polymerase was made. Each DNA sample was added in the correct PCR tube and this tubes were put in the PCR thermocycler. Tests protocols are available and can be found in Appendix B. Primers were used and a positive and negative PCR control was added to ensure the test was working properly. The PCR was done twice on each DNA extraction from ticks to ensure the outcome was correct. The PCR of DNA extraction from dogs and the second generation nymphs was done once, due to time constraints.

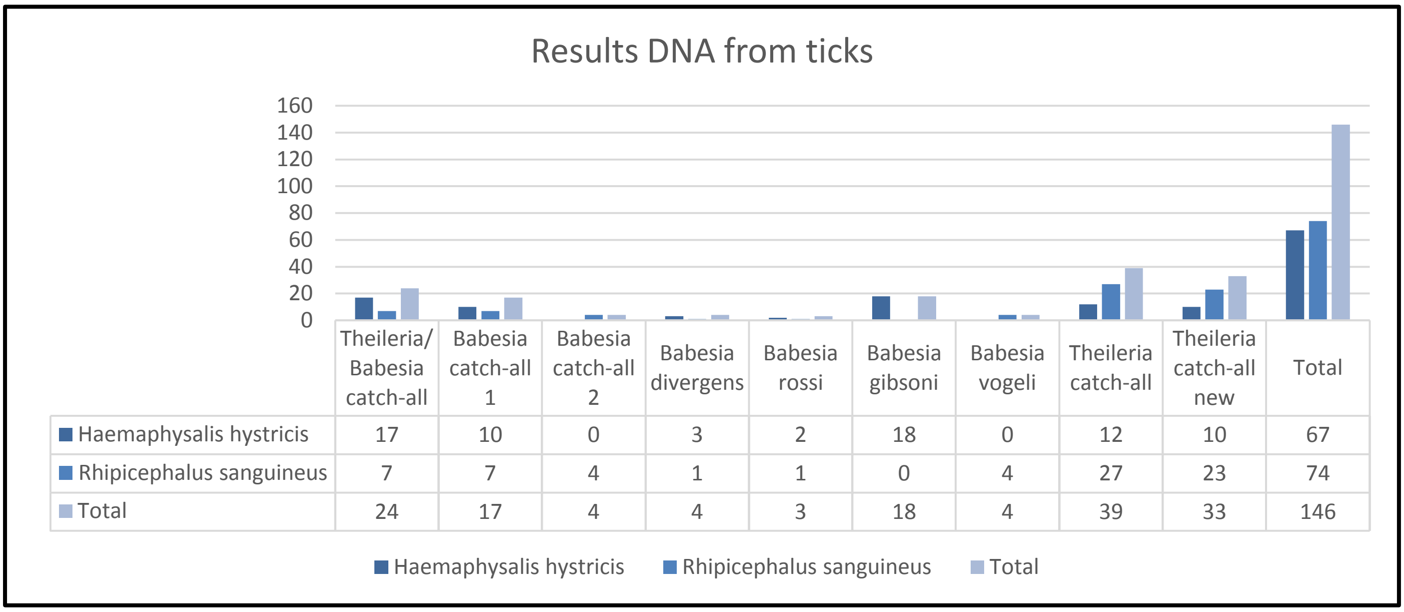
3.8 Reverse Line Blot Hybridization (RLB)

The final test used is the RLB. An RLB analyses multiple samples at the same time, therefore a wide variety of pathogens can be detected quickly. The samples were first tested on Membrane ID 7&8&14&15 for *Anaplasma*, *Theileria*, *Babesia*, *Borrelia* and *Ricketssia* species. Later on, there was only tested for *Babesia* and *Theileria* species on Membrane ID 12&13. The Membrane ID's can be found in Appendix E. For this test a protocol is available which has been attached in Appendix C. The RLB of the PCR products of DNA extraction from ticks was done twice to ensure the outcome was correct. RLB's of the PCR products of DNA extraction from dogs and the second generation nymphs were done once, due to time constraints.

4 Results

4.1 DNA extraction from ticks

The positive reactions for several pathogens are shown in figure 4. The samples without positive reactions are not included in the figure. Most samples showed positive reactions for multiple pathogens, this information is not included in the figure. Moreover, *B. vogeli* has been found in *R. sanguineus* adults and not in *H. hystricis* ticks, as seen in figure 4.

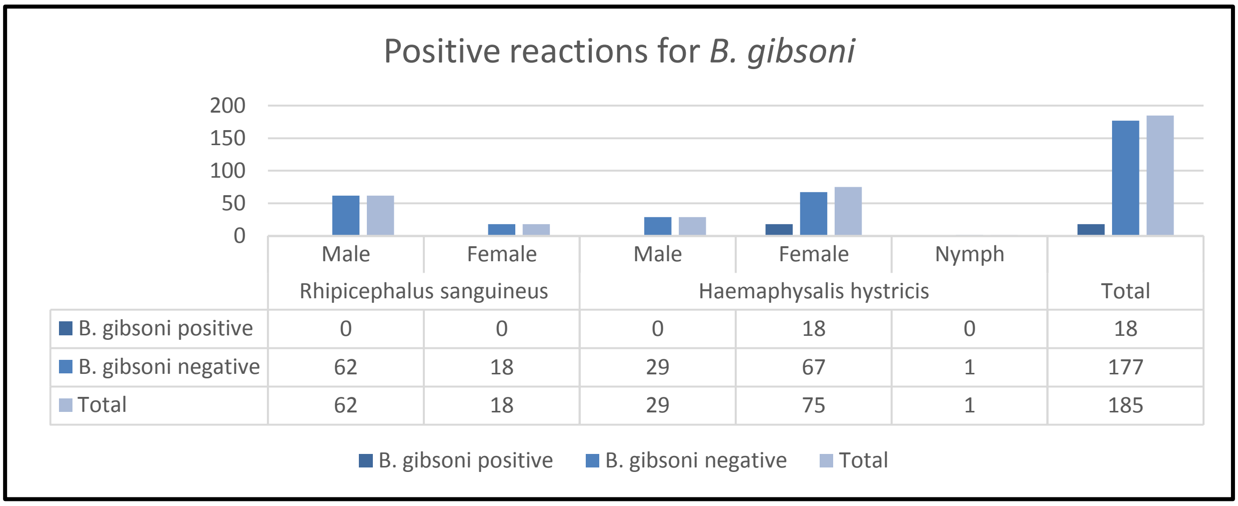


	Theileria/ Babesia catch-all	Babesia catch-all 1	Babesia catch-all 2	Babesia divergens	Babesia rossi	Babesia gibsoni	Babesia vogeli	Theileria catch-all	Theileria catch-all new	Total
Haemaphysalis hystricis	17	10	0	3	2	18	0	12	10	67
Rhipicephalus sanguineus	7	7	4	1	1	0	4	27	23	74
Total	24	17	4	4	3	18	4	39	33	146

Figure 4: An overview of the positive reactions of DNA from ticks

4.2 Infection with *B. gibsoni* in ticks

B. gibsoni infection was detected in 17,3% (18/104) of *H. hystricis* ticks and in 0,0% (0/80) of *R. sanguineus* ticks. Only female *H. hystricis* adults were tested positive for *B. gibsoni*. Figure 5 and Figure 6 provide an overview of positive reactions and infection rate for *B. gibsoni*. The RLB results are shown in Appendix F.



	Rhipicephalus sanguineus		Haemaphysalis hystricis			Total
	Male	Female	Male	Female	Nymph	
B. gibsoni positive	0	0	0	18	0	18
B. gibsoni negative	62	18	29	67	1	177
Total	62	18	29	75	1	185

Figure 5: Overview of positive reactions for B. gibsoni

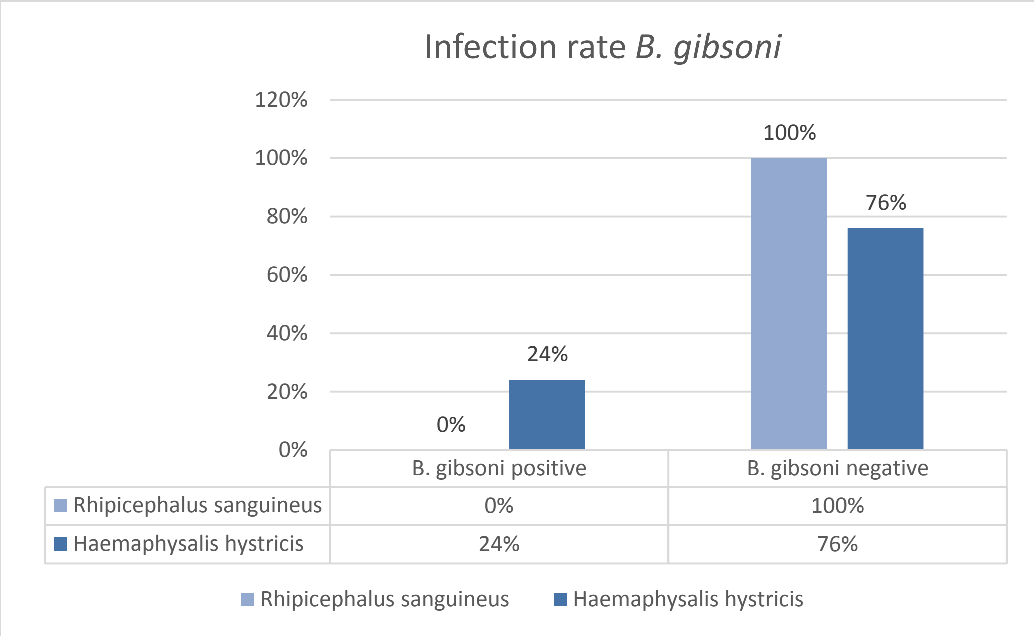


Figure 6: Infection rate of B. gibsoni in R. sanguineus and H. hystricis ticks

4.3 DNA extraction from dogs

Figure 7 shows the positive reactions of DNA from dogs for different pathogens. Not all DNA samples from dogs showed positive reactions for pathogens, these are not included in the figure. A total of 20,5% (25/122) samples showed a positive reaction for *B. gibsoni* Japan. The numbers of these samples are: c104-022, c104-023, c104-024, c104-086, c104-093, c104-094, c104-097, c104-103, c104-104, c104-002, c104-003, c104-004, c104-009, c104-010, c104-011, c104-012, c104-013, c104-014, c104-016, c104-019, c104-033, c104-046, c104-047, c104-048 and c104-052.

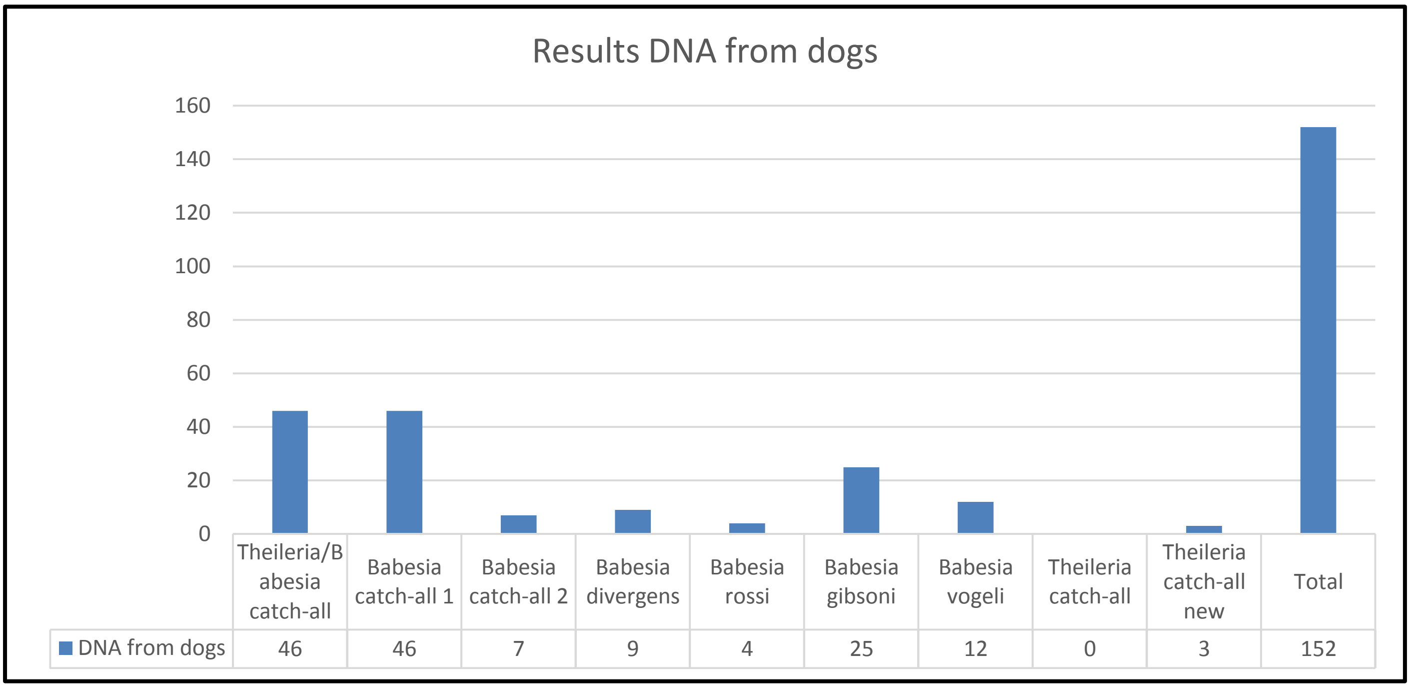


Figure 7: Overview of positive reactions of DNA from dogs

4.4 Overview of number of ticks on dogs with positive reactions for *B. gibsoni*

The ticks received from Taiwan from one dog were of the same species. Table 2 shows the positive reactions for *B. gibsoni* in ticks and dogs.

Dog ID	Infection with *B. gibsoni*	Tick species found on dogs	Ticks *B. gibsoni* positive
C104-009	*B. gibsoni*	*R. sanguineus*	0,0% (0/3)
C104-010	*B. gibsoni*	*R. sanguineus*	0,0% (0/2)
C104-016	*B. gibsoni*	*H. hystricis*	0,0% (0/3)
C104-017	-	*H. hystricis*	20,0% (3/15)
C104-018	-	*H. hystricis*	14,3% (1/7)
C104-019	-	*H. hystricis*	36,4% (4/11)
C104-020	*B. gibsoni*	*H. hystricis*	0,0% (0/6)
C104-022	*B. gibsoni*	*H. hystricis*	20,0% (5/25)
C104-024	*B. gibsoni*	*H. hystricis*	0,0% (0/2)
C104-047	*B. gibsoni*	*R. sanguineus*	0,0% (0/1)
C104-052	*B. gibsoni*	*H. hystricis*	0,0% (0/1)
C104-086	*B. gibsoni*	*H. hystricis*	0,0% (0/1)
C104-097	*B. gibsoni*	*H. hystricis*	50,0% (1/2)
C104-103	*B. gibsoni*	*H. hystricis*	100,0% (3/3)
C104-119	-	*H. hystricis*	20,0% (1/5)

Table 2: Positive reactions for B. gibsoni in ticks and dogs

4.5 Infection with *B. gibsoni* in the second generation *H. hystricis* nymphs

No infection with *B. gibsoni* was found in the second generation *H. hystricis* nymphs.

5 Discussion

There is published evidence for the transmission of *B. gibsoni* via the vector ticks, such as *R. sanguineus* and *H. longicornis*, but there is no information available about the vector capacity of *H. hystricis* for *B. gibsoni* (Shimada et al. 2003; Mandal et al. 2015; Taylor et al. 2007).

It has been assumed that *R. sanguineus* can transmit *B. gibsoni* (Taylor et al. 2007). However, convincing experimental data to support this hypothesis are lacking (Irwin 2010). Previous research was done about the development of *B. gibsoni* in the salivary glands of *R. sanguineus* species. *R. sanguineus* was fed on *B. gibsoni* infected dogs, which became positive for *B. gibsoni*. The transmission of *B. gibsoni* however was not examined, which does not proof that *R. sanguineus* can transmit this pathogen (Higuchi et al. 1995). Therefore, further research about this transmission is required.

Furthermore, *B. gibsoni* is reported to be transmitted by *Haemaphysalis* tick species (Irwin 2010). Related research about the development of *B. gibsoni* in the gut of *H. longicornis* has been done. *H. longicornis* was also fed on *B. gibsoni* infected dogs, which became positive for this pathogen. The transmission of *B. gibsoni* via *H. longicornis* was also not examined, which makes it uncertain if *H. longicornis* can transmit the infection (Higuchi et al. 1991). Furtermore, the disease relationship of *H. hystricis* has not been studied (Geevarghese & Mishra 2011). This makes it uncertain if *H. hystricis* has a vector capacity for different diseases including *B. gibsoni*. Also, no evidence has been found to support the hypothesis that *H. hystricis* can cause transmission of *B. gibsoni*. Therefore, the vector capacity of *H. hystricis* and *H. longicornis* requires further study.

This study was designed to investigate the vector capacity of *H. hystricis* and *R. sanguineus* for *B. gibsoni*. *H. hystricis* appeared the most likely vector for this pathogen, while it was thought *R. sanguineus* was the most important vector for *B. gibsoni*. *B. gibsoni* was not found in *R. sanguineus*, while this pathogen was found in *H. hystricis*. This suggests vector capacity of *H. hystricis* for *B. gibsoni*.

Transovarial transmission of *B. gibsoni* was suspected in *H. hystricis* ticks. Therefore, a selection of 40 second generation nymphs was made from one collection positive first generation ticks. The second generation nymphs were all negative for *B. gibsoni*, suggesting no transovarial transmission had occured. Positive results in the second generation ticks may be seen when the selection was made from different collections. More second generation ticks from different collections should be tested to proof transmission.

Some ticks were positive for *B. gibsoni* while the matching dogs were negative, which can be a result of removing the positive ticks from dogs too early. Therefore, the ticks were unable to transmit the pathogen on dogs which can result in a negative outcome from these dogs.

Removing ticks from dogs too early can also result in negative ticks while the matching dogs were positive for *B. gibsoni*, because the ticks were unable to engorge properly. It may be that more positive results could have been obtained when the ticks were removed from dogs less early.

B. vogeli was only found in *R. sanguineus* adults and not in *H. hystricis* ticks, while most positive tested dogs for *B. vogeli* only carried the *H. hystricis* species. This supports the hypothesis that *R. sanguineus* is the vector for *B. vogeli*.

In Taiwan tests have been done to detect *B. gibsoni* and *B. canis* in dogs. DNA samples from the dogs were also sent to the UCTD where a PCR and RLB was done. The results obtained at the UCTD did not always match with the results in Taiwan, which need to be further examined by both groups.

In addition, the DNA samples from dogs and the second generation *H. hystricis* nymphs were tested once. Different fragments of DNA were used on each PCR and RLB, which can cause a variety of positive reactions shown on the RLB. It can be that a second test of DNA samples from dogs and the second generation nymphs shows other positive results. Therefore, these DNA samples should be tested again to ensure all positive reactions are obtained.

Finally, during the survey problems arose in the laboratory. A contamination with Anaplasma/Ehrlichia controls was observed. Therefore, the tests for Anaplasma/Ehrlichia were no longer done in this survey and the RLB tests were effectuated on Membrane ID 12 instead of Membrane ID 8. The different membranes are shown in Appendix E. Co-infection with *Babesia* species could occur, which makes it uncertain if the first batches of RLB on Membrane ID 8 are sure for positive reactions for *Babesia* species.

6 Conclusion

In contrast of what has been assumed in the literature, it is likely that *B. gibsoni* is transmitted by *H. hystricis* and not by *R. sanguineus* ticks.

7 Acknowledgements

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Appendix A: DNA extraction protocol

DNA EXTRACTION FROM TICKS

Sample description	
Number of samples	

Wear gloves and use filter pipet tips

Strictly follow the one-way route: Clean room → Dirty room → PCR room

		Done
1	Clean workspace with sodium hypochlorite.	
2	Turn on a water bath at 56°C.	
3	Take the proteinase K solution from the freezer and store at 4°C.	
4	Wash the ticks in a ultrasonic bath with demineralized water for up to 30 seconds.	
5	Put the ticks, with cleaned forceps, in 1.5ml tubes with 70% ethanol and vortex for several seconds.	
6	Wash the forceps in 70% ethanol followed by washing in demineralized water after each tick.	
7	Take the ticks from the tubes and let it dry on a clean tissue paper and place the dried ticks in a sterile 2ml tube with 180µl T1 lysis buffer.	
8	Freeze the samples at -80°C for 15 minutes.	
9	Add a 5 or 7mm (depending on tick size) metal bead to the frozen samples.	
10	Disrupt the ticks in the TissueLyser LT at 50 oscillations per second for 3 minutes.	
11	Briefly spin down the tubes. **1000x g maximum!**	
12	Add 25µl proteinase K and vortex.	
13	Prelyse the samples at 56°C in a water bath for 3 hours and vortex every hour.	
14	**During the incubation**; empty and clean the ultrasonic bath.	
15	**During the last incubation hour** ; turn on the heating block at 70°C and preheat the BE buffer.	
16	Briefly spin down the tubes. **1000x g maximum!**	
17	Add 200µl B3 buffer and vortex.	
18	Incubate the tubes at 70°C for 15 minutes.	

19	Briefly spin down the tubes. **1000x g maximum!**	
20	Add 210µl 96% ethanol, vortex and briefly spin down the tubes. **1000x g maximum!**	
21	Transfer the supernatant to new sterile 1.5ml tubes. (Tick parts are allowed to be transferred.)	
22	Centrifuge the tubes at 11,000x g for 2 minutes.	
23	Transfer the supernatant to spin columns. Avoid pipetting tick parts, as it can block the spin column.	
24	Centrifuge the columns at 11,000x g for 1 minute. Discard the flow through.	
25	Add 500µl BW buffer and centrifuge the columns at 11,000x g for 1 minute. Discard the flow through.	
26	Add 600µl B5 buffer and centrifuge the columns at 11,000x g for 1 minute. Discard the flow through.	
27	Centrifuge the columns at 11,000x g for 1 minute.	
28	Place the spin columns in sterile 1.5ml tubes. **Label the tubes accordingly.**	
29	Add 100µl preheated BE buffer directly on the membrane of the spin columns and incubate at room temperature for 1 minute.	
30	Centrifuge the columns at 11,000x g for 1 minute. Discard the spin columns.	
31	Store the DNA samples at 4°C for use within the next few days or store at -20°C for long term preservation.	
32	Turn off all equipment and clean working space with sodium hypochlorite.	

DNA extraction done:

by ______________________________ on ______________

Comments:

Appendix B: PCR amplification protocol

PCR FOR RLB

Sample description			
Number of samples		Number of controls	

Wear (green) gloves and use filter pipet tips

Strictly follow the one-way route: Clean room → Dirty room → PCR room

Primers:	*Anaplasma Ehrlichia*	*Babesia Theileria*	*Borrelia*	*Rickettsia*	Other:

Reagent	1x	Number of samples + controls + 10%	
PCR grade H_2O	15.875µl		
5x Phire reaction buffer	5.0µl		
10mM dNTPs	0.5µl		
Forward primer (20pmol/µl)	0.5µl		
Reverse primer (20pmol/µl)	0.5µl		
2U/µl Phire Hot Start II DNA polymerase	0.125µl		

		Done
1	Put DNA samples a (few) day(s) before the PCR at 4°C.	
2	Calculate the needed volumes of each reagent.	
3	Turn on the DNA workstations in the clean room and the dirty room.	
4	Clean workspace in both DNA workstations with sodium hypochlorite.	
5	Label the PCR and Eppendorf tubes and put them in the DNA workstation in the clean room	
6	Turn on the UV-light in both DNA workstations for 20 minutes.	
7	**During the UV-light**; thaw the PCR reagents at room temperature, except the polymerase.	
8	Prepare the PCR mix in the Eppendorf tube(s).	

9	Pipet the master mix gently up and down to mix well.	
10	Pipet 22,5µl master mix to each PCR tube and add the leftover mix to an additional tube which will be the negative PCR control.	
11	Close the PCR tubes and remove them from the workstation, clean the workspace with sodium hypochlorite and turn on the UV-light for 20 minutes.	
12	Take the closed PCR tubes to the dirty room and place them in the workstation.	
13	Vortex the DNA samples and controls, spin them down briefly at 11,000x g and place them in the workstation.	
14	Add 2.5µl DNA sample to the corresponding PCR tube.	
15	Add 2.5µl of the positive control (, corresponding to the PCR to be performed,) to the positive PCR control tube.	
16	Vortex and spin down briefly.	
17	Clean the workstation with sodium hypochlorite and turn on the UV-light for 20 minutes.	
18	Run the corresponding PCR program.	
19	Store the PCR products at 4°C for use within the next few days or store at -20°C for long term preservation.	
20	Turn off both DNA workstations after the UV-light is switched off.	

PCR done:

by ____ on ________________________

Comments:

Appendix C: RLB protocol

REVERSE LINE BLOT HYBRIDIZATION

Sample description	
Number of samples	
Membrane ID	

Wear gloves and use filter pipet tips

Strictly follow the one-way route: Clean room → Dirty room → PCR room

		Done
1	Clean workspace with sodium hypochlorite.	
2	Turn on a heating block at 100°C.	
3	Turn on the hybridization oven at 42°C en preheat 50ml 2x SSPE/0.5% SDS solution.	
4	Turn on the water bath at 50°C en preheat the bottle with 2x SSPE/0.5% SDS solution.	
5	Combine and dilute the PCR products per DNA sample in a 1.5ml tube. Take 10µl of every PCR product and add 2x SSPE/0.1% SDS to a final volume of 160µl. (10µl *Anaplasma/Ehrlichia* PCR + 10µl *Babesia/Theileria* PCR + 140µl 2x SSPE/0.1% SDS.)	
6	Take 10µl of the RLB positive controls and add 150µl 2x SSPE/0.1% SDS to a 1.5ml tube,	
7	Denature the diluted PCR samples and controls at 100°C for 10 minutes.	
8	**During the denaturation step**; wash the membrane at room temperature with 2X 2SSPE/0.1% SDS for 5 minutes under gentle shaking and fill a bucket with ice.	
9	Immediately transfer the samples in order on ice after the denaturation.	
10	Prepare the miniblotter by placing the membrane on the lanes, with the line pattern of the membrane perpendicular to the lanes of the blotter. Place de support cushion on the membrane followed by the other half of the blotter. Turn the blotter right-side up without moving the membrane and turn the screws hand-tight,	
11	Remove residual fluid in the slots by aspiration.	
12	Briefly spin down the tubes at 4°C and place them back on ice in order.	
13	Fill the slots with the samples (150µl) and fill the first, last and other empty slots with 2x SSPE/0.1% SDS. **Avoid air bubbles.**	
14	Hybridize the blotter at 42°C for 60 minutes in the hybridization oven without shaking.	
15	Remove the samples by aspiration.	
16	Dissemble the blotter and remove the membrane from the blotter.	

17	Wash the membrane **twice** with preheated 2x SSPE/0.5% SDS at 50°C for 10 minutes under gentle shaking.		
18	**During the washing step**; clean the blotter and the support cushion.		
19	Incubate the membrane with 50ml 2x SSPE/0.5% SDS + 5µl streptavidin at 42°C for 30 minutes in the hybridization oven under gentle shaking. **Discard the streptavidin solution in a tube and into the bio-waste bin. Do not pour it in the sink.**		
20	**During the streptavidin hybridization**; change the water bath temperature to 42°C and preheat the bottle with 2x SSPE/0.5% SDS solution. Keep the lid open.		
21	Wash the membrane **twice** with preheated 2x SSPE/0.5% SDS solution at 42°C for 10 minutes under gentle shaking.		
22	Change the water bath temperature to 80°C and preheat the bottle with 1% SDS solution.		
23	Wash the membrane **twice** with 2x SSPE at room temperature for 5 minutes, under gentle shaking.		
24	**During the washing step**; prepare the foil and film cassette and check if the developing machine is on (5th floor).		
25	Add 10ml ECL (5ml ECL1 + 5ml ECL2) to the membrane and gently shake by hand until the whole membrane is covered. **Discard the ECL in a tube and into the bio-waste bin. Do not pour it in the sink.**		
26	Cover the membrane in foil and place it in the film cassette. **Avoid air bubbles.**		
27	Go to the dark room and expose a film to the membrane for 10 minutes.		
28	Develop the film with the developing machine.		
29	Remove the foil and wash the membrane **twice** with preheated 1% SDS at 80°C for 30 minutes under gentle shaking.		
30	Wash the membrane with 20mM EDTA at room temperature for 15 minutes under gentle shaking.		
31	Store the membrane in a seal bag with 20mM EDTA at 4°C.		
32	Turn off all equipment and clean workspace with sodium hypo chloride.		

RLB done:

by ____ on ____________________

Comments:

Appendix D: Sample information

Consignment		Date of shipment	Received	No.	Sexe	Number	Tick identification (Taiwan)
1	**I**	19-5-2015	26-5-2015	c104-008	Female + eggs	1x	NE
				c104-008	Female + eggs	1x	NE
				c104-009	Female + eggs	1x	NE
				c104-010	Female + eggs	1x	NE
				c104-008	Female + eggs	1x	NE
				c104-010	Female + eggs	1x	NE
				c104-009	Female + eggs	1x	NE
				c104-009	Female + eggs	1x	NE
2	**II**	19-5-2015	26-5-2015				
3	**III**	26-4-2015	30-5-2015	c104-001	Female + eggs + larvae	1x	NE
				c104-001	Female	1x	NE
				c104-001	Female + eggs	1x	NE
				c104-001	Female + eggs	1x	NE
				c104-001	Female + eggs	1x	NE
				c104-001	Female + eggs	1x	NE
4	**IV**	11-6-2015	17-6-2015	c104-016	Female + eggs	1x	Haemaphysalis
				c104-016	Female + eggs	1x	Haemaphysalis
5	**V**	12-6-2015	17-6-2015	c104-016	Female	1x	Haemaphysalis
				c104-016	Female	1x	Haemaphysalis
				c104-016	Female	1x	Haemaphysalis
				c104-016	Female	1x	Haemaphysalis
				c104-016	Female + eggs	1x	Haemaphysalis
				c104-016	Female	1x	Haemaphysalis
				c104-016	Female	1x	Haemaphysalis
				c104-016	Female + eggs	1x	Haemaphysalis
				c104-016	Female + eggs	1x	Haemaphysalis
		Tweede verpakking in dezelfde doos					
		12-6-2015	17-6-2015	c104-016	Female	1x	Haemaphysalis
				c104-016	Female	1x	Haemaphysalis
				c104-016	Female	1x	Haemaphysalis
				c104-016	Female	1x	Haemaphysalis
				c104-016	Female + eggs	1x	Haemaphysalis

Tick identification (Utrecht)	Saved in	Region	PCR (Taiwan)
R. sanguineus	International tick collection 2015	Luzhu Dist., Taoyuan City	*B. canis*
R. sanguineus	International tick collection 2015	Luzhu Dist., Taoyuan City	*B. canis*
R. sanguineus	International tick collection 2015	Dayuan Dist., Taoyuan City	*B. canis*
R. sanguineus	Stove 1: 28°C	Dayuan Dist., Taoyuan City	*B.gibsoni*
R. sanguineus	Stove 1: 28°C	Luzhu Dist., Taoyuan City	*B. canis*
R. sanguineus	Stove 1: 28°C	Dayuan Dist., Taoyuan City	*B. gibsoni*
R. sanguineus	Stove 1: 28°C	Dayuan Dist., Taoyuan City	*B. canis*
R. sanguineus	Stove 1: 28°C	Dayuan Dist., Taoyuan City	*B. canis*
R. sanguineus	International tick collection 2015	Tamsui Dist., New Taipei City	Negative
R. sanguineus	International tick collection 2015	Tamsui Dist., New Taipei City	Negative
R. sanguineus	International tick collection 2015	Tamsui Dist., New Taipei City	Negative
R. sanguineus	International tick collection 2015	Tamsui Dist., New Taipei City	Negative
R. sanguineus	International tick collection 2015	Tamsui Dist., New Taipei City	Negative
R. sanguineus	International tick collection 2015	Tamsui Dist., New Taipei City	Negative
H. hystricis	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1 till 28-07-2015, then moved to stove 3: 12°C (Because of larvae)	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1 till 28-07-2015. then moved to stove 3: 12°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1 till 28-07-2015. then moved to stove 3: 12°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1 till 28-07-2015. then moved to stove 3: 12°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1 till 28-07-2015. then moved to stove 3: 12°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1 till 28-07-2015. then moved to stove 3: 12°C	Christ's College, Beitou Dist., Taipei City.	Negative

Microscopic exam - parasite (Taiwan)	Microscopic exam - Microfilaria (Taiwan)	PCV (Taiwan)	Clinical signs	Concurrent diseases
Negative	Negative	33,3	Clinically healthy, stray dog	
Negative	Negative	33,3	Clinically healthy, stray dog	
Negative	Negative	29,3	Clinically healthy, stray dog	
Negative	Negative	34,5	Clinically healthy, stray dog	
Negative	Negative	33,3	Clinically healthy, stray dog	
Negative	Negative	34,5	Clinically healthy, stray dog	
Negative	Negative	29,3	Clinically healthy, stray dog	
Negative	Negative	29,3	Clinically healthy, stray dog	
			Clinically healthy, stray dog	
Negative	NE	25,6	Clinically healthy, stray dog	Splenomegaly, anemia
Negative	NE	25,6	Clinically healthy, stray dog	Splenomegaly, anemia
Negative	NE	25,6	Clinically healthy, stray dog	Splenomegaly, anemia
Negative	NE	25,6	Clinically healthy, stray dog	Splenomegaly, anemia
Negative	NE	25,6	Clinically healthy, stray dog	Splenomegaly, anemia
Negative	NE	25,6	Clinically healthy, stray dog	Splenomegaly, anemia
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease
Negative	NE	33	Clinically healthy, stray dog	Heart worm disease

Check 7-9-15	**RLB Utrecht Ticks *B. gibsoni***	**RLB dogs Utrecht *B. gibsoni***
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	*Babesia gibsoni* positive
International tick collection 2015	Negative	*Babesia gibsoni* positive
International tick collection 2015	Negative	Negative
	Negative	*Babesia gibsoni* positive
International tick collection 2015	Negative	*Babesia gibsoni* positive
International tick collection 2015	Negative	*Babesia gibsoni* positive
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
		Babesia gibsoni positive
		Babesia gibsoni positive
		Babesia gibsoni positive
		Babesia gibsoni positive
		Babesia gibsoni positive
		Babesia gibsoni positive
International tick collection 2015	Negative	*Babesia gibsoni* positive
		Babesia gibsoni positive
		Babesia gibsoni positive
		Babesia gibsoni positive
		Babesia gibsoni positive
		Babesia gibsoni positive
		Babesia gibsoni positive
International tick collection 2015	Negative	*Babesia gibsoni* positive
International tick collection 2015	Negative	*Babesia gibsoni* positive
		Babesia gibsoni positive

Consignment		Date of shipment	Received	No.	Sexe	Number	Tick identification (Taiwan)
6	VI	16-6-2015	22-6-2015	c104-020	Female	1x	*Haemaphysalis*
				c104-022	Female	1x	*Haemaphysalis*
				c104-022	Female	1x	*Haemaphysalis*
				c104-019	Female	5x	*Haemaphysalis*
				c104-022	Female	1x	*Haemaphysalis*
				c104-022	Female	1x	*Haemaphysalis*
				c104-022	Female	1x	*Haemaphysalis*
				c104-019	Female	3x	*Haemaphysalis*
				c104-019	Male	1x	*Haemaphysalis*
				c104-019	Female	1x	*Haemaphysalis*
				c104-019	Female	1x	*Haemaphysalis*
				c104-022	Female	1x	*Haemaphysalis*
				c104-022	Female	1x	*Haemaphysalis*
				c104-021	Female	1x	*Haemaphysalis*
				c104-021	Female	1x	*Haemaphysalis*
				c104-022	Female	1x	*Haemaphysalis*
				c104-022	Female	2x	*Haemaphysalis*
				c104-022	Male	4x	*Haemaphysalis*
				c104-017	Female	7x	*Haemaphysalis*
				c104-017	Male	6x	*Haemaphysalis*
				c104-021	Female	3x	*Haemaphysalis*
				c104-021	Male	6x	*Haemaphysalis*
				c104-018	Female	2x	*Haemaphysalis*
				c104-018	Male	1x	*Haemaphysalis*
				c104-020	Female	3x	*Haemaphysalis*
				c104-020	Male + Nymph	1x + 1x	*Haemaphysalis*
				c104-017	Female	1x	*Haemaphysalis*
				c104-017	Female	1x	*Haemaphysalis*
				c104-018	Female	2x	*Haemaphysalis*
				c104-018	Male	2x	*Haemaphysalis*
				c104-022	Female	8x	*Haemaphysalis*
				c104-022	Male	5x	*Haemaphysalis*

Tick identification (Utrecht)	Saved in	Region	PCR (Taiwan)
H. hystricis	Stove 1: 28°C	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	Stove 1: 28°C	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	Stove 1: 28°C	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	Stove 1: 28°C	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	Stove 1: 28°C	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	Stove 1: 28°C	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	Stove 1: 28°C	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	Stove 1: 28°C	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	Stove 1: 28°C	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	Stove 1: 28°C	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	Stove 1: 28°C	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	Stove 1: 28°C	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	Stove 1: 28°C	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	Stove 1: 28°C	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	Stove 1: 28°C	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*
H. hystricis	International tick collection 2015	Soochow University, Shihlin Dist, Taipei City	*B. gibsoni*

Microscopic exam-Parasite (Taiwan)	Microscopic exam-Microfilaria (Taiwan)	PCV (Taiwan)	Clinical signs	Concurrent diseases
B. gibsoni	Positive	24,4	Swollen mammary glands with milk production, stray dog	Heart worm disease
B. gibsoni	Positive	20,8	Clinically healthy, stray dog	Heart worm disease
B. gibsoni	Positive	20,8	Clinically healthy, stray dog	Heart worm disease
B. gibsoni	Negative	39	Clinically healthy, stray dog	
B. gibsoni	Positive	20,8	Clinically healthy, stray dog	Heart worm disease
B. gibsoni	Positive	20,8	Clinically healthy, stray dog	Heart worm disease
B. gibsoni	Positive	20,8	Clinically healthy, stray dog	Heart worm disease
B. gibsoni	Negative	39	Clinically healthy, stray dog	
B. gibsoni	Negative	39	Clinically healthy, stray dog	
B. gibsoni	Negative	39	Clinically healthy, stray dog	
B. gibsoni	Negative	39	Clinically healthy, stray dog	
B. gibsoni	Positive	20,8	Clinically healthy, stray dog	Heart worm disease
B. gibsoni	Positive	20,8	Clinically healthy, stray dog	Heart worm disease
Negative	Positive	28,8	Clinically healthy, stray dog	Heart worm disease
Negative	Positive	28,8	Clinically healthy, stray dog	Heart worm disease
B. gibsoni	Positive	20,8	Clinically healthy, stray dog	Heart worm disease
B. gibsoni	Positive	20,8	Clinically healthy, stray dog	Heart worm disease
B. gibsoni	Positive	20,8	Clinically healthy, stray dog	Heart worm disease
B. gibsoni	Negative	33	Clinically healthy, stray dog	
B. gibsoni	Negative	33	Clinically healthy, stray dog	
Negative	Positive	28,8	Clinically healthy, stray dog	Heart worm disease
Negative	Positive	28,8	Clinically healthy, stray dog	Heart worm disease
B. gibsoni	Negative	38,5	Clinically healthy, stray dog	
B. gibsoni	Negative	38,5	Clinically healthy, stray dog	
B. gibsoni	Positive	24,4	Swollen mammary glands with milk production, stray dog	Heart worm disease
B. gibsoni	Positive	24,4	Swollen mammary glands with milk production, stray dog	Heart worm disease
B. gibsoni	Negative	33	Clinically healthy, stray dog	
B. gibsoni	Negative	33	Clinically healthy, stray dog	
B. gibsoni	Negative	38,5	Clinically healthy, stray dog	
B. gibsoni	Negative	38,5	Clinically healthy, stray dog	
B. gibsoni	Positive	20,8	Clinically healthy, stray dog	Heart worm disease
B. gibsoni	Positive	20,8	Clinically healthy, stray dog	Heart worm disease

Consignment		Date of shipment	Received	No.	Sexe	Number	Tick identification (Taiwan)
7	VII	29-6-2015	2-7-2015	c104-026	Female	1x	*Haemaphysalis*
				c104-025	Female	1x	*Haemaphysalis*
				c104-025	Female	1x	*Haemaphysalis*
				c104-024	Female	1x	*Haemaphysalis*
				c104-024	Female	?	*Haemaphysalis*
8	VIII	13-7-2015	22-7-2015	c104-034	Female + eggs	1x	*Rhipicephalus*
				c104-037	Female	1x	*Rhipicephalus*
				c104-040	Female + eggs	1x	*Rhipicephalus*
				c104-039	Female + eggs	1x	*Rhipicephalus*
				c104-034	Female + eggs	1x	*Rhipicephalus*
				c104-039	Female + eggs	1x	*Rhipicephalus*
				c104-034	Female + eggs	1x	*Rhipicephalus*
				c104-039	Female + eggs	1x	*Rhipicephalus*
				c104-034	Female + eggs	1x	*Rhipicephalus*
				c104-034	Female + eggs	1x	*Rhipicephalus*
				c104-034	Female + eggs	1x	*Rhipicephalus*
				c104-037	Female	1x	*Rhipicephalus*
				c104-031	Male	7x	*Haemaphysalis*
				c104-031	Male	5x	*Haemaphysalis*
				c104-031	Male	6x	*Haemaphysalis*
				c104-031	?	1x	*?*
9	IX	22-7-2015	28-7-2015	c104-045	Female	1x	*Haemaphysalis*
10	X	27-7-2015	3-8-2015	c104-062	Female + eggs	1x	*Rhipicephalus*
				c104-051	Female + eggs	1x	*Rhipicephalus*
				c104-077?	Female + eggs	3x	*Rhipicephalus*
				c104-060	Female	1x	*Rhipicephalus*
				c104-066	Female	3x	*Rhipicephalus*
				c104-058	Female + eggs	3x	*Rhipicephalus*
				c104-051	Female + eggs	1x	*Rhipicephalus*
				c104-056	Female	1x	*Rhipicephalus*
				c104-060	Female + eggs	3x	*Rhipicephalus*
				c104-052	Female	1x	*Haemaphysalis*
				c104-057	Female	1x	*Rhipicephalus*
				c104-057	Female	4x	*Rhipicephalus*
				c104-077	Female + eggs	3x	*Rhipicephalus*

Tick identification (Utrecht)	Saved in	Region	PCR (Taiwan)
H. hystricis	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Negative
H. hystricis	Stove 1: 28°C	Shen Mei elementary school, Sinyi District, Keelung City	*B. gibsoni*
H. hystricis	Stove 1: 28°C	Shen Mei elementary school, Sinyi District, Keelung City	*B. gibsoni*
R. sanguineus	Stove 1: 28°C	Sanxia Dist., New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Ren'ai Rd., Jiaoxi Township, Yilan County	Pending
R. sanguineus	Stove 1: 28°C	Annong Rd., Dongshan Township, Yilan County	Pending
R. sanguineus	Stove 1: 28°C	Xianmin Blvd., Zhuangwei Township, Yilan County	Pending
R. sanguineus	Stove 1: 28°C	Sanxia Dist., New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Xianmin Blvd., Zhuangwei Township, Yilan County	Pending
R. sanguineus	Stove 1: 28°C	Sanxia Dist., New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Xianmin Blvd., Zhuangwei Township, Yilan County	Pending
R. sanguineus	Stove 1: 28°C	Sanxia Dist., New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Sanxia Dist., New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Sanxia Dist., New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Ren'ai Rd., Jiaoxi Township, Yilan County	Pending
R. sanguineus	International tick collection 2015	Dongshan Township, Yilan County	Pending
R. sanguineus	International tick collection 2015	Dongshan Township, Yilan County	Pending
R. sanguineus	Stove 1: 28°C	Dongshan Township, Yilan County	Pending
?	International tick collection 2015	Dongshan Township, Yilan County	Pending
H. hystricis	Stove 1: 28°C	Yushan Rd., Xindian Dist., New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Xinwu Dist., Taoyuan City	Pending
H. hystricis	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Pending
H. hystricis	Stove 1: 28°C	Xinwu Dist., Taoyuan City	Pending
R. sanguineus	Stove 1: 28°C	Xinwu Dist., Taoyuan City	Pending
R. sanguineus	Stove 1: 28°C	Jhongshan S. Rd., Dayuan Dist., Taoyuan City	Pending
R. sanguineus	1x Stove 1: 28°C 2x International tick collection 2015	Xinwu Dist., Taoyuan City	Pending
R. sanguineus	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Pending
R. sanguineus	Stove 1: 28°C	Xinwu Dist., Taoyuan City	Pending
R. sanguineus	Stove 1: 28°C	Xinwu Dist., Taoyuan City	Pending
H. hystricis	International tick collection 2015	Anxiang Rd., Xindian Dist., New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Guanyin Dist., Taoyuan City	Pending
R. sanguineus	Stove 1: 28°C	Guanyin Dist., Taoyuan City	Pending
R. sanguineus	Stove 1: 28°C	Xinwu Dist., Taoyuan City	Pending

Microscopic exam - parasite (Taiwan)	Microscopic exam - Microfilaria (Taiwan)	PCV (Taiwan)	Clinical signs	Concurrent diseases
Negative	Positive	42,2	Clinically healthy, stray dog	Heart worm disease
Negative	Negative	31,1	Clinically healthy, stray dog	
Negative	Negative	31,1	Clinically healthy, stray dog	
NE	NE	NE	Clinically healthy, stray dog	
NE	NE	NE	Clinically healthy, stray dog	
Negative	Negative	31,8	Clinically healthy, stray dog	
Negative	Negative	39,4	Clinically healthy, free ranging	
Negative	Negative	28,5	Clinically healthy, free ranging	
Negative	Negative	38,3	Clinically healthy, free ranging	
Negative	Negative	31,8	Clinically healthy, stray dog	
Negative	Negative	38,3	Clinically healthy, free ranging	
Negative	Negative	31,8	Clinically healthy, stray dog	
Negative	Negative	38,3	Clinically healthy, free ranging	
Negative	Negative	31,8	Clinically healthy, stray dog	
Negative	Negative	31,8	Clinically healthy, stray dog	
Negative	Negative	31,8	Clinically healthy, stray dog	
Negative	Negative	39,4	Clinically healthy, free ranging	
Negative	Negative	36,4	Clinically healthy, stray dog	
Negative	Negative	36,4	Clinically healthy, stray dog	
Negative	Negative	36,4	Clinically healthy, stray dog	
Negative	Negative	36,4	Clinically healthy, stray dog	
Negative	Negative	38,2	Panting, coughing	Thyroid mass
Negative	Negative	35,9	Clinically healthy, free ranging	
Negative	Positive	30,2	Clinically healthy, stray dog	
Negative	Negative	38	Clinically healthy, free ranging	
Negative	Negative	31,9	Clinically healthy, free ranging	
Negative	Negative	32	Clinically healthy, free ranging	
Negative	Negative	34,6	Clinically healthy, free ranging	
Negative	Positive	30,2	Clinically healthy, stray dog	
Negative	Negative	34,9	Clinically healthy, free ranging	
Negative	Negative	31,9	Clinically healthy, free ranging	
B. gibsoni	Negative	17,2	Decreased activity and appetite	
Negative	Negative	32,5	Clinically healthy, free ranging	
Negative	Negative	32,5	Clinically healthy, free ranging	
Negative	Negative	38	Clinically healthy, free ranging	

Check 7-9-15	**RLB Utrecht Ticks *B. gibsoni***	**RLB dogs Utrecht *B. gibsoni***
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	*Babesia gibsoni* positive
International tick collection 2015	Negative	*Babesia gibsoni* positive
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
Stove 1: 28°C		Negative
International tick collection 2015	Negative	Negative
Stove 1: 28°C		Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
Stove 1: 28°C		Negative
Stove 1: 28°C		Negative
Stove 1: 28°C		Negative
Stove 1: 28°C		Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
Stove 1: 28°C	Negative	Negative
Stove 1: 28°C		Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
Stove 1: 28°C		Negative
International tick collection 2015	Negative	Negative
Stove 1: 28°C		Negative
International tick collection 2015	Negative	Negative
Stove 1: 28°C	Negative	Negative
International tick collection 2015	Negative	*Babesia gibsoni* positive
International tick collection 2015	Negative	Negative
Stove 1: 28°C	Negative	Negative
Stove 1: 28°C	Negative	Negative

Consignment		Date of shipment	Received	No.	Sexe	Number	Tick identification (Taiwan)
11	XI	3-8-2015	6-8-2015	c104-079	Female + eggs	1x	Rhipicephalus
				c104-078	Female + eggs	1x	Rhipicephalus
				c104-080	Female + eggs	1x	Rhipicephalus
				c104-080	Female + eggs	3x	Rhipicephalus
				c104-078	Female + eggs	2x	Rhipicephalus
				c104-047	Female + eggs	1x	Rhipicephalus
				c104-049	Female	3x	
				c104-064	Female	4x	Rhipicephalus
				c104-075	Female + eggs	5x	
12	XII	13-8-2015	18-8-2015	c104-086	Female	1x	Haemaphysalis
				c104-083	Nymph	2x	Haemaphysalis
13	XIII	24-8-2015	27-8-2015	c104-097	Female	1x	Haemaphysalis
				c104-100	Female	1x	Haemaphysalis
				c104-101	Female	1x	
				c104-102	Female	1x	
				c104-100	Female	1x	Haemaphysalis
				c104-099	Female	1x	
				c104-097	Female + eggs	1x	Haemaphysalis
				c104-102	Female	3x	
				c104-092	Female + eggs	3x	Rhipicephalus
14	XIV	31-8-2015	3-9-2015	c104-103	Female	1x	Haemaphysalis
				c104-103	Female	1x	Haemaphysalis
				c104-106	Female	2x	Rhipicephalus
				c104-103	Female	1x	Haemaphysalis
15	XV	10-9-2015	15-9-2015	c104-110	Female + eggs	1x	
				c104-113	Female	1x	

Tick identification (Utrecht)	Saved in	Region	PCR (Taiwan)
H. hystricis	Stove 1: 28°C	?	Pending
R. sanguineus	Stove 1: 28°C	Taoyuan City	Pending
R. sanguineus	Stove 1: 28°C	Sansia Dist., New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Sansia Dist., New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Taoyuan City	Pending
R. sanguineus	Stove 1: 28°C	Xinliao Rd., Dongshan Township, Yilan County	Pending
R. sanguineus	International tick collection 2015	?	?
R. sanguineus	International tick collection 2015	Xinwu Dist., Taoyuan City	Pending
R. sanguineus	Stove 1: 28°C	?	?
H. hystricis	Stove 1: 28°C	Shihlin Dist, Taipei City	Pending
H. hystricis	Stove 1: 28°C	Jhongshan Rd., Bali Dist., New Taipei City	Pending
H. hystricis	Stove 1: 28°C	Sansia Dist., New Taipei City	Pending
H. hystricis	Stove 1: 28°C	Yingge Dist., New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Shijian St., Beitou Dist., Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Shipai Rd., Beitou Dist., Taipei City	Pending
H. hystricis	Stove 1: 28°C	Yingge Dist., New Taipei City	Pending
H. hystricis	Stove 1: 28°C	?	?
H. hystricis	Stove 1: 28°C	Sansia Dist., New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Shipai Rd., Beitou Dist., Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Xindian Rd., Xindian Dist., New Taipei City	Pending
H. hystricis	International tick collection 2015	Sec. 2, Shuiyuan Rd., Xizhi Dist., New Taipei City	Pending
H. hystricis	International tick collection 2015	Sec. 2, Shuiyuan Rd., Xizhi Dist., New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Dongshan Township, Yilan County	Pending
H. hystricis	Stove 1: 28°C	Sec. 2, Shuiyuan Rd., Xizhi Dist., New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Tai'an St., Guanyin Dist., Taoyuan City	Pending
R. sanguineus	Stove 1: 28°C	Guanyin Dist., Taoyuan City	Pending

Microscopic exam - parasite (Taiwan)	Microscopic exam - Microfilaria (Taiwan)	PCV (Taiwan)	Clinical signs	Concurrent diseases
Negative	Negative	42,2	Clinically healthy, stray dog	
Negative	Negative	30,7	Clinically healthy, stray dog	
Negative	Negative	39,9	Clinically healthy, stray dog	
Negative	Negative	39,9	Clinically healthy, stray dog	
Negative	Negative	30,7	Clinically healthy, stray dog	
B. gibsoni	Negative	24,4	Clinically healthy, stray dog	
?	?	?	?	
Negative	Negative	36,9	Clinically healthy, free ranging	
?	?	?	?	
B. gibsoni	Negative	26,2	Clinically healthy, stray dog	
Negative	Negative	46,7	Clinically healthy, stray dog	
Negative	Negative	28,6	Clinically healthy, stray dog	
Negative	Negative	42,1	Clinically healthy, stray dog	
Negative	Negative	42,4	Severe tick infection	
Negative	Negative	30,3	Severe tick infection	
Negative	Negative	42,1	Clinically healthy, stray dog	
?	?	?	Clinically healthy, stray dog	
Negative	Negative	28,6	Clinically healthy, stray dog	
Negative	Negative	30,3	Severe tick infection	
Negative	Negative	31,8	Clinically healthy, stray dog	
Negative	Negative	21,9	Clinically healthy, stray dog	
Negative	Negative	21,9	Clinically healthy, stray dog	
Negative	Negative	24,9	Clinically healthy, stray dog	
Negative	Negative	21,9	Clinically healthy, stray dog	
Negative	Negative	33,4	Clinically healthy, free ranging	
Hepatozoon canis	Negative	39,5	Clinically healthy, free ranging	

Check 7-9-15	**RLB Utrecht Ticks *B. gibsoni***	**RLB dogs Utrecht *B. gibsoni***
International tick collection 2015	Negative	Negative
Stove 1: 28°C		Negative
Stove 1: 28°C		Negative
Stove 1: 28°C		Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	*Babesia gibsoni* positive
International tick collection 2015		Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	Negative
International tick collection 2015	Negative	*Babesia gibsoni* positive
Stove 1: 28°C	Negative	Negative
International tick collection 2015	*Babesia gibsoni* positive	*Babesia gibsoni* positive
International tick collection 2015	Negative	Negative
Stove 1: 28°C		Negative
Stove 1: 28°C		Negative
International tick collection 2015	Negative	Negative
Stove 1: 28°C	Negative	Negative
International tick collection 2015	Negative	*Babesia gibsoni* positive
Stove 1: 28°C		Negative
	Negative	Negative
International tick collection 2015	*Babesia gibsoni* positive	*Babesia gibsoni* positive
International tick collection 2015	*Babesia gibsoni* positive	*Babesia gibsoni* positive
International tick collection 2015	Negative	Negative
International tick collection 2015	*Babesia gibsoni* positive	*Babesia gibsoni* positive
	Negative	Negative
	Negative	Negative

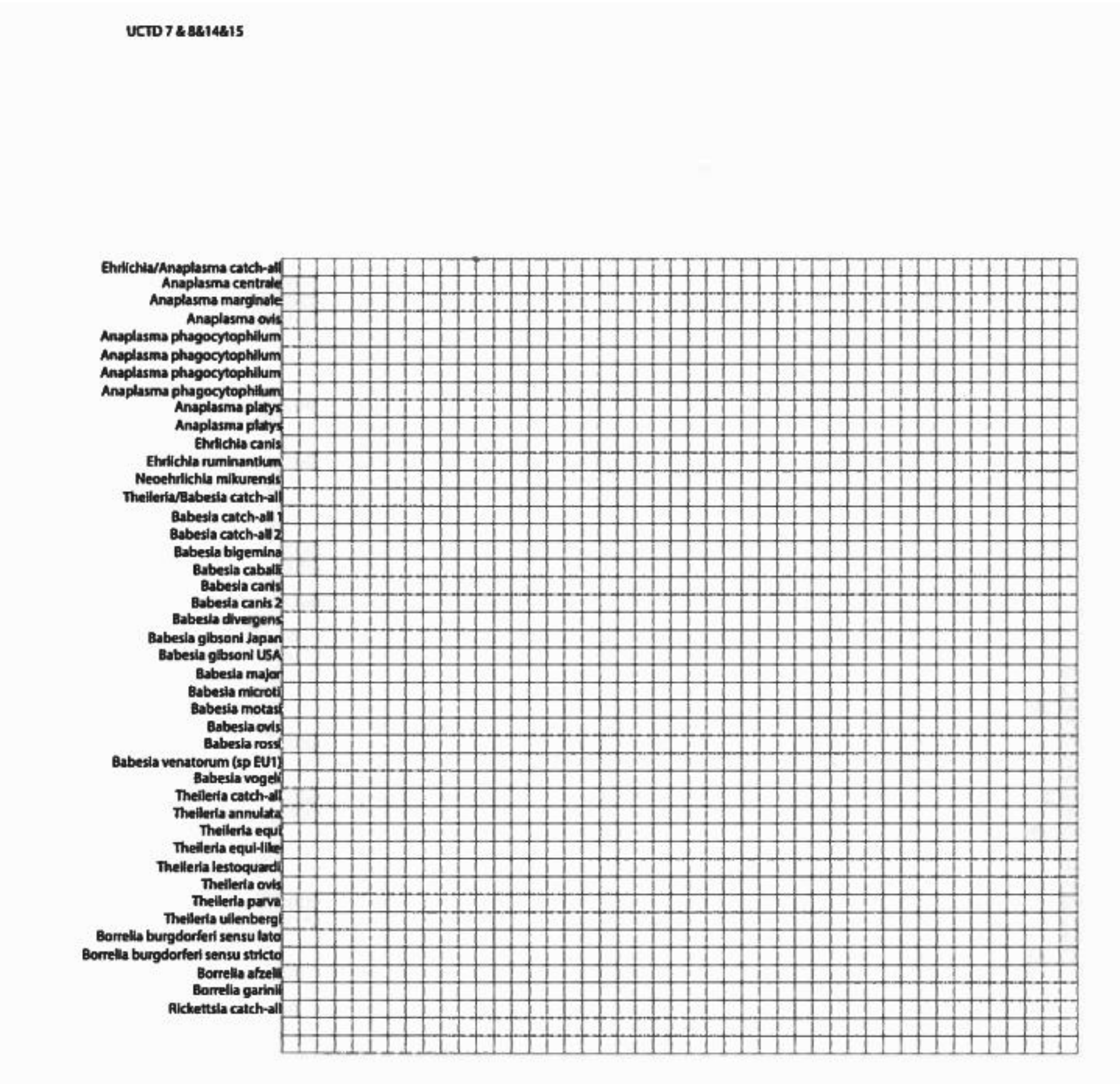
Consignment		Date of shipment	Received	No.	Sexe	Number	Tick identification (Taiwan)
16	XVI	14-9-2015	17-9-2015	c104-119	Female + eggs	1x	Haemaphysalis
				c104-119	Female + eggs	1x	Haemaphysalis
				c104-119	Female + eggs	1x	Haemaphysalis
				c104-119	Female + eggs	1x	Haemaphysalis
				c104-119	Female + eggs	1x	Haemaphysalis
17	XVII	18-9-2015	23-9-2015	c104-120	Female + eggs	1x	Haemaphysalis
				c104-120	Female + eggs	1x	Haemaphysalis
				c104-120	Female + eggs	1x	Haemaphysalis
				c104-120	Female + eggs	1x	Haemaphysalis
				c104-120	Female + eggs	1x	Haemaphysalis
				c104-120	Female + eggs	1x	Haemaphysalis
18	XVIII	2-10-2015	8-10-2015	c104-123	Female	1x	
				c104-123	Female	1x	
				c104-123	Female	1x	
				c104-123	Female	1x	
				c104-123	Female	1x	
19	XIX	12-10-2015	15-10-2015	c104-125	Female	1x	
				c104-125	Female + eggs	1x	
				c104-125	Female + eggs	1x	

Tick identification (Utrecht)	Saved in	Region	PCR (Taiwan)
H. hystricis	Stove 1: 28°C	Menghuan Lake Wetland, Beitou Dist., Taipei City.	Pending
H. hystricis	Stove 1: 28°C	Menghuan Lake Wetland, Beitou Dist., Taipei City.	Pending
H. hystricis	Stove 1: 28°C	Menghuan Lake Wetland, Beitou Dist., Taipei City.	Pending
H. hystricis	Stove 1: 28°C	Menghuan Lake Wetland, Beitou Dist., Taipei City.	Pending
H. hystricis	Stove 1: 28°C	Menghuan Lake Wetland, Beitou Dist., Taipei City.	Pending
R. sanguineus	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Pending
R. sanguineus	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Pending
R. sanguineus	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Pending
R. sanguineus	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Pending
R. sanguineus	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Pending
R. sanguineus	Stove 1: 28°C	Christ's College, Beitou Dist., Taipei City.	Pending
R. sanguineus	Stove 1: 28°C	Bali township, New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Bali township, New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Bali township, New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Bali township, New Taipei City	Pending
R. sanguineus	Stove 1: 28°C	Bali township, New Taipei City	Pending
R. sanguineus	International tick collection 2015		
R. sanguineus	Stove 1: 28°C		
R. sanguineus	Stove 1: 28°C		

Microscopic exam - parasite (Taiwan)	Microscopic exam - Microfilaria (Taiwan)	PCV (Taiwan)	Clinical signs	Concurrent diseases
Negative	Negative	21,2	Clinically healthy, stray dog	
Negative	Negative	21,2	Clinically healthy, stray dog	
Negative	Negative	21,2	Clinically healthy, stray dog	
Negative	Negative	21,2	Clinically healthy, stray dog	
Negative	Negative	21,2	Clinically healthy, stray dog	
Negative	Negative	41,9	Clinically healthy, stray dog	
Negative	Negative	41,9	Clinically healthy, stray dog	
Negative	Negative	41,9	Clinically healthy, stray dog	
Negative	Negative	41,9	Clinically healthy, stray dog	
Negative	Negative	41,9	Clinically healthy, stray dog	
Negative	Negative	41,9	Clinically healthy, stray dog	
Negative	Negative	36,2	Clinically healthy, stray dog	
Negative	Negative	36,2	Clinically healthy, stray dog	
Negative	Negative	36,2	Clinically healthy, stray dog	
Negative	Negative	36,2	Clinically healthy, stray dog	
Negative	Negative	36,2	Clinically healthy, stray dog	

Check 7-9-15	RLB Utrecht Ticks *B. gibsoni*	RLB dogs Utrecht *B. gibsoni*
	Negative	Negative
	Babesia gibsoni positive	Negative
	Negative	Negative
	Negative	Negative
	Negative	Negative
		Negative
		Negative
		Negative
		Negative
		Negative
		Negative

Appendix E: Membrane ID's used

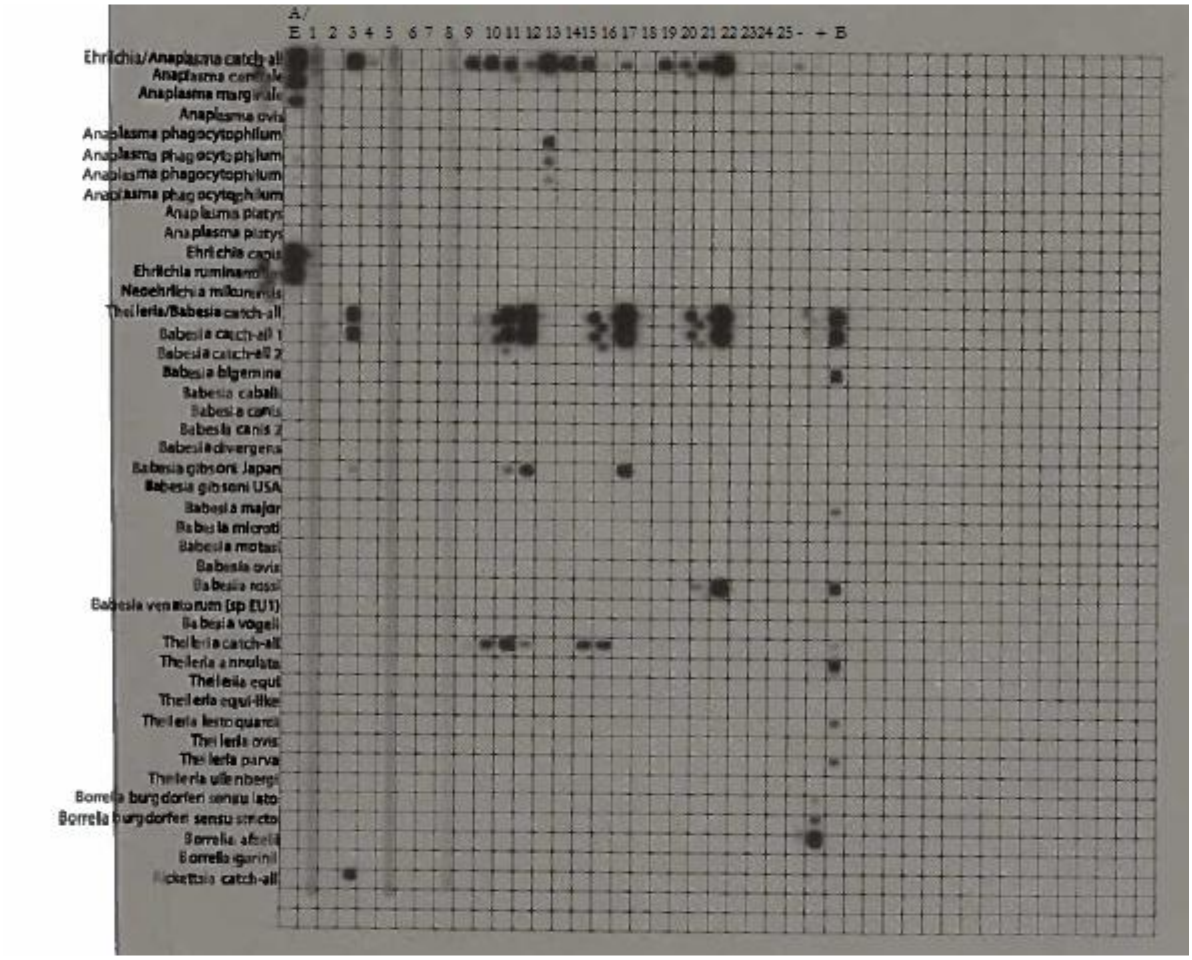


UCTD-12&13

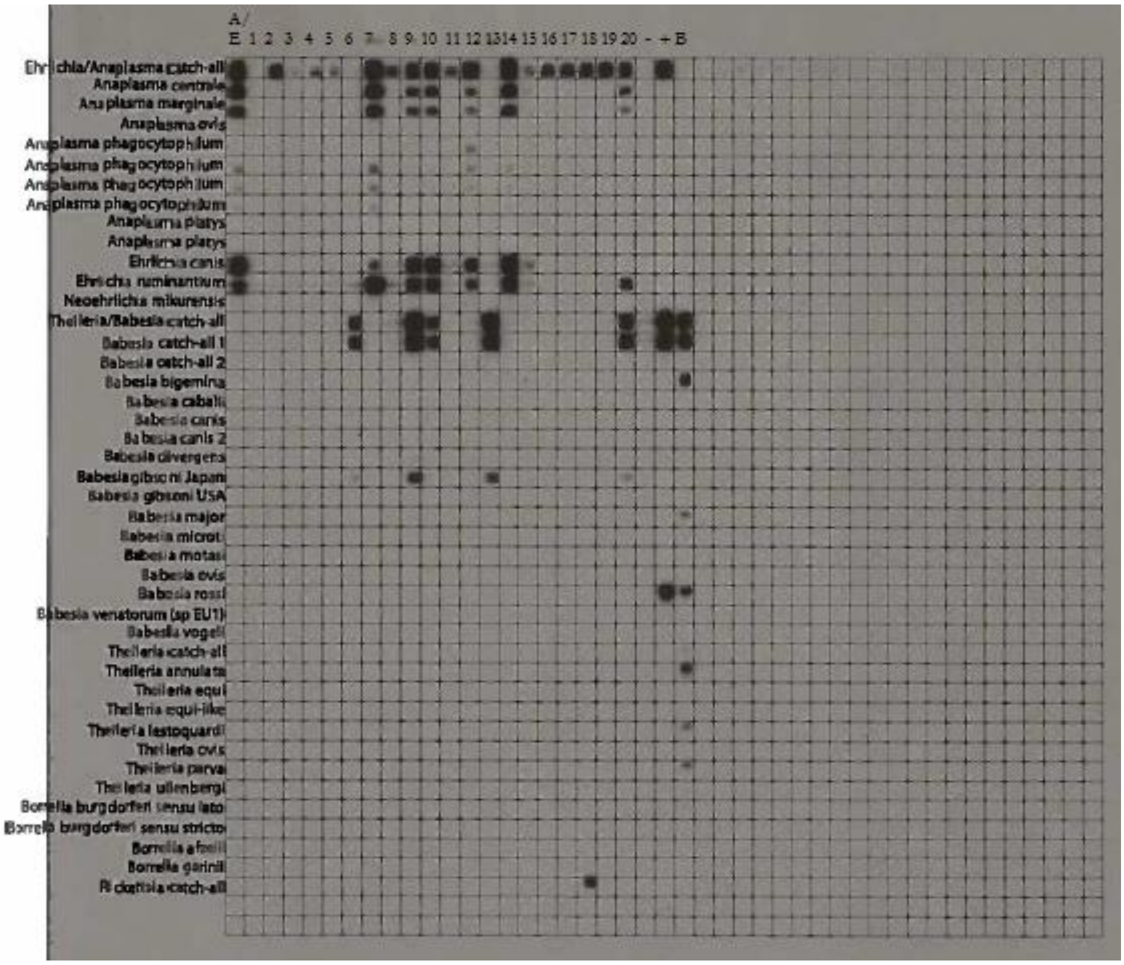
Theileria/Babesia catch-all
Babesia catch-all 1
Babesia catch-all 2
Babesia bicornis
Babesia bigemina
Babesia bovis
Babesia caball
Babesia capreoli
Babesia canis
Babesia canis2
Babesia divergens
Babesia divergens new
Babesia felis
Babesia gibsoni Japan
Babesia gibsoni USA
Babesia major
Babesia microti
Babesia microti new
Babesia motasi
Babesia ovis
Babesia rossi
Babesia venatorum (sp EU1)
Babesia vogeli
Theileria catch-all
Theileria catch-all new
Theileria annulata
Theileria annulata new
Theileria buffeli
Theileria equi
Theileria equi-like
Theileria lestoquardi
Theileria mutans
Theileria ovis
Theileria ovis new
Theileria parva
Theileria sp. (buffalo)
Theileria sp. (duike
Theileria sp. (kudu
Theileria sp. (sable)
Theileria sp. (waykambas)
Theileria taurotragi
Theileria uilenbergi
Theileria velifera

Appendix F: RLB results

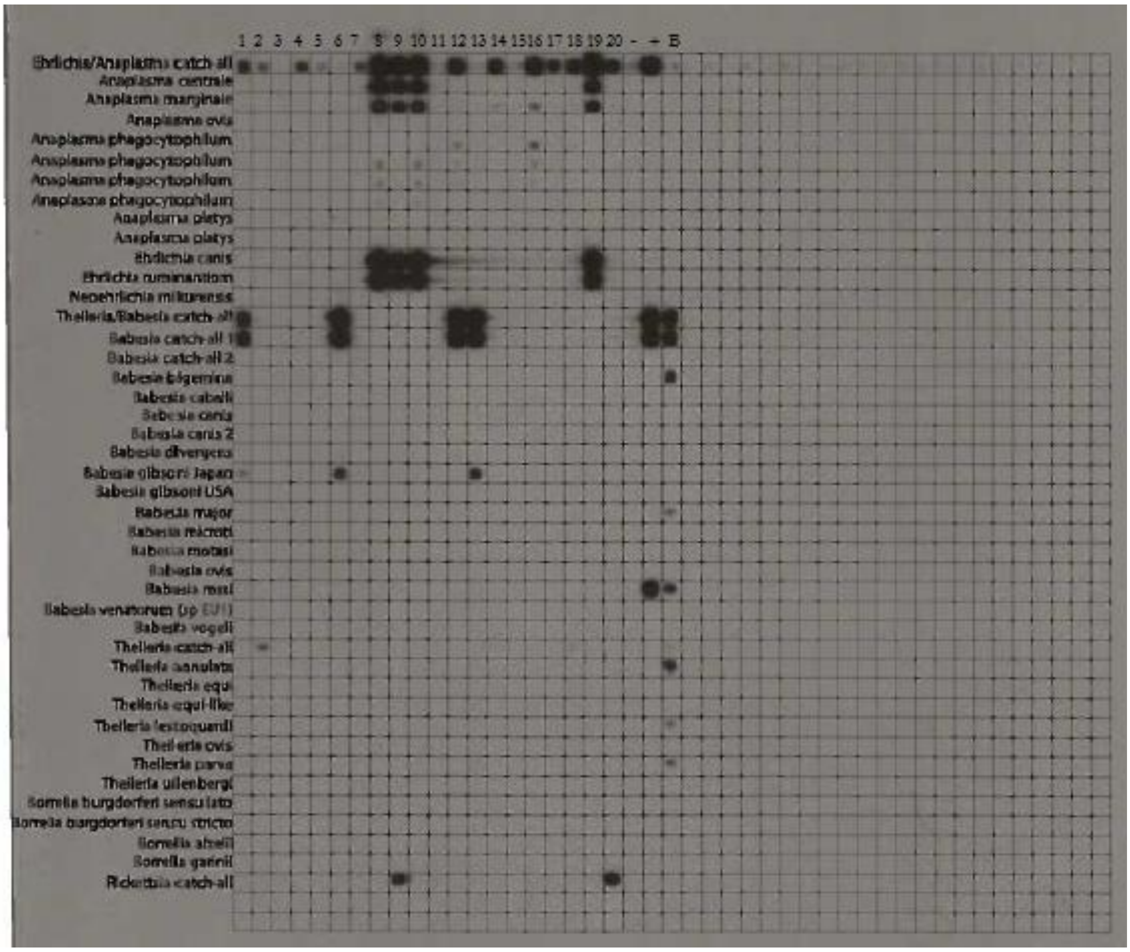
DNA extraction from ticks I – first test



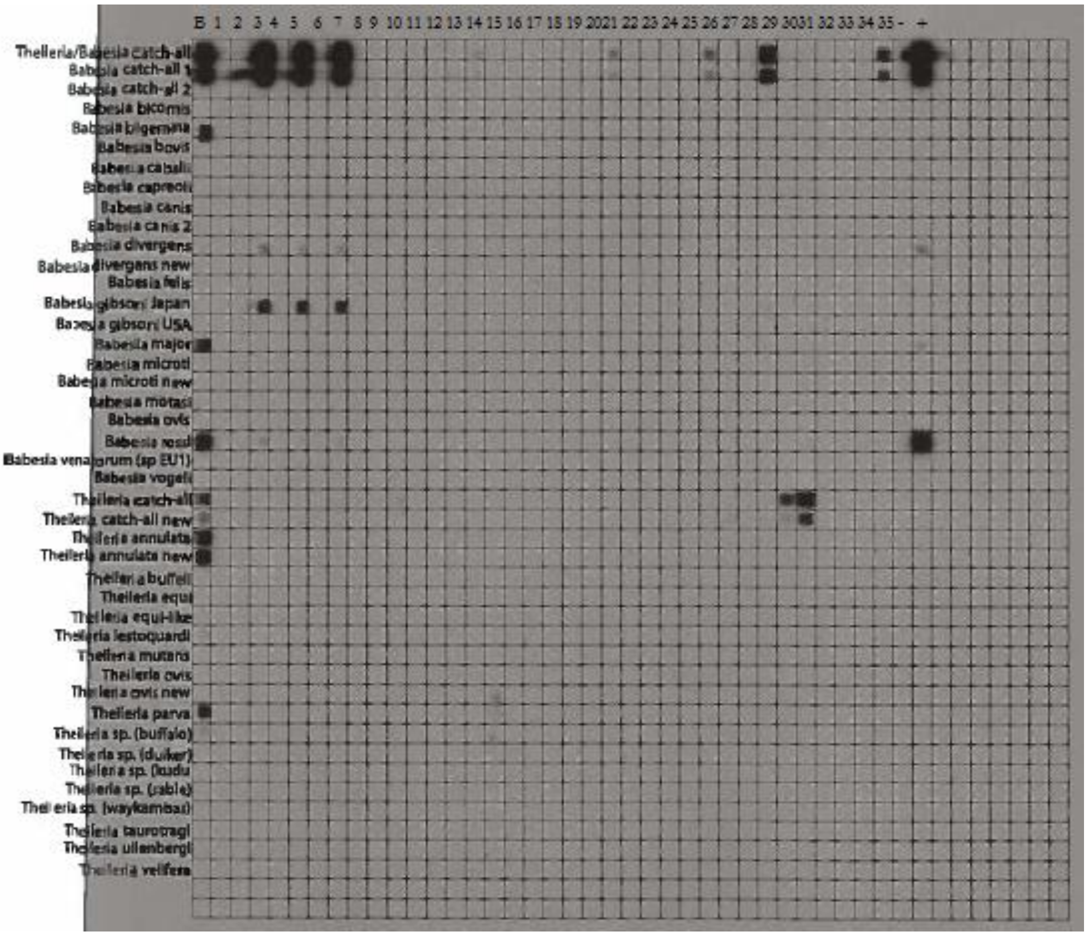
DNA extraction from ticks II – first test



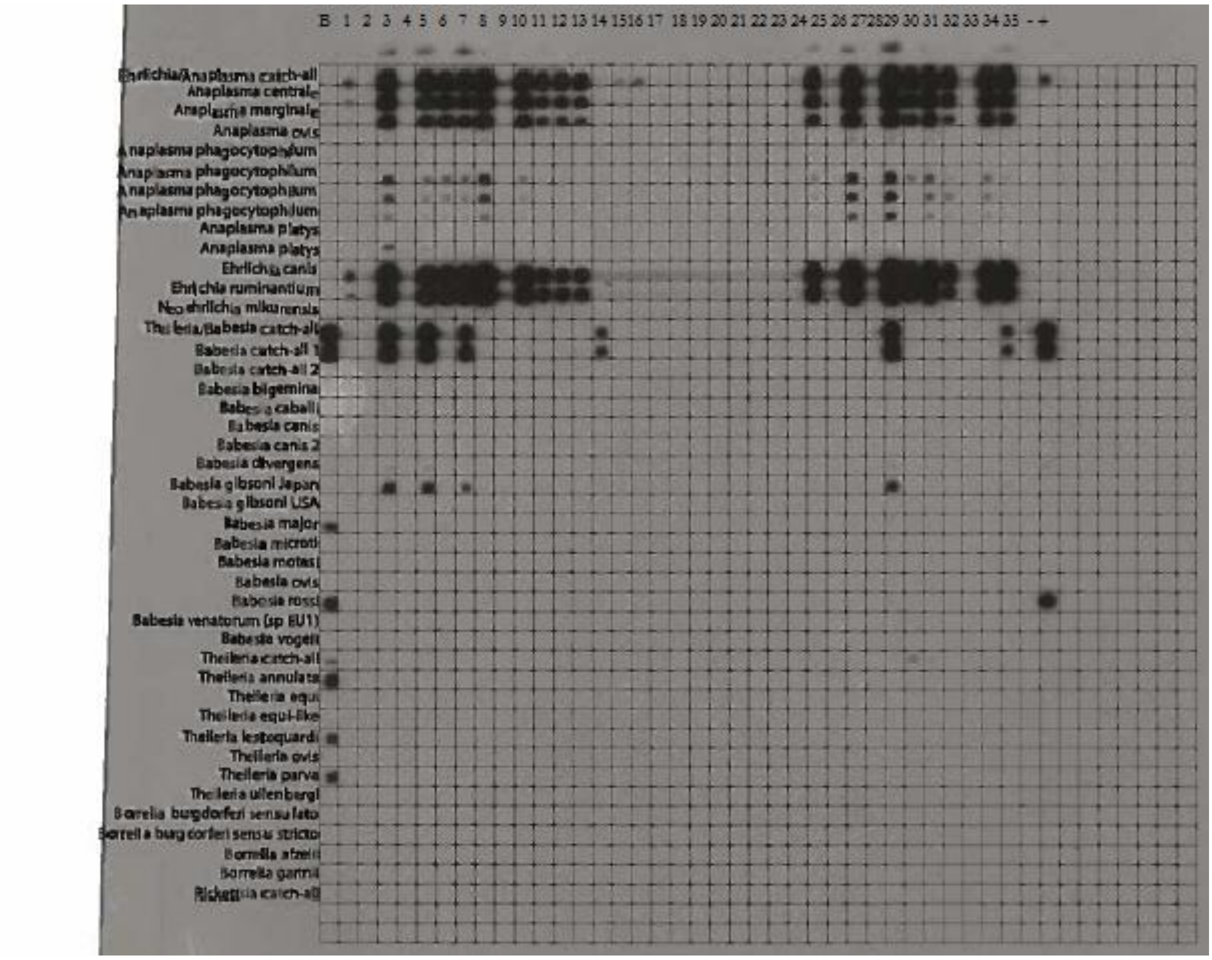
DNA extraction from ticks II – second test



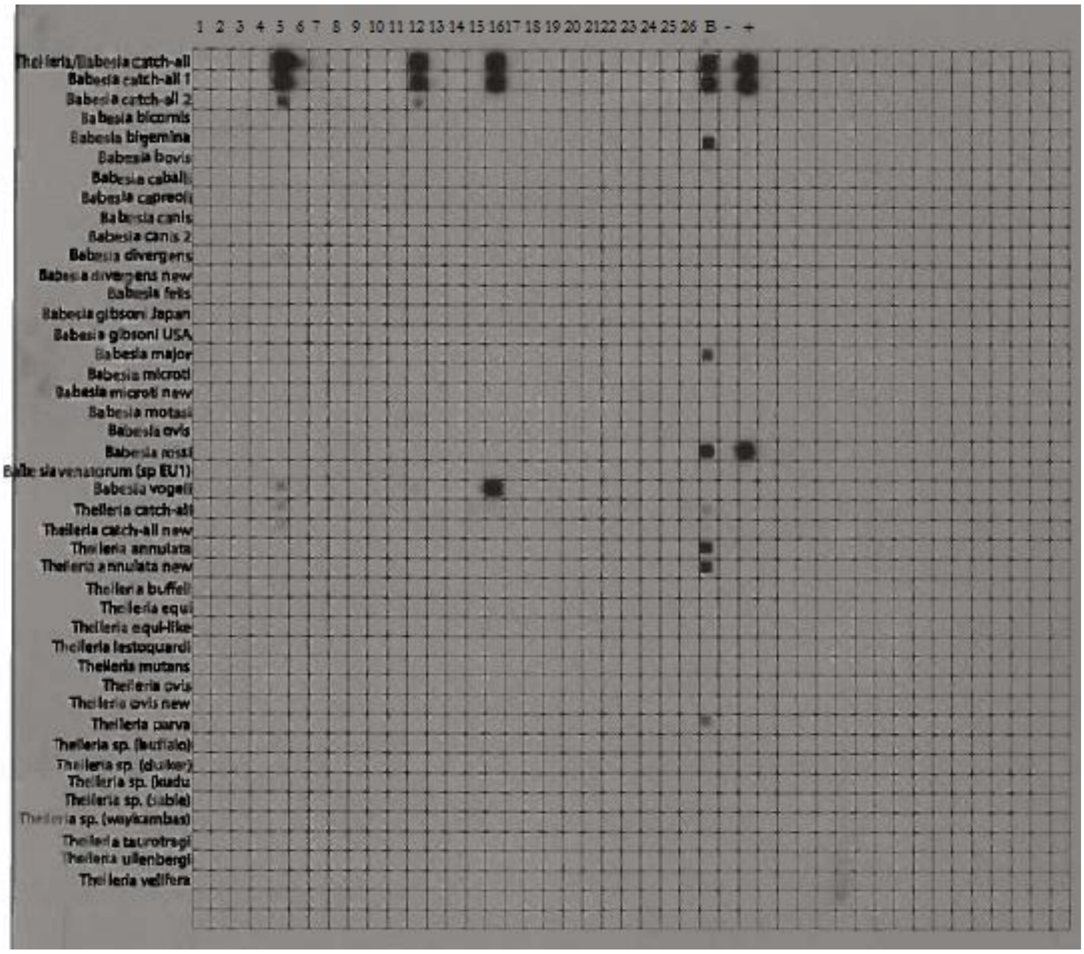
DNA extraction from ticks III – first test



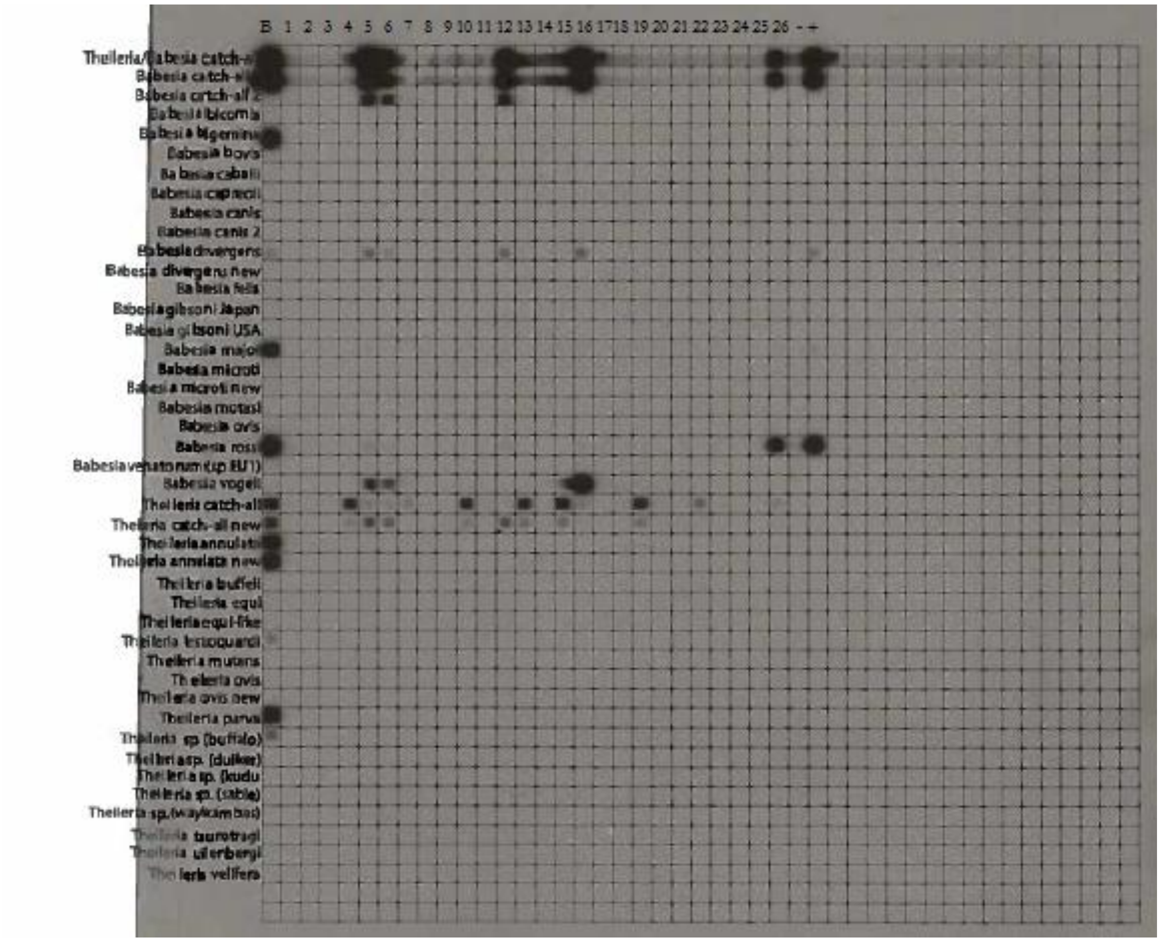
DNA extraction from ticks III – second test



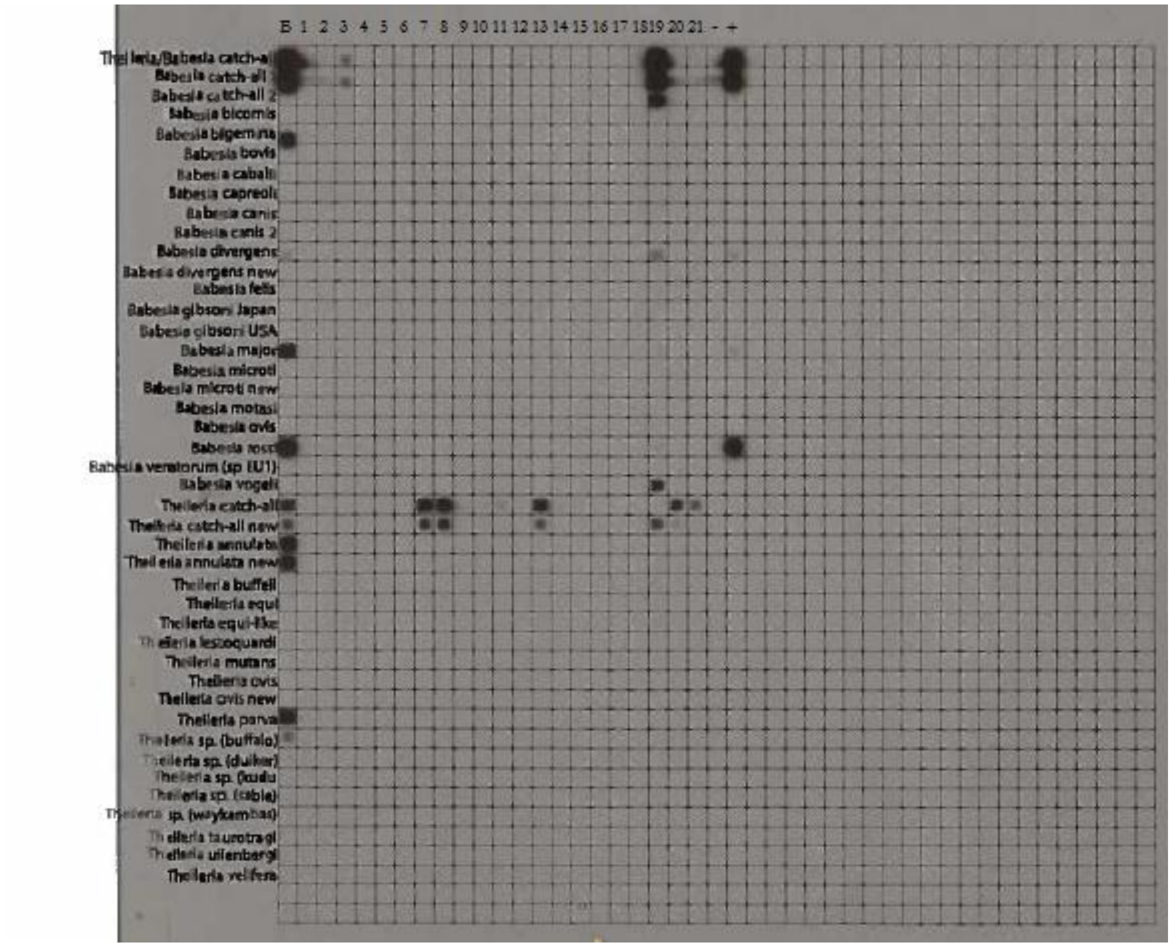
DNA extraction from ticks IV – first test



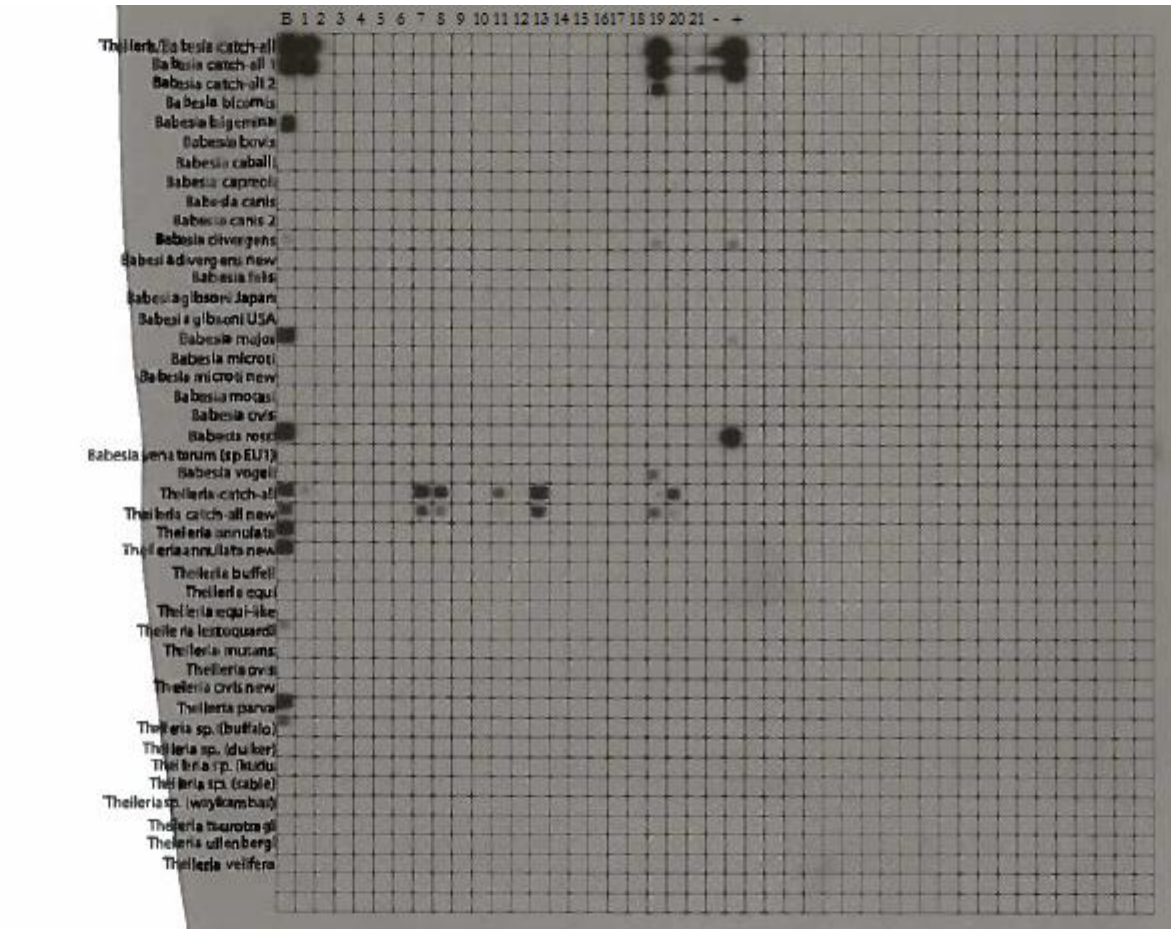
DNA extraction from ticks IV – second test



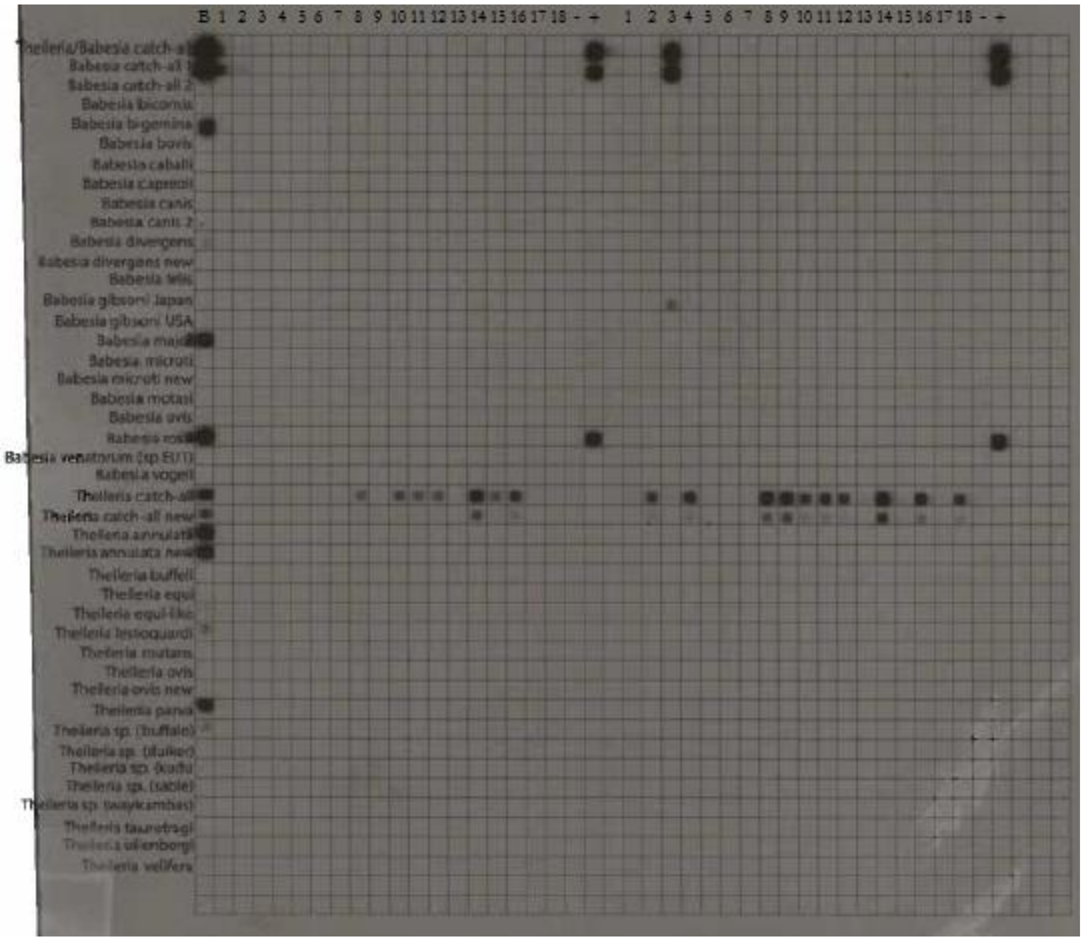
DNA extraction from ticks V – first test



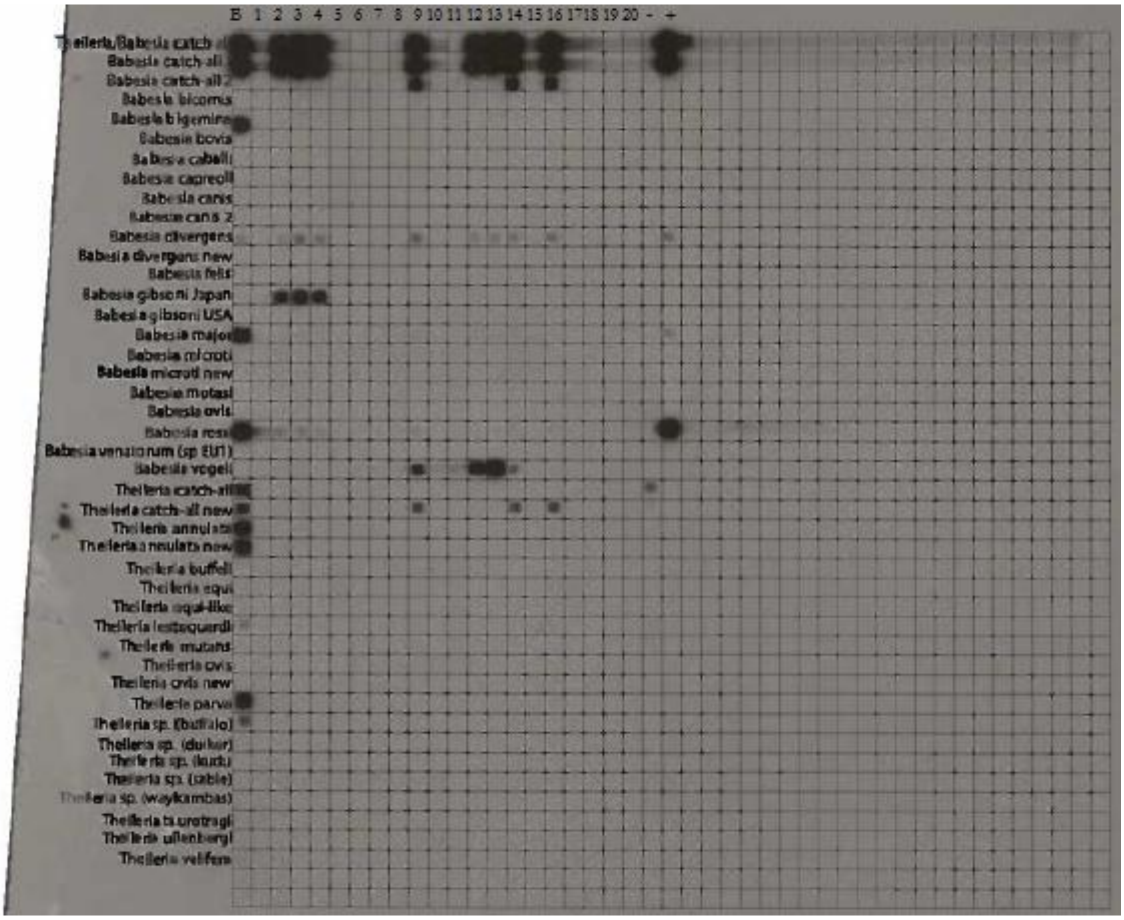
DNA extraction from ticks V – second test



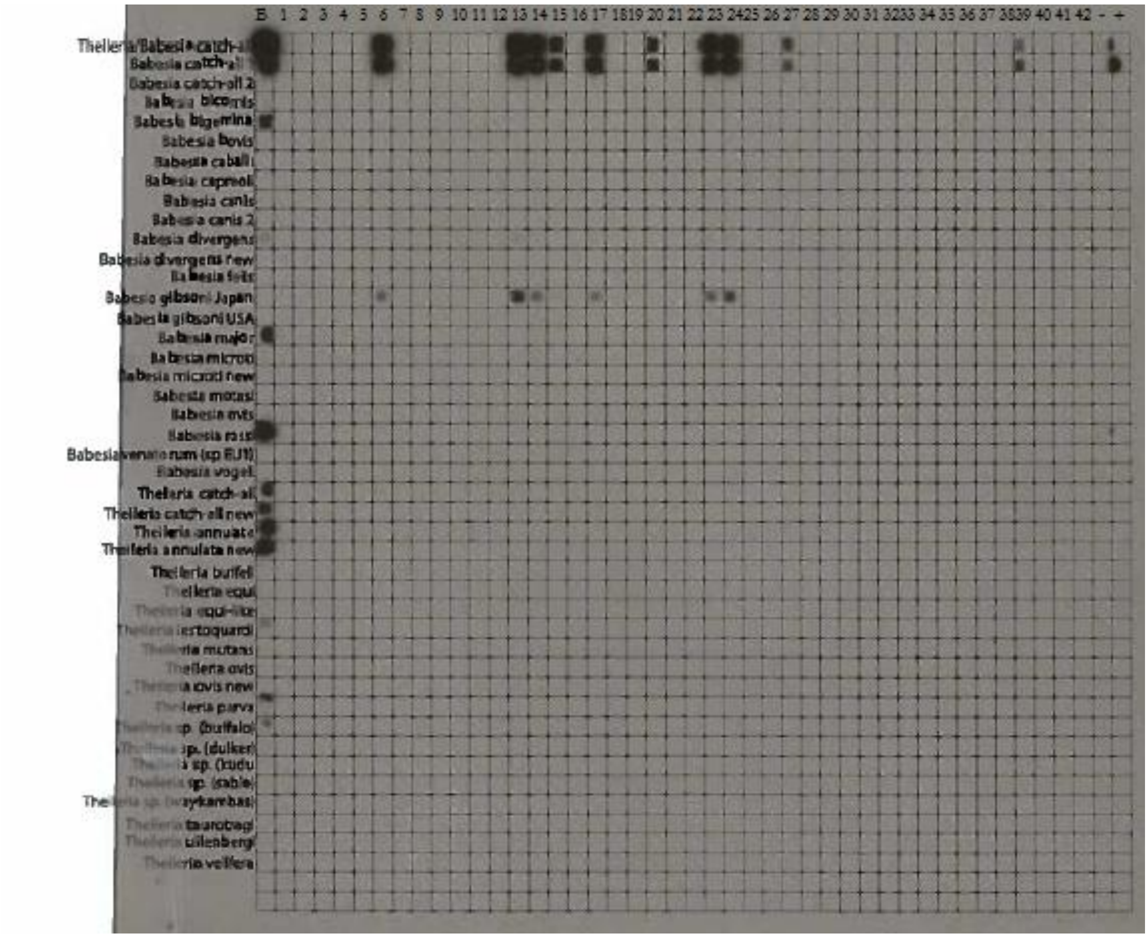
DNA extraction from ticks VI – first & second test



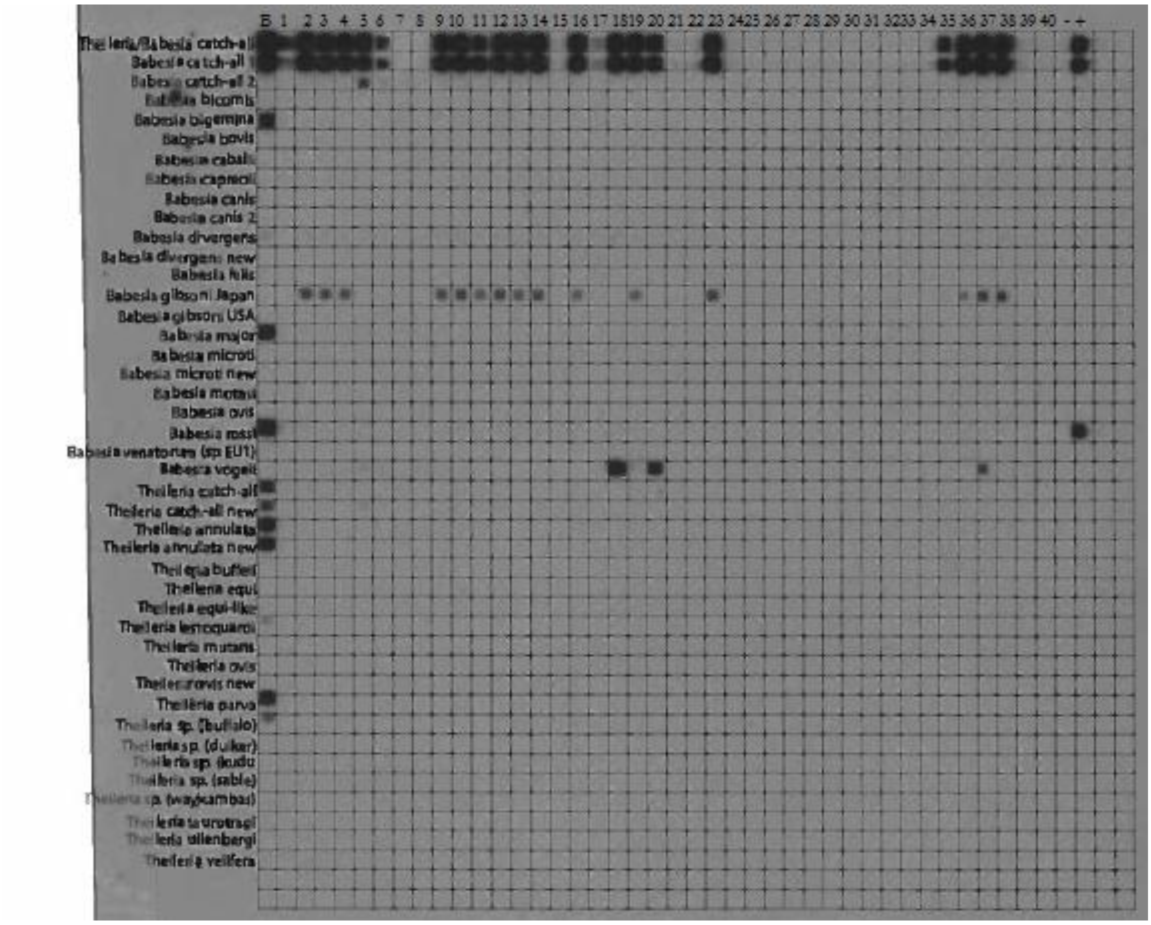
DNA extraction from dogs I



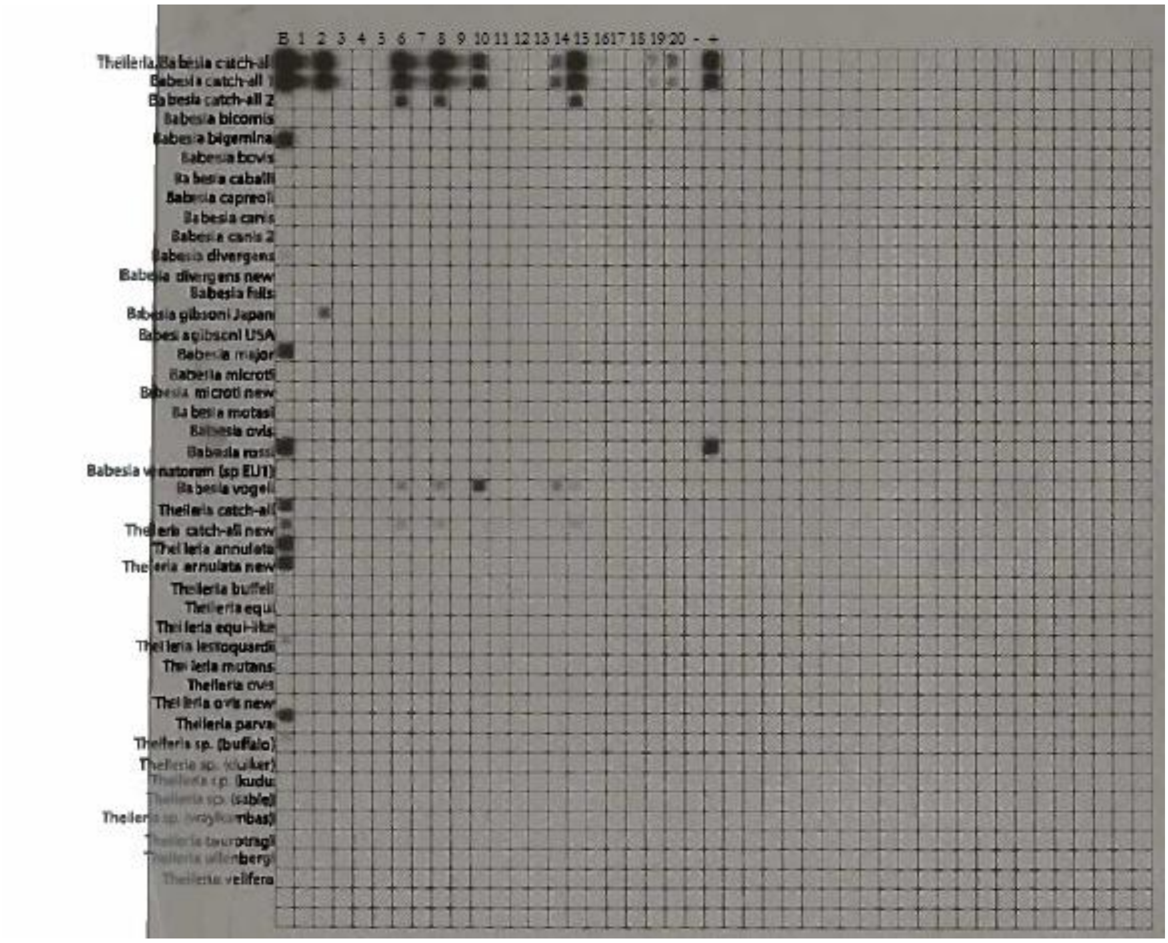
DNA extraction from dogs II



DNA extraction from dogs III



DNA extraction from dogs IV



Appendix G: Sample information of RLB results

DNA extraction from ticks I

Number	Species	Stage	Description
1	*Haemaphysalis hystricis*	Female 1x	VI 1 c104-020
2	*Haemaphysalis hystricis*	Female 1x	VI 2 c104 -022
3	*Haemaphysalis hystricis*	Female 1x	VI 3 c104-022
4	*Haemaphysalis hystricis*	Female 1x	VI 7 c104-022
5	*Haemaphysalis hystricis*	Female 1x	VI 10 c104-019
6	*Haemaphysalis hystricis*	Female 1x	VI 11 c104-019
7	*Haemaphysalis hystricis*	Female 1x	VI 12 c104-022
8	*Haemaphysalis hystricis*	Female 1x	VI 13 c104-022
9	*Haemaphysalis hystricis*	Female 1x	VI 14 c104-021
10	*Haemaphysalis hystricis*	Female 1x	VI 15 c104-021
11	*Haemaphysalis hystricis*	Female 1x	VI 16 c104-022
12	*Haemaphysalis hystricis*	Female 1x	VI 27 c104-017
13	*Haemaphysalis hystricis*	Female 1x	VI 28 c104-017
14	*Haemaphysalis hystricis*	Female 1x	V 12 c104-016
15	*Haemaphysalis hystricis*	Female 1x	V 5 c104-016
16	*Haemaphysalis hystricis*	Female 1x	VII 5 c104-024
17	*Haemaphysalis hystricis*	Female 1x	XIV 4 c104-103
18	*Haemaphysalis hystricis*	Female 1x	X 3 c104-077
19	*Haemaphysalis hystricis*	Female 1x	X 3 c104-077
20	*Haemaphysalis hystricis*	Female 1x	X 3 c104-077

DNA extraction from ticks II

Number	Species	Stage	Description
1	*Haemaphysalis hystricis*	Female 3x	VI 4 c104-019
2	*Haemaphysalis hystricis*	Female 1x	VI 4 c104-019
3	*Haemaphysalis hystricis*	Female 1x	VI 4 c104-019
4	*Haemaphysalis hystricis*	Female 1x	VI 31 c104-022
5	*Haemaphysalis hystricis*	Female 1x	VI 31 c104-022
6	*Haemaphysalis hystricis*	Female 1x	VI 31 c104-022
7	*Haemaphysalis hystricis*	Female 1x	VI 21 c104-021
8	*Haemaphysalis hystricis*	Female 2x	VI 19 c104-017
9	*Haemaphysalis hystricis*	Female 2x	VI 19 c104-017
10	*Haemaphysalis hystricis*	Female 2x	VI 19 c104-017
11	*Haemaphysalis hystricis*	Female 1x	VI 19 c104-017
12	*Haemaphysalis hystricis*	Female 2x	VI 8 c104-019
13	*Haemaphysalis hystricis*	Female 1x	VI 8 c104-019
14	*Haemaphysalis hystricis*	Female 1x	VI 17 c104-022
15	*Haemaphysalis hystricis*	Female 1x	VI 17 c104-022
16	*Haemaphysalis hystricis*	Female 1x	VI 17 c104-022
17	*Haemaphysalis hystricis*	Female 1x	VI 31 c104-022
18	*Haemaphysalis hystricis*	Female 1x	VI 31 c104-022
19	*Haemaphysalis hystricis*	Female 1x	VI 31 c104-022
20	*Haemaphysalis hystricis*	Female 1x	VI 31 c104-022

DNA extraction from ticks III

Number	Species	Stage	Description
1	*Haemaphysalis hystricis*	Male 2x	VI 18 c104-022
2	*Haemaphysalis hystricis*	Male 2x	VI 18 c104-022
3	*Haemaphysalis hystricis*	Female 1x	XIV 1 c104-103
4	*Haemaphysalis hystricis*	Female 2x	VI 21 c104-021
5	*Haemaphysalis hystricis*	Female 1x	XIV 2 c104-103
6	*Haemaphysalis hystricis*	Female 1x	VI 23 c104-018
7	*Haemaphysalis hystricis*	Female 1x	VI 23 c104-018
8	*Haemaphysalis hystricis*	Female 1x	VI 25 c104-020
9	*Haemaphysalis hystricis*	Female 1x	VI 25 c104-020
10	*Haemaphysalis hystricis*	Female 1x	VI 25 c104-020
11	*Haemaphysalis hystricis*	Female 1x	X 10 c104-052
12	*Haemaphysalis hystricis*	Female 1x	VI 29 c104-018
13	*Haemaphysalis hystricis*	Female 1x	VI 29 c104-018
14	*Haemaphysalis hystricis*	Nymph 1x	VI 26 c104-020
15	*Haemaphysalis hystricis*	Male 1x	VI 26 c104-020
16	*Haemaphysalis hystricis*	Male 2x	VI 30 c104-018
17	*Haemaphysalis hystricis*	Male 1x	VI 24 c104-018
18	*Haemaphysalis hystricis*	Male 1x	VI 9 c104-019
19	*Haemaphysalis hystricis*	Male 3x	VI 32 c104-022
20	*Haemaphysalis hystricis*	Male 2x	VI 32 c104-022
21	*Haemaphysalis hystricis*	Male 3x	VI 22 c104-021
22	*Haemaphysalis hystricis*	Male 3x	VI 22 c104-021
23	*Haemaphysalis hystricis*	Male 3x	VI 20 c104-017
24	*Haemaphysalis hystricis*	Male 3x	VI 20 c104-017
25	*Haemaphysalis hystricis*	Female 1x	V 13 c04-016
26	*Haemaphysalis hystricis*	Female 1x	XII 1 c104-086
27	*Haemaphysalis hystricis*	Female 1x	VII 2 c104-025
28	*Haemaphysalis hystricis*	Female 1x	VII 3 c104-025
29	*Haemaphysalis hystricis*	Female 1x	XIII 1 c104-097
30	*Haemaphysalis hystricis*	Female 1x	VII 1 c104-026
31	*Haemaphysalis hystricis*	Female 1x	VII 4 c104-024
32	*Haemaphysalis hystricis*	Female 1x	XIII 2 c104-100
33	*Haemaphysalis hystricis*	Female 1x	IX 1 c104-045
34	*Haemaphysalis hystricis*	Female 1x	XIII 7 c104-097
35	*Haemaphysalis hystricis*	Female 1x	XIII 5 c104-100

DNA extraction from ticks IV

Number	Species	Stage	Description
1	*Rhipicephalus sanguineus*	Female 1x	X 8 c104-056
2	*Rhipicephalus sanguineus*	Female 1x	X 4 c104-060
3	*Rhipicephalus sanguineus*	Female 1x	X 11 c104-057
4	*Rhipicephalus sanguineus*	Female 1x	X 11 c104-057
5	*Rhipicephalus sanguineus*	Female 1x	X 6 x104-058
6	*Rhipicephalus sanguineus*	Female 1x	XIII 9 c104-092
7	*Rhipicephalus sanguineus*	Female 1x	XIII 9 c104-092
8	*Rhipicephalus sanguineus*	Female 1x	XIII 9 c104-092
9	*Rhipicephalus sanguineus*	Female 1x	I + II 4 c104-010
10	*Rhipicephalus sanguineus*	Female 1x	I + II 7 c104-009
11	*Rhipicephalus sanguineus*	Female 1x	I + II 8 c104-009
12	*Rhipicephalus sanguineus*	Female 1x	XI 1 c104-079
13	*Rhipicephalus sanguineus*	Female 2x	XI 5 c104-078
14	*Rhipicephalus sanguineus*	Female 1x	I + II 5 c104-008
15	*Rhipicephalus sanguineus*	Female 1x	XI 6 c104-047
16	*Rhipicephalus sanguineus*	Female 2x	XI 9 c104-075
17	*Rhipicephalus sanguineus*	Female 2x	XI 9 c104-075
18	*Rhipicephalus sanguineus*	Female 1x	VIII 2 c104-037
19	*Rhipicephalus sanguineus*	Female 1x	VIII 1 c104-034
20	*Rhipicephalus sanguineus*	Female 1x	VIII 4 c104-039
21	*Rhipicephalus sanguineus*	Female 2x	XIV 3 c104-106
22	*Rhipicephalus sanguineus*	Female 1x	VIII 12 c104-037
23	*Rhipicephalus sanguineus*	Male 3x	VIII 15 c104-031
24	*Rhipicephalus sanguineus*	Male 3x	VIII 15 c104-031
25	*Rhipicephalus sanguineus*	Female 1x	VIII 6 c104-039
26	*Rhipicephalus sanguineus*	Female 1x	VIII 7 c104-034

DNA extraction from ticks V

Number	Species	Stage	Description
1	*Rhipicephalus sanguineus*	Female 1x	III 6 c104-001
2	*Rhipicephalus sanguineus*	Female 1x	III 2 c104-001
3	*Rhipicephalus sanguineus*	Male 4x	VIII 13 c104-031
4	*Rhipicephalus sanguineus*	Male 3x	VIII 13 c104-031
5	*Rhipicephalus sanguineus*	Female 2x	XI 8 c104-064
6	*Rhipicephalus sanguineus*	Female 2x	XI 8 c104-064
7	*Rhipicephalus sanguineus*	Female 1x	III 1 c104-001
8	*Rhipicephalus sanguineus*	Female 1x	I + II 2 c104-008
9	*Rhipicephalus sanguineus?*	Female 1x	VIII 16 c104-031
10	*Rhipicephalus sanguineus*	Female 1x	I + II 1 c104-008
11	*Rhipicephalus sanguineus*	Male 3x	VIII 14 c104-031
12	*Rhipicephalus sanguineus*	Male 2x	VIII 14 c104-031
13	*Rhipicephalus sanguineus*	Female 2x	XI 1 c104-049
14	*Rhipicephalus sanguineus*	Female 1x	XI 1 c104-049
15	*Rhipicephalus sanguineus*	Female 1x	I + II 6 c104-010
16	*Rhipicephalus sanguineus*	Female 1x	III 5 c104-001
17	*Rhipicephalus sanguineus*	Female 1x	X 6
18	*Rhipicephalus sanguineus*	Female 1x	X 6
19	*Rhipicephalus sanguineus*	Female 1x	III 3 c104-001
20	*Rhipicephalus sanguineus*	Female 1x	I + II 3 c104-009
21	*Rhipicephalus sanguineus*	Female 1x	III 4 c104-001

DNA extraction from ticks VI

Number	Species	Stage	Description
1	*Haemaphysalis hystricis*	Female 1x	XVI 5 c104-119
2	*Haemaphysalis hystricis*	Female 1x	XVI 1 c104-119
3	*Haemaphysalis hystricis*	Female 1x	XVI 2 c104-119
4	*Haemaphysalis hystricis*	Female 1x	XVI 4 c104-119
5	*Haemaphysalis hystricis*	Male 3x	XII 2 c104-083
6	*Haemaphysalis hystricis*	Female 1x	XIII 6 c104-099
7	*Haemaphysalis hystricis*	Female 1x	XVI 3 c104-119
8	*Haemaphysalis hystricis*	Nymphe 5x	Babesia pos 1 29-8-15
9	*Rhipicephalus sanguineus*	Female 1x	I + II c104-006
10	*Rhipicephalus sanguineus*	Female 1x	XV 2 c104-113
11	*Rhipicephalus sanguineus*	Female 2x	X 9 c104-060
12	*Rhipicephalus sanguineus*	Female 1x	X 9 c104-060
13	*Rhipicephalus sanguineus*	Female 1x	XV 1 c104-110
14	*Rhipicephalus sanguineus*	Female 3x	X 12 c104-059
15	*Rhipicephalus sanguineus*	Female 2x	X 12 c104-059
16	*Rhipicephalus sanguineus*	Female 2x	X 13 c104-099
17	*Rhipicephalus sanguineus*	Female 1x	X 13 c104-099
18	*Rhipicephalus sanguineus*	Female 1x	X 1 c104-062

DNA extraction from dogs I

Number	Description
1	c104-021 B
2	c104-022 B
3	c104-023 B
4	c104-024 B
5	c104-025 B
6	c104-026 B
7	c104-027 B
8	c104-028 B
9	c104-029 B
10	c104-030 B
11	c104-061 B
12	c104-062 B
13	c104-063 B
14	c104-064 B
15	c104-065 B
16	c104-066 B
17	c104-067 B
18	c104-068 B
19	c104-069 B
20	c104-070 B

DNA extraction from dog II

Number	Description
1	c104-081
2	c104-082
3	c104-083
4	c104-084
5	c104-085
6	c104-086
7	c104-087
8	c104-088
9	c104-089
10	c104-090
11	c104-091
12	c104-092
13	c104-093
14	c104-094
15	c104-095
16	c104-096
17	c104-097
18	c104-098
19	c104-099
20	c104-100
21	c104-101
22	c104-102
23	c104-103
24	c104-104
25	c104-105
26	c104-106
27	c104-107
28	c104-108
29	c104-109
30	c104-110
31	c104-111
32	c104-112
33	c104-113
34	c104-114
35	c104-115
36	c104-116
37	c104-117
38	c104-118
39	c104-119
40	c104-120
41	c104-121
42	c104-122

DNA extraction from dogs III

Number	Description
1	c104-001
2	c104-002
3	c104-003
4	c104-004
5	c104-005
6	c104-006
7	c104-007
8	c104-008
9	c104-009
10	c104-010
11	c104-011
12	c104-012
13	c104-013
14	c104-014
15	c104-015
16	c104-016
17	c104-017
18	c104-018
19	c104-019
20	c104-020
21	c104-031
22	c104-032
23	c104-033
24	c104-034
25	c104-035
26	c104-036
27	c104-037
28	c104-038
29	c104-039
30	c104-040
31	c104-041
32	c104-042
33	c104-043
34	c104-044
35	c104-045
36	c104-046
37	c104-047
38	c104-048
39	c104-049
40	c104-050

DNA extraction from dogs IV

Number	Description
1	c104-051
2	c104-052
3	c104-053
4	c104-054
5	c104-055
6	c104-056
7	c104-057
8	c104-058
9	c104-059
10	c104-060
11	c104-071
12	c104-072
13	c104-073
14	c104-074
15	c104-075
16	c104-076
17	c104-077
18	c104-078
19	c104-079
20	c104-080