Prevalence of *Babesia canis* in *Dermacentor reticulatus* ticks in the Netherlands and Belgium

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Abstract

Dermacentor reticulatus is an important vector of several pathogens, including *Babesia canis*. Currently, *D. reticulatus* ticks appear in the vegetation of the Netherlands and Belgium. Adult ticks were collected from the vegetation of three places including Panne, Belgium (*n*=208), the Dintelse Gorzen, the Netherlands (*n*=40) and Rozenburg, the Netherlands (*n*=40). The ticks were screened for the presence of *Babesia canis* and also for other *Babesia* and *Theileria* species, using Reverse Line Blot (RLB) and PCR techniques. *D. reticulatus* ticks were infected with *Theileria equi-like* (17.1%), *Babesia* sp. (6.8%) and *Babesia canis* (3.5%). We concluded that a broader spectrum of tick-borne pathogens is present in *D. reticulatus* ticks from the vegetation of the Netherlands and Belgium than previously thought.

Keywords: *Dermacentor reticulatus*; *Babesia canis*; the Netherlands; Belgium

1. Introduction

Ticks are of great veterinary and medical importance. They are vectors for several pathogens. *Dermacentor reticulatus* (Fabricius, 1794), also known as the ornate dog tick, is the most important vector of *Babesia canis*. *D. reticulatus* is distributed in Europe and Asia (Solano-Gallego et al. 2008, Anderson, Magnarelli 2008) and can cause besides canine babesiosis several other diseases like equine babesiosis (*Babesia caballi*), Siberian tick typhus (*Rickettsia sibirica*) and human rickettsiosis (*Rickettsia slovaka*)(Jongejan, Uilenberg 2004). Recently, *D. reticulatus* has also been found as a vector of tick-borne encephalitis virus (TBEV) (Wojcik-Fatla et al. 2011). *D. reticulatus* ticks have a number of characteristics. They are medium of size (4 to 5 mm), their mouthparts are short and anterior, they have large eyes, enamel on the scutum and the first pair of coxae have paired spurs (Estrada-Peña et al. 2004). *D. reticulatus* is a three host tick, meaning that each stage feeds only once on the host and the ecdysis occurs in the environment. Only the adult stage uses the dog as host (Matjila et al. 2005). Larvae and nymphs feed on small rodents and birds, while adult ticks can, besides dogs, feed on ponies and large ruminants such as roe deer and cattle (Bodaan et al. 2007).

During the feeding, *D. reticulatus* can transmit *B. canis* to the dog. As a result, the dog can develop the disease canine babesiosis. This disease can be recognized by fever, anaemia, dark urine and kidney failure (Bodaan et al. 2007). The clinical signs in dogs vary from a mild transient disease to an acute illness, caused by severe haemolysis, quickly resulting in death (Jongejan et al. 2011). Without treatment, babesiosis is fatal for the dog (Bodaan et al. 2007). *B. canis* has been distributed worldwide in Europe, but was previously relatively rare in cold continental climates (Beugnet, Marie 2009).

Recent research has demonstrated that *D. reticulatus* ticks have been established in the Netherlands (Nijhof et al. 2007). The distribution of *D. reticulatus* ticks has been caused by import of dogs and climate changes, especially the shorter winters and increasing minimum temperatures. Besides this, travelling with dogs also plays a role in the spread of *D. reticulatus* (Irwin 2010). The presence of *D. reticulatus* in the entire Europe increases the risk of canine babesiosis in many countries (Beugnet, Marie 2009). Babesiosis is

endemic in France, Switzerland, Hungary, Serbia, Croatia, northern Italy and northern Spain. However babesiosis was less common in Belgium, Germany, Poland and the Netherlands. But since 2000 a clear expansion takes place in these countries (Beugnet, Marie 2009). Six locations have been recognised in the Netherlands where *D.* reticulatus ticks are found in large numbers in the vegetation. These are the Dintelse Gorzen, St. Philipsland, Slikken van de Heen, nature area the Piet at the lake of the Veer in Zeeland, the Maashorst in Brabant and St. Maartenszee in North Holland (Bodaan et al. 2007). Because of the establishment of *D. reticulatus* in the Netherlands, canine babesiosis can become endemic in this country.

In this survey *D. reticulatus* ticks have been screened by Reverse Line Blot (RLB) of the presence of *B. canis*. Those ticks have been collected during field surveys in the Netherlands and Belgium. The aim of this survey is to determine the prevalence of *B. canis* and other *Babesia* and *Theileria* species in *D. reticulatus* ticks. To discover more places where *D. reticulatus* ticks lives in the vegetation. And to prove that *B. canis* actually exist in *D. reticulatus* ticks in the field in the Netherlands and Belgium.

2. Materials and methods

2.1 Collection of ticks

Ticks were collected in different areas in the Netherlands and Belgium by dragging the vegetation with flannel. In total, three areas have been dragged, Rozenburg (A), Dintelse Gorzen (B) in the Netherlands and Panne (C) in Belgium (Fig. 1 and Table 4). In the laboratory the ticks were kept in a stove at 27 degrees and at 80% relative humidity. For the research they were slain in 70% alcohol.

Figure 1. Map of the Netherlands and Belgium showing the localities where *D. reticulatus* was found in the vegetation. The letters correspond to the locations presented in Table 4.

2.2 DNA extraction

DNA was extracted from the collected *D. reticulatus* ticks. The NucleoSpin® Tissue Kit (Art. No. 740952.10/.50/.250, Macherey-Nagel) was used for this extraction. Surface sterilised ticks were cut into four pieces, triturated and further treated according to the manufacturer's protocols. The DNA samples were stored at -20 °C.

2.3 Polymerase Chain Reaction (PCR)

The obtained DNA samples can then be used in certain provisions, including Reserve Line Blot (RLB) hybridisation. For this, a Polymerase Chain Reaction (PCR) should be done with a PCR primer pair, so that specific DNA fragments cause, which can be demonstrated by the RLB. For this survey, PCR was performed with a primer for *Babesia/Theileria* species (Table 1).

Table 1. Sequence primers.

Pathogen	Primer	Sequence	Orientation	T*m* (°C)
Babesia	RLB-F2	5'-GAC ACA GGG AGG TAG TGA CAA G	+	57.9
	RLB-R2	5'-Biotin-CTA AGA ATT TCA CCT CTG ACA GT	-	53.7

A special master mix for PCR had been made (Table 2). This PCR mix contains a buffer, dNTPS, the two primers for *Babesia*, DNA polymerase and H_2O. The master mix then had to be pipetted over the individual tubes. Each tube had a final volume of 25 µl (22.5 µl mix + 2.5 µ DNA product). A special thermocycler PCR touchdown program was performed in the thermocycler (Table 3). During the 10 cycles program, the temperature was one degree lower each round, starting with 67 degree and ending at 57 degree.

Table 2. PCR mix.

Total master mix for 1 reaction	
5.0 µl	5x Phire reaction buffer
0.5 µl	10 mM dNTPS
0.5 µl	F primer (20pmol/µl)
0.5 µl	R primer (20pmol/µl)
0.125 µl	2U/µl Phire Hot Start II DNA polymerase
15.875 µl	H_2O
2.5 µl	DNA

Table 3. Temperature cycle of PCR program

Number of cycles	Time	Temperature
1 cycle	30 sec	98 °C
	5 sec	98 °C
10 cycles	5 sec	67 -> 57 °C
	7 sec	72 °C
	5 sec	98 °C
50 cycles	5 sec	57 °C
	7 sec	72 °C
1 cycle	60 sec	72 °C

2.4 Agarose gel electrophoresis

To verify that the PCR was carried out properly, an agarose gel with the positive and negative control of the PCR was run. A 1.125 % gel was made using the protocol. The positive and negative samples were mixed with a 6x DNA loading dye and loaded into the sample wells. A reference 100bp DNA ladder was also loaded into the sample wells. The gel was run for 30 to 45 minutes. After sufficient migration, the gel was observed under an UV-illuminator. The PCR was performed correctly if the positive control sample was visible with a fragment around 500bp size and the negative sample showed no amplification. In Figure 2, the result of an agarose gel electrophoresis is shown.

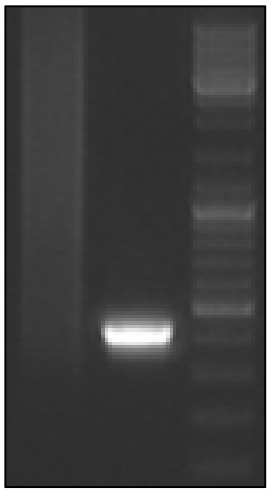

Figure 2. Agarose gel electrophoresis

2.5 Reserve line blot (RBL) hybridisation

After PCR amplification, the PCR products are hybridised on a blot on which a specific oligonucleotide for each (known) *Theileria* and *Babesia* species has been covalently linked. The PCR products are applied to the membrane, using a miniblotter, so that the direction of the PCR products is perpendicular to the direction of the species-specific oligonucleotides. Two control oligonucleotides, namely AE30 and B100, are also applied to the membrane. To remove unbound PCR products, the membrane has been stringent washed. After this, the hybridised PCR products are visualised using chemiluminescence. Visualisation makes use of a biotin label attached to the PCR primer. The biotin label is incubated with a streptavidin + HRP conjugate. Incubation of the blot with the peroxidase substrate, ECL, results in a reaction producing light. The light can be detected on a suitable film by 10 minutes incubation. After development of the film, spots occur at the sites where species-specific oligonucleotides and PCR products hybridised. The identity of the *Theileria* and *Babesia* species in the sample can then be identified (Isogen Life Science, 2004). Fig. 3 and 4 gives a schematic representation of the hybridisation principle and of the RLB assay.

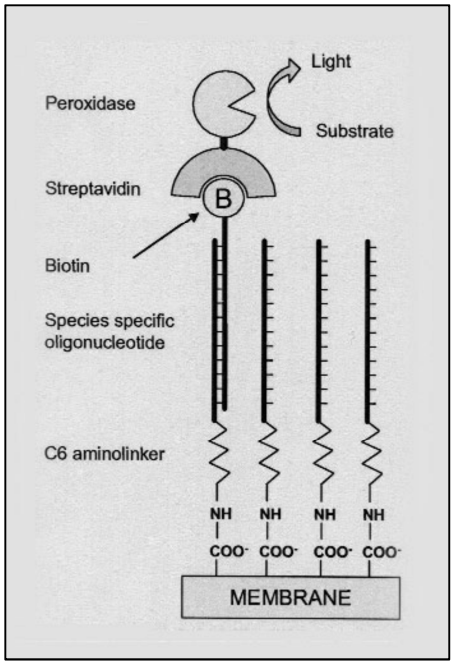

Figure 3. Schematic representation of the hybridisation principle.

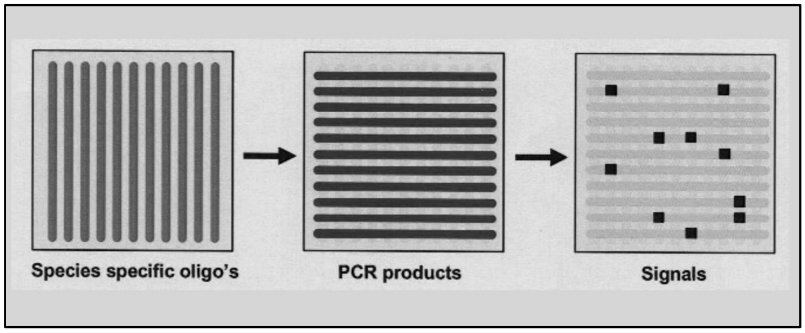

Figure 4. Schematic representation of the RLB assay.

3. Results

3.1 Submitted ticks

A total of 288 adult ticks, 117 males and 131 females and 40 unknown sexes, were collected alive from the vegetation in different areas in the Netherlands and Belgium during the autumn and winter of 2011. In total, three areas have been surveyed (Fig. 1 and Table 4) including Panne, the Dintelse Gorzen and Rozenburg. Panne is a nature area in Belgium 500 meters from the beach with dune grass and long reed-like grass. In this area, cattle and wild horses are grazing. In Panne, 208 adult ticks, 97 males and 111 females, were collected. The Dintelse Gorzen (Fig. 5) is a fresh water nature area in the Netherlands with moist soil and enough vegetation for shelter against extreme environmental conditions (Bodaan et al. 2007). In the Dintelse Gorzen, 40 adult ticks, many males as females, were collected. Rozenburg (Fig. 6) is a wild dune-like landscape. In Rozenburg, 40 adult ticks (sex unknown), were collected. Moreover, 40 adult ticks, one male and 39

females, were collected from cattle. Those cattle were from a farm in the Netherlands. Also, 40 adult ticks, many males as females, were obtained from a laboratory colony of South Africa. Furthermore, a blood sample of a horse from the Netherlands has been screened for *Babesia* and *Theileria* species.

Table 4. Localities in the Netherlands and Belgium where *D. reticulatus* was found in the vegetation.

Location	Name	Country	Date of inspection	Number and stage of collected ticks
A	Rozenburg	Netherlands	September 2011	40, sex unknown
B	Dintelse Gorzen	Netherlands	November 2011	40, 20 males and 20 females
C	Panne	Belgium	October 2011	208, 97 males and 111 females

Figure 5. The Dintelse Gorzen

Figure 6. Rozenburg

3.2 Pathogen detection

This is a summary of the RLB results which can be found in the appendix.

a. Ticks from the vegetation of the Netherlands and Belgium

A total of 288 adult ticks from the vegetation of the Netherlands and Belgium were screened by RLB for the presence of *Babesia* and *Theileria* (Table 5 and Fig. 7). 117 (40.6%) of the ticks were male and 131 (45.5%) were female. 40 (13.9%) ticks were used whose gender is unknown. An amount of 45 ticks (15.6%) were positive for *Theileria equi-like*. Fifteen ticks (5.2%) were positive for *Babesia* catch-all, but no specific *Babesia* species was found in this case. Five ticks (1.7%) were positive for *Theileria equi-like* and also for *Babesia* catch-all, all collected in Panne. Four ticks (1.4%) were positive for *Theileria/Babesia* catch-all and *B. canis*, all collected in Rozenburg. Six ticks (2.1%) were positive for *Theileria/Babesia* catch-all, *B. canis* and *B. divergens*, all collected in Rozenburg too. An amount of 213 ticks (74.0%) were negative for any *Babesia* or *Theileria* species.

Table 5. Pathogens detected in *D. reticulatus* ticks from the vegetation of the Netherlands and Belgium by RLB screening.

	Amount	Proportion
Total number of ticks	288	100%
Males	117	40.6%
Females	131	45.5%
Sex unknown	40	13.9%
Theileria equi-like positive	45	15.6%
Babesia catch-all positive	15	5.2%
Theileria equi-like and *Babesia* catch-all positive	5	1.7%
Theileria/Babesia catch-all and *B. canis* positive	10	3.5%
Negative	213	74.0%

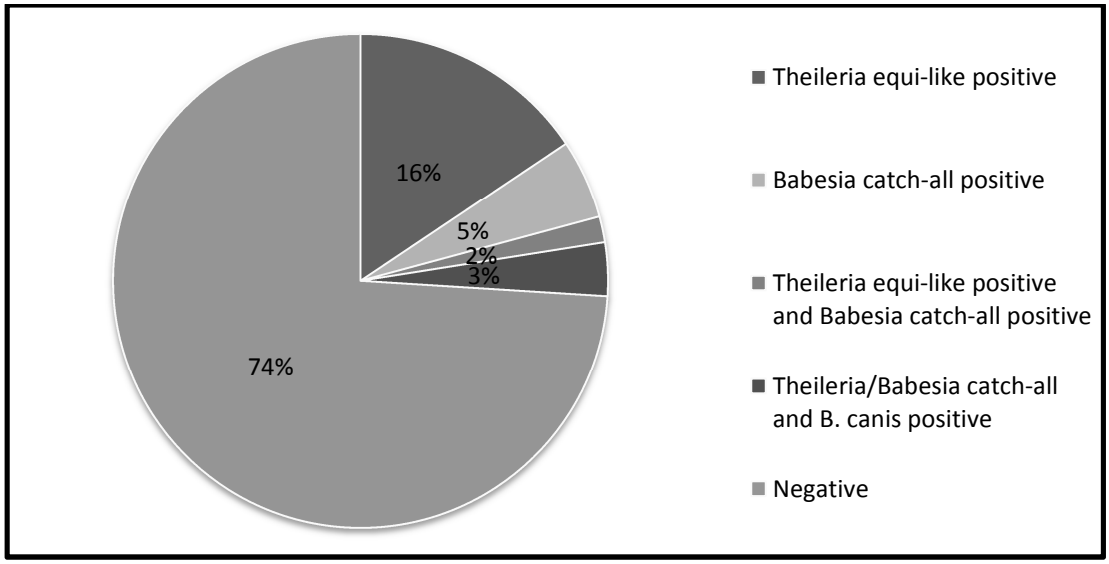

Figure 7. Pathogens detected in *D. reticulatus* ticks from the vegetation of the Netherlands and Belgium by RLB screening.

b. Ticks from the vegetation of Panne, Belgium

In Panne, a total of 208 adult ticks were collected from the vegetation and screened by RLB for the presence of *Babesia* and *Theileria* species (Table 6 and Fig. 8). 97 (46.6%) of the ticks were male and 111 (53.4%) were female. An amount of 41 ticks (19.7%) were positive for *T. equi-like*. Eleven ticks (5.3%) were positive for *Babesia* catch-all, but no specific *Babesia* species was found in this case. Five ticks (2.4%) were positive for *T. equi-like* and also for *Babesia* catch-all. An amount of 151 ticks (72.6%) were negative for any *Babesia* or *Theileria* species.

Table 6. Pathogens detected in *D. reticulatus* ticks from the vegetation of Panne, Belgium by RLB screening.

	Amount	**Proportion**
Total number of ticks	208	100%
Males	97	46.6%
Females	111	53.4%
Theileria equi-like positive	41	19.7%
Babesia catch-all positive	11	5.3%
Theileria equi-like and *Babesia* catch-all positive	5	2.4%
Negative	151	72.6%

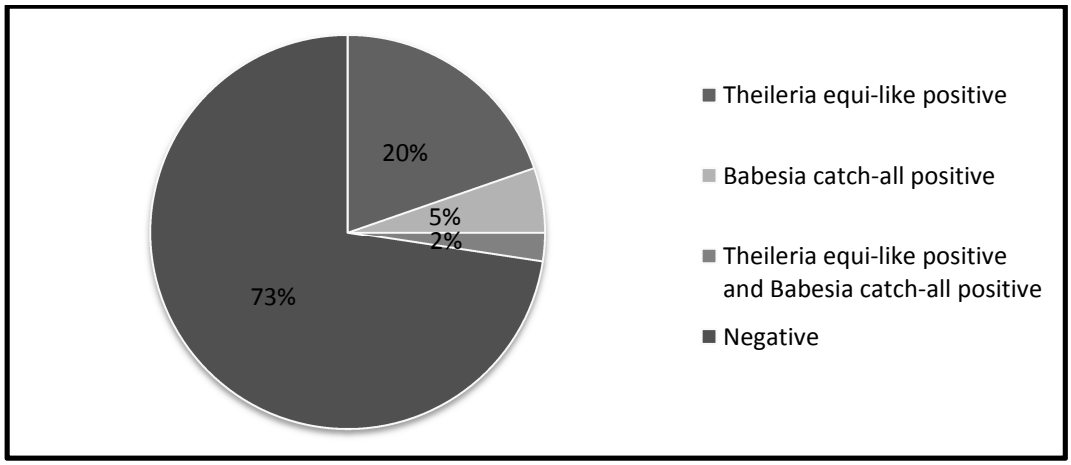

Figure 8. Pathogens detected in *D. reticulatus* ticks from the vegetation of Panne, Belgium by RLB screening.

c. Ticks from the vegetation of Dintelse Gorzen, the Netherlands

In the Dintelse Gorzen, a total of 40 adult ticks were collected from the vegetation and screened by RLB for the presence of *Babesia* and *Theileria* species (Table 7 and Fig. 9). Half of the ticks were male and half of the ticks were female. Four ticks (10%) were positive for *T. equi-like*. Two ticks (5%) were positive for *Babesia* catch-all, but no specific *Babesia* species was found in this case. The rest of the ticks (85%) were negative for any *Babesia* or *Theileria* species.

Table 7. Pathogens detected in *D. reticulatus* ticks from the vegetation of Dintelse Gorzen, the Netherlands by RLB screening.

	Amount	Proportion
Total number of ticks	40	100%
Males	20	50%
Females	20	50%
Theileria equi-like positive	4	10%
Babesia catch-all positive	2	5%
Negative	34	85%

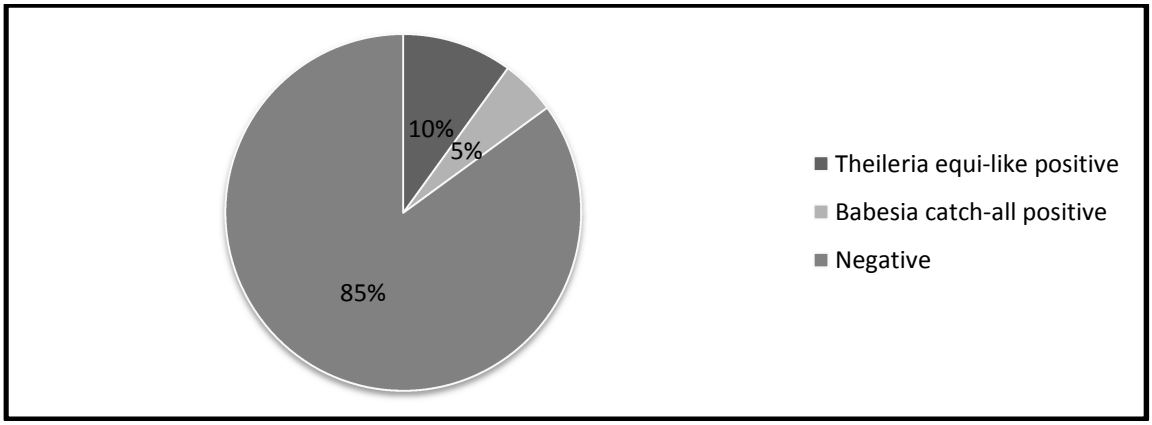

Figure 9. Pathogens detected in *D. reticulatus* ticks from the vegetation of Dintelse Gorzen, the Netherlands by RLB screening.

d. Ticks from the vegetation of Rozenburg, the Netherlands

In Rozenburg, a total of 40 adult ticks (sex unknown) were collected from the vegetation and screened by RLB for the presence of *Babesia* and *Theileria* species (Table 8 and Fig. 10). Two ticks (5%) were positive for *Babesia* catch-all, but no specific *Babesia* species was found in this case. Four ticks (10%) were positive for *Theileria/Babesia* catch-all and *B. canis*. Six ticks (15%) were positive for *Theileria/Babesia* catch-all, *B. canis* and *B. divergens*. The rest of the ticks (70%) were negative for any *Babesia* or *Theileria* species.

Table 8. Pathogens detected in *D. reticulatus* ticks from the vegetation of Rozenburg, the Netherlands by RLB screening.

	Amount	Proportion
Total number of ticks	40	100%
Babesia catch-all positive	2	5%
Theileria/Babesia catch-all and *B. canis* positive	10	25%
Negative	28	70%

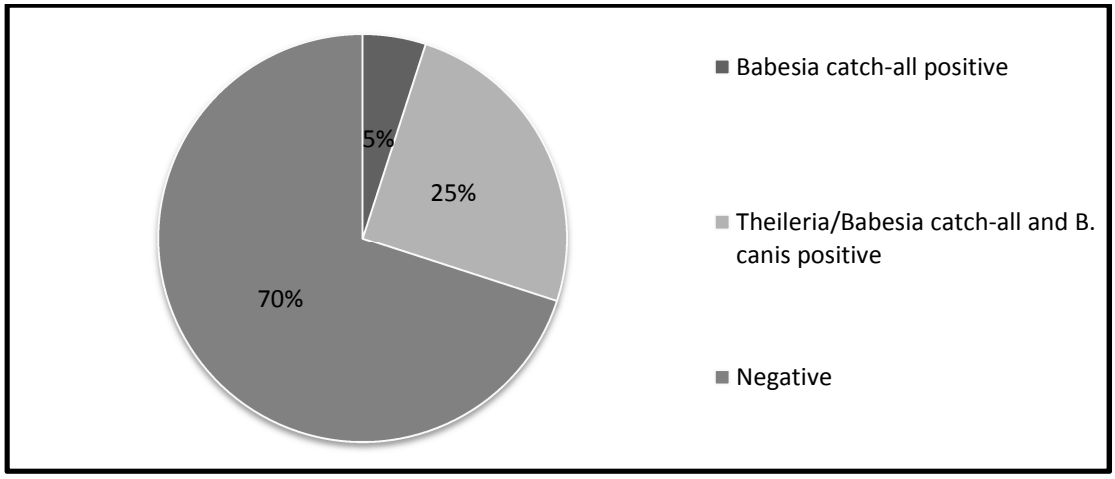

Figure 10. Pathogens detected in *D. reticulatus* ticks from the vegetation of Rozenburg, the Netherlands by RLB screening.

e. Ticks from cattle of a farm in the Netherlands

In addition, 40 ticks removed of cattle from a farm in the Netherlands are also screened on pathogens by RLB (Table 9 and Fig. 11). Two ticks (5%) were positive for *T. equi-like*. The other 38 ticks (95%) were negative for any *Babesia* or *Theileria* species.

Table 9. Pathogens detected in *D. reticulatus* ticks from cattle of a farm in the Netherlands by RLB screening.

	Amount	Proportion
Total number of ticks	40	100%
Males	1	2.5%
Females	39	97.5%
Theileria equi-like positive	2	5%
Negative	38	95%

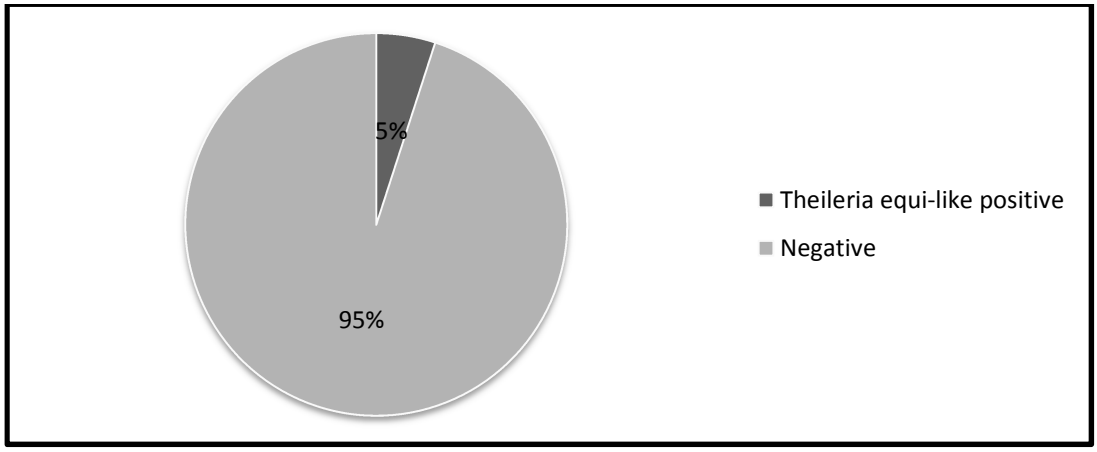

Figure 11. Pathogens detected in D. reticulatus ticks from cattle of a farm in the Netherlands by RLB screening.

f. Ticks from a laboratory colony of South Africa

From a laboratory colony of South Africa, a total of 40 adult ticks were screened by RLB for the presence of *Babesia* and *Theileria* species (Table 10 and Fig. 12). Half of the ticks were male and half of the ticks were female. One tick (2.5%) was positive for *T. equi-like*. Fifteen ticks (37.5%) were positive for *B. canis*. Five ticks (12.5%) were positive for both *T. equi* and *B. canis*. Three ticks (7.5%) were positive for *T. equi-like*, *B. canis* and *B. divergens*. Also three ticks (7.5%) were positive for three kinds of *Babesia*, namely *B. canis*, *B. divergens* and *B. venatorum*. One tick

(2.5%) was positive for *T. equi-like*, *B. canis*, *B. divergens* and *B. venatorum*. The rest of the ticks (30%) were negative for any *Babesia* or *Theileria* species.

Table 10. Pathogens detected in *D. reticulatus* ticks from a laboratory colony of South Africa by RLB screening.

	Amount	Proportion
Total number of ticks	40	100%
Males	20	50%
Females	20	50%
T. equi-like positive	1	2.5%
B. canis positive	18	45%
T. equi-like and *B. canis* positive	9	22.5%
Negative	12	30%

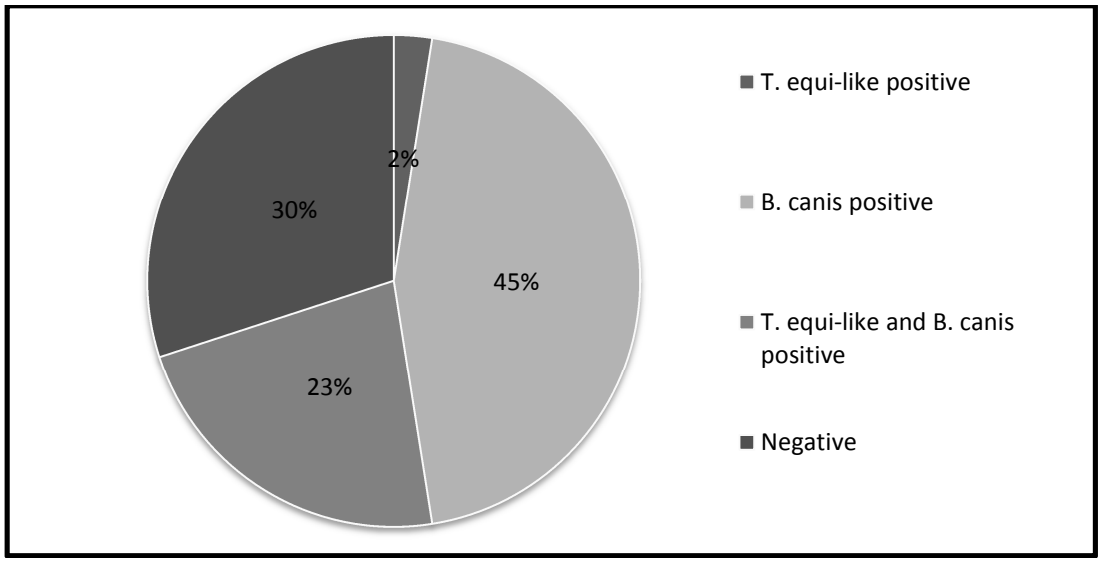

Figure 12. Pathogens detected in *D. reticulatus* ticks from a laboratory colony of South Africa by RLB screening.

g. Blood sample of horse from the Netherlands

A horse coming from Loosdrecht, the Netherlands, is been tested for pathogens by RLB screening. The five years old horse had no history of foreign countries and did no ticks pesticide used. The blood sample of this horse was screened for *Babesia* and *Theileria* species and came out to be positive for *T. equi-like*. This is fed back to the owner and treatment has been set.

4. Discussion

The presence of *D. reticulatus* ticks in the Netherlands has been observed earlier in 2004 and 2007. In 2004, *D. reticulatus* ticks could only be found on dogs, but in 2007, they were found also in the vegetation (Matjila et al. 2005, Nijhof et al. 2007). *Dermacentor reticulates* ticks were found in six localities, including the Dintelse Gorzen. During that research, *Rickettsia raoultii* was found in 33 ticks from the vegetation and one tick was infected with *Babesia afzelii and Babesia burgdorferi* (Nijhof et al. 2007). In this survey, again *D. reticulates* ticks were found in the Dintelse Gorzen and also in Panne and Rozenburg. The pathogens that were found in previous studies were not being found this time, but a few other interesting discoveries were made.

B. canis, the causal agent of canine babesiosis, was only found in *D. reticulatus* ticks from Rozenburg. The infection rate of *B. canis* in Rozenburg was 15%, but in all *D. reticulatus* ticks that were investigated for this study, the infection rate of *B. canis* was much lower (3.5%). Infection rates of *B. canis* in *D. reticulatus* ticks reported from other European

countries, like France (6% and 13.3%), Russia (4.2%), Germany (0%), Poland (11%), Slovakia (3.2%) (Beugnet, Marie 2009, Rar et al. 2005, Dautel et al. 2006, Zygner, Jaros & Wedrychowicz 2008, Kubelova et al. 2011), were variably. This is in accordance with our results. The infection rate of *B. canis* in the Netherlands and Belgium in the present study is low, suggesting that the pathogen is still more restricted than its potential vector, *D. reticulatus*.

Interestingly, *T. equi-like* was detected in 15.6% of *D. reticulatus* ticks. This has not been reported so far in the Netherlands and Belgium. Also two *D. reticulatus* ticks removed from cattle were positive for *T. equi-like*. In Panne, the area were most of the positive *T. equi-like D. reticulatus* ticks were found, wild horses graze in the vegetation. Maybe there is a connection between those horses and the positive result for *T. equi-like*. Both *T. equi* and *T. equi-like* can cause equine piroplasmosis. Clinical signs include fever, ataxia, mucus membrane paleness, haematuria and thrombocytopenia (Adaszek et al. 2011). Recent studies have also reported *Theileria equi* infection in dogs in Spain (Criado-Fornelio et al. 2007). So this pathogen can be a risk for horses and dogs. In the Netherlands and Belgium, the prevalence and incidence of equine piroplasmosis are unknown. More survey should be done to increase our knowledge about this.

Also *B. divergens* was found during this study in 2.1% of *D. reticulatus* ticks from the Netherlands and Belgium. In the *D. reticulatus* ticks from Rozenburg the prevalence was 15% and in the laboratory colony of South Africa, the prevalence was 17.5%. This specie of *Babesia* can also cause canine babesiosis. In Belgium, *B. divergens* was found recently in *Ixodes ricinus* ticks from wild cervids (Lempereur et al. 2012). The prevalence of *B. divergens* was 0.78%, a little below our results, suggesting that this *Babesia* specie is less common in the Netherlands and Belgium. *Babesia venatorum* was only found in the laboratory colony of South Africa. *B. venatorum* can cause bovine babesiosis in cattle. In our research *B. venatorum* wasn't found in *D. reticulatus* ticks collected from cattle. The laboratory colony of South Africa was officially pathogen-free and only infected with *Babesia canis* under laboratory conditions. Remarkably, in the RLB results also two other *Babesia* species staining positive. This can be explained by the fact that very strong positive signals can create other additional results. Those results should not be taken into account. Therefore, the two other *Babesia* species from Rozenburg and the laboratory colony of South Africa, *B. divergens* and *B. venatorum*, are not included in the results of this survey.

Remarkable is the fact that more female than male ticks are collected during this survey. Whereas the expectation is that this number should be equal. But the numbers are not far from each other, namely 47.2% males and 52.8% females. Most of the ticks are collected in Panne, so that the results of this had got the greatest impact on the overall results. Several other places will also be explored to create a better picture of the situation. The use of an RLB for *Babesia* and *Theileria* species makes it possible to detect pathogens that were not anticipated. Therefore *T. equi-like* was discovered by change in this survey. In this survey 5.2% of *D. reticulatus* ticks from the vegetation of the Netherlands and Belgium were positive for *Babesia* catch-all, but no specific *Babesia* species was found in this case. It is possible that none of the investigated *Babesia* species appeared in this and that perhaps a new *Babesia* species has been discovered.

As previously told in the introduction, *D. reticulatus* is also a vector of *B. caballi*, *Rickettsia sibirica* and *Rickettsia slovaka*. In this study no *B. caballi* is found. The prevalence of *R. sibirica* and *R. slovaka* has not been studied. In a subsequent study, these pathogens can also be taken into account. Further surveillance is necessary to determine the vector role of *D. reticulatus* ticks. This includes the vector capacity of *D. reticulates* ticks for *B. canis*, *T. equi-like* and especially for tick-borne encephalitis virus (TBEV), since recent research has detected a seropositive dog in Belgium (Roelandt et al. 2011).

5. Conclusion

This study confirmed that *D. reticulatus* is the vector of *B. canis* in the field in the Netherlands and Belgium. The prevalence of *Babeisa canis* (3.5%) and other *Babesia* and *Theileria* species, including *T. equi-like* (17.1%) and *Babesia* sp. (6.8%) has been determined. Also more places in the Netherlands and Belgium where *D. reticulates* ticks lives in the vegetation have been discovered. *D. reticulatus* ticks are also present on cattle in the Netherlands. In conclusion, further surveillance is important to further improve the knowledge about the role of *D. retiuclatus* ticks as vector of pathogens.

6. Acknowledgements

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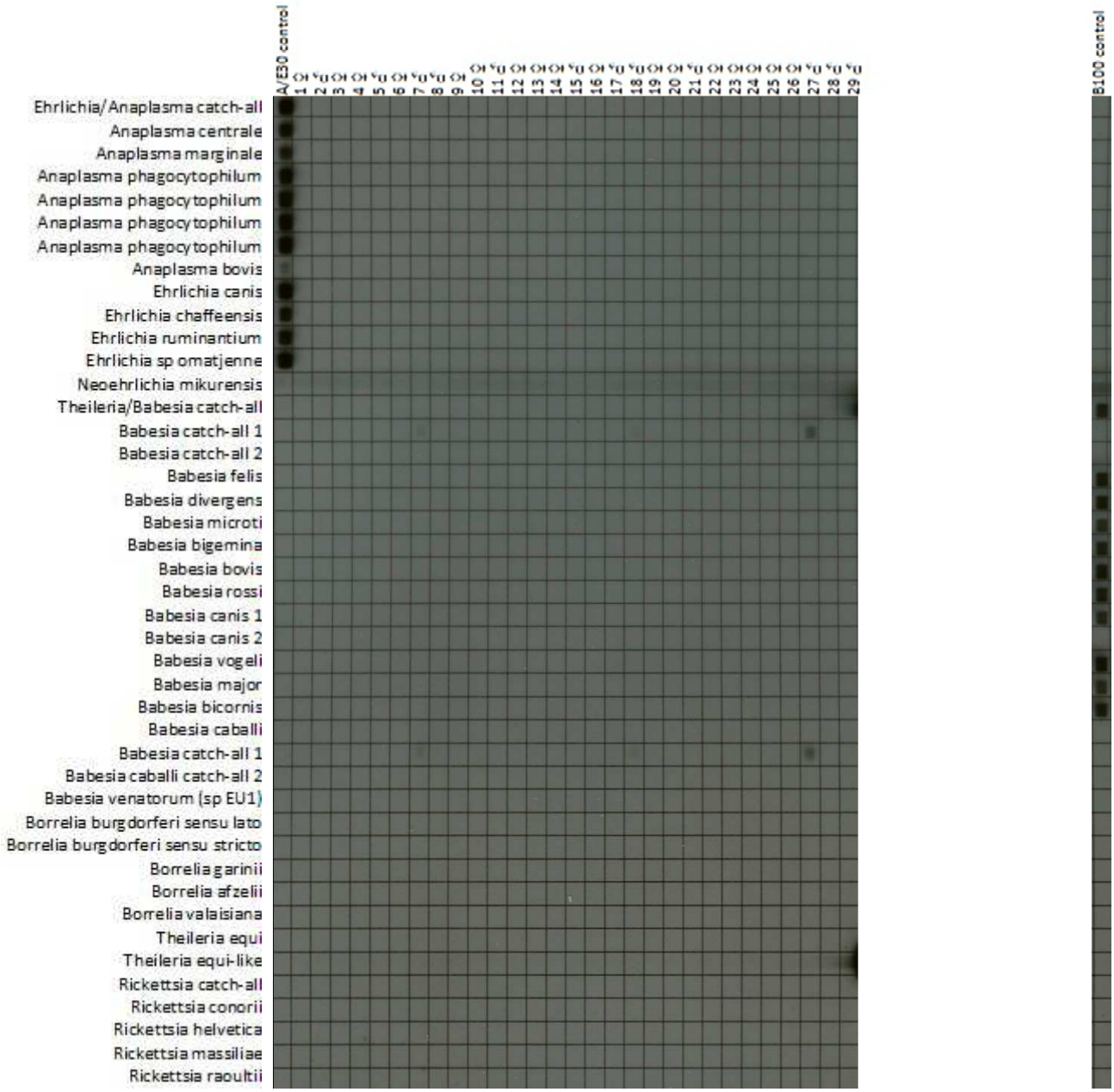
Appendix

Number ticks	Place
1-208	Panne, the Netherlands
D1-D40	Dintelse Gorzen, the Netherlands
R1-R40	Rozenburg, Belgium
N1-N40	Cattle of farm from the Netherlands
Z1-Z40	Laboratory colony of South Africa

RLB 19-10-2011 Panne



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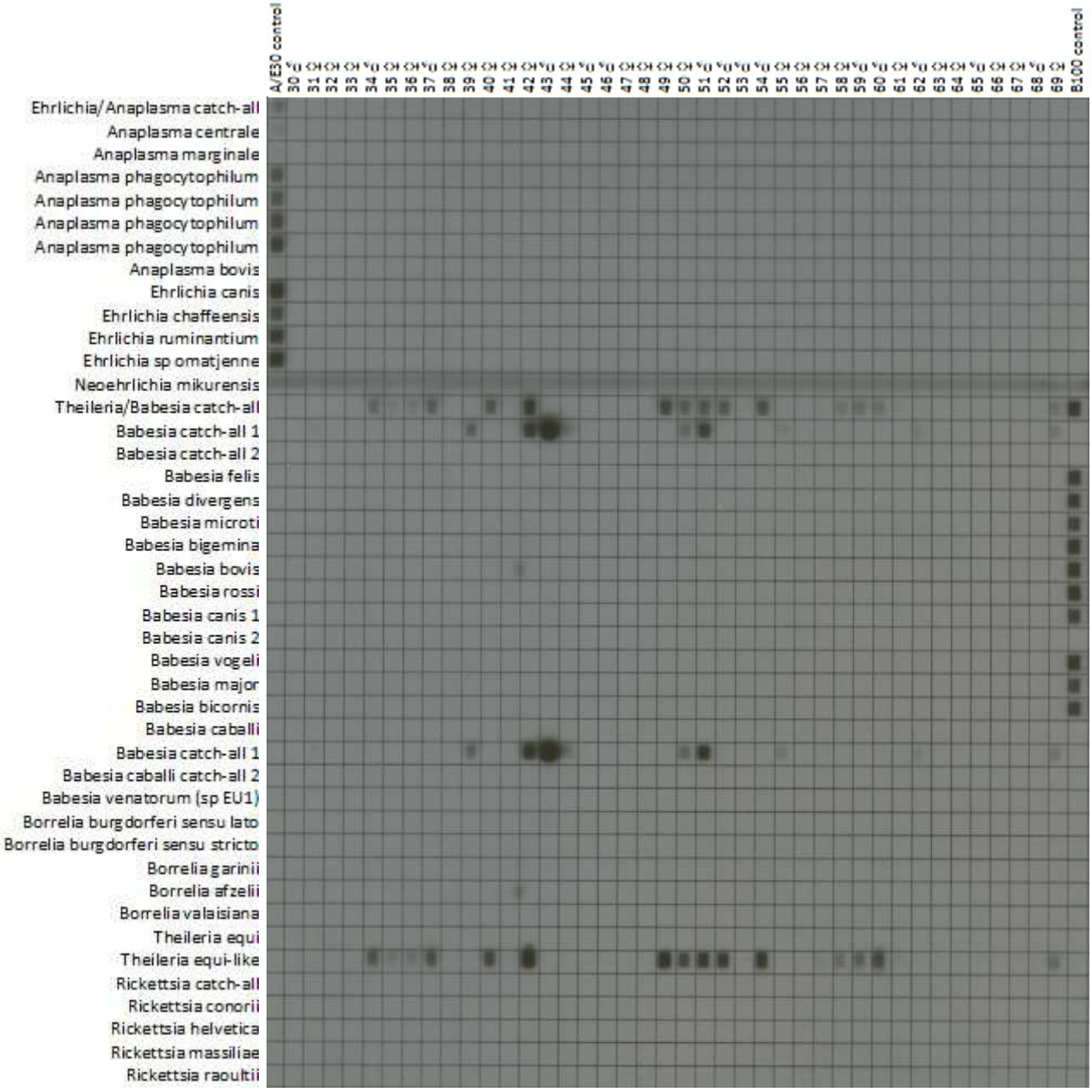
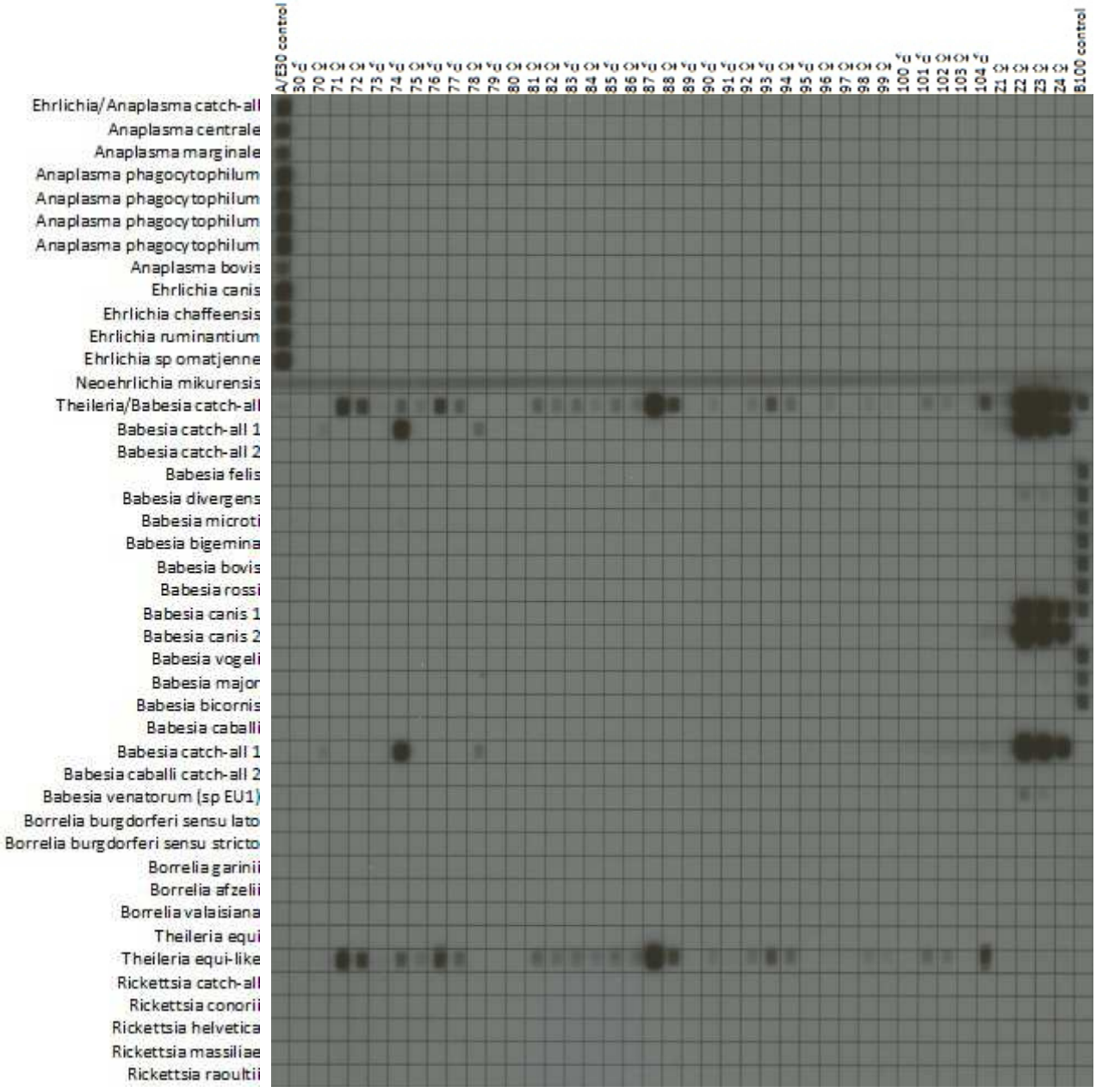
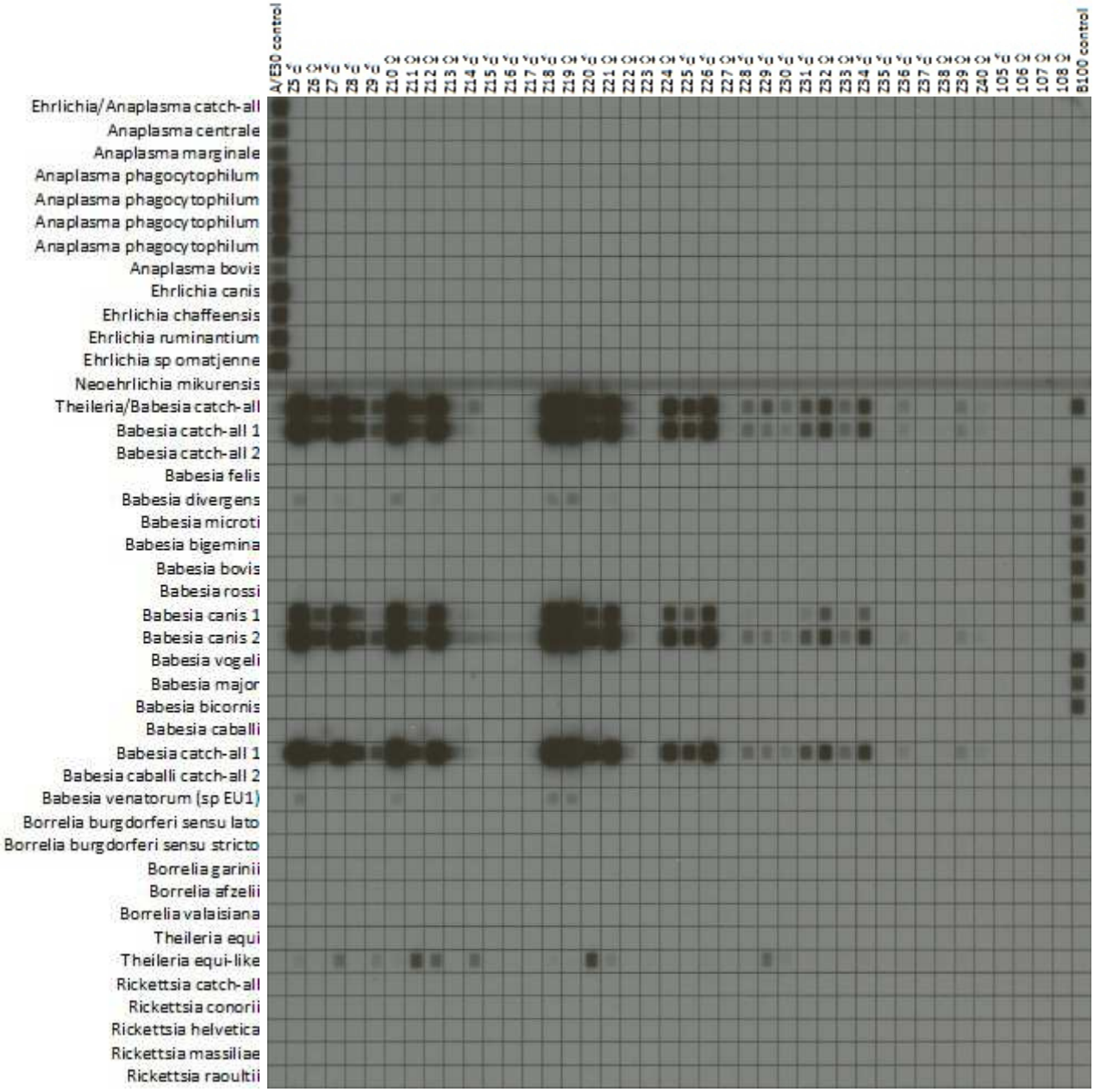


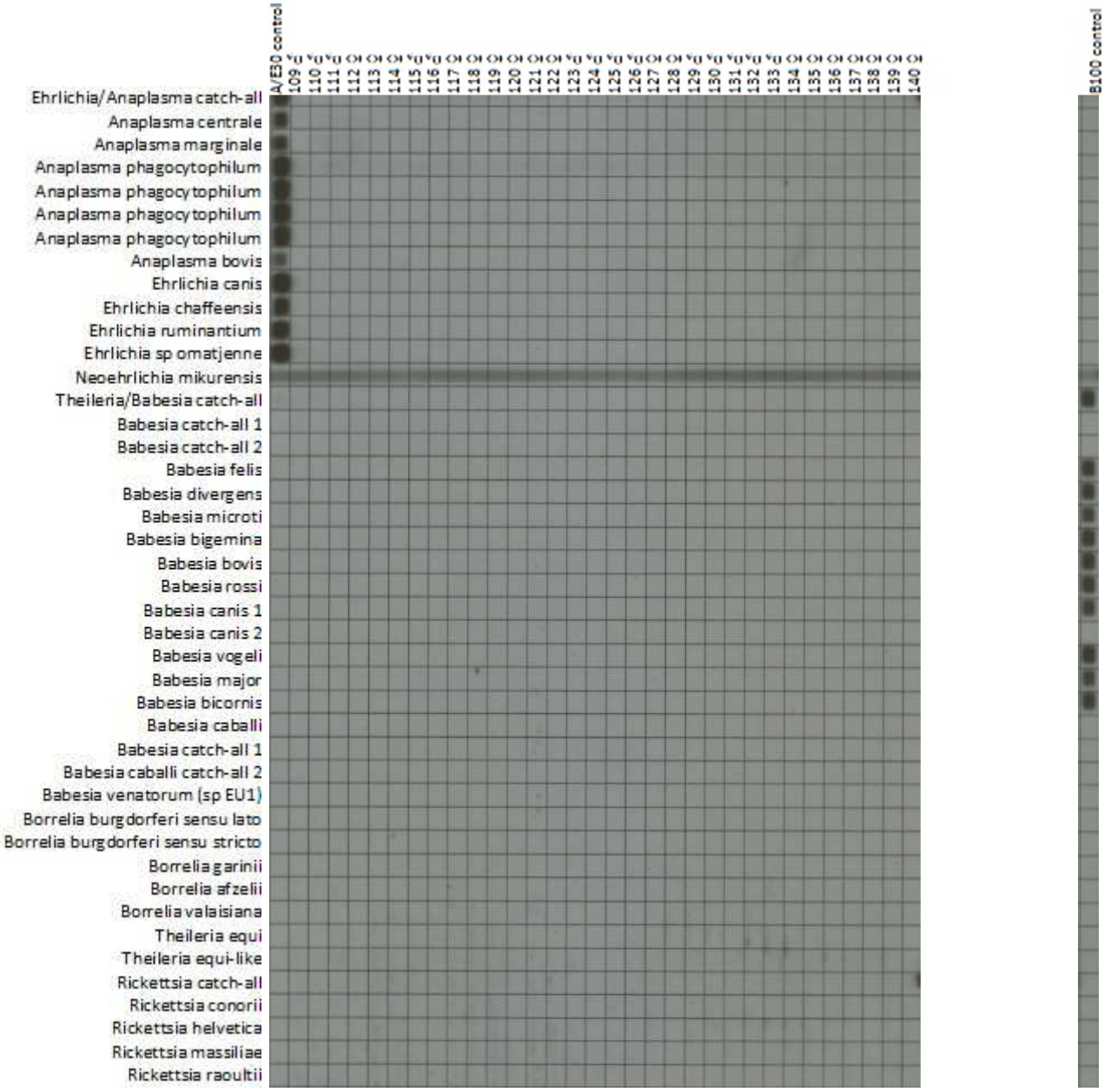
RLB 1-11-2011 Panne and South Africa



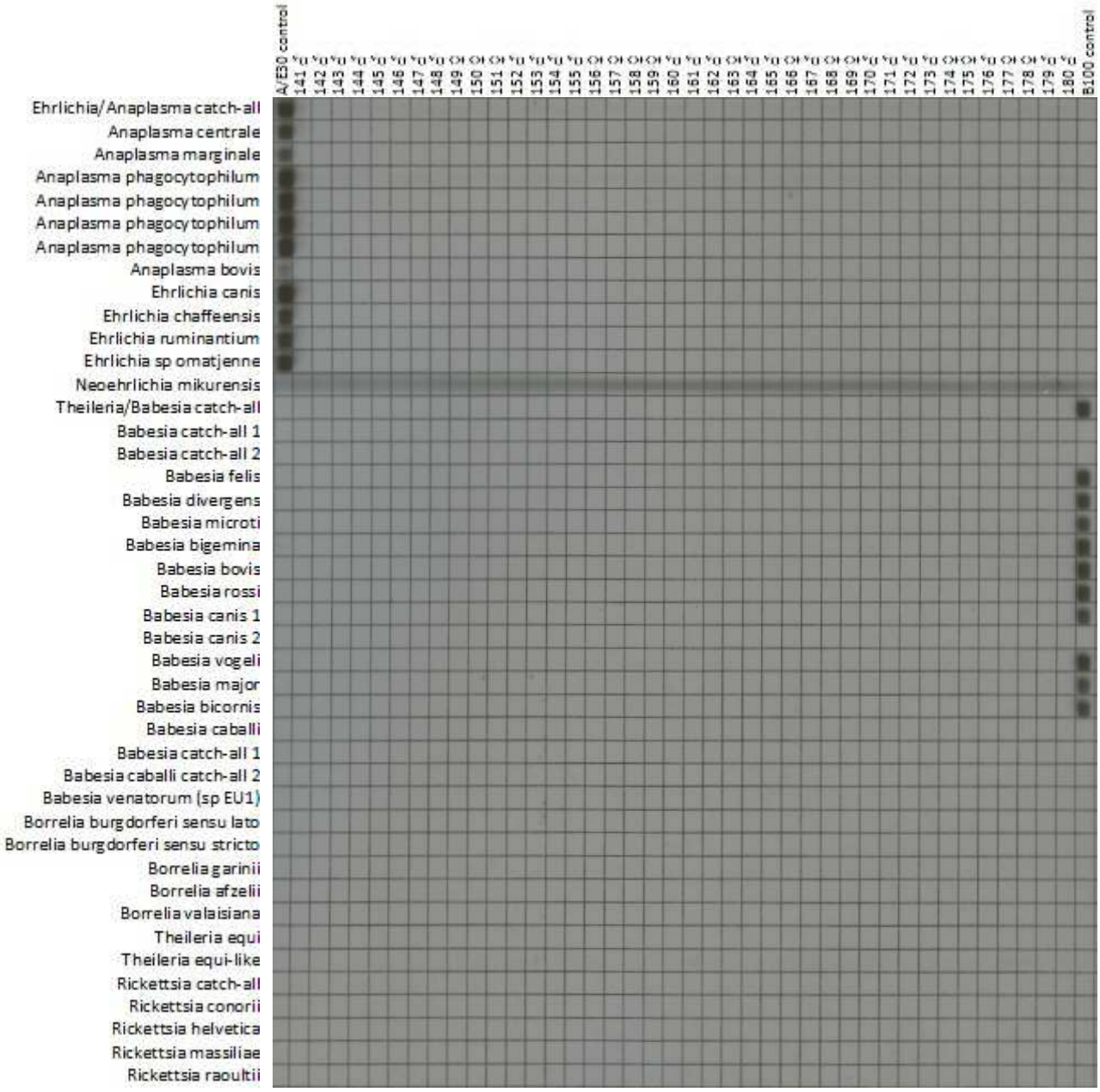
RLB 4-11-2011 South Africa and Panne



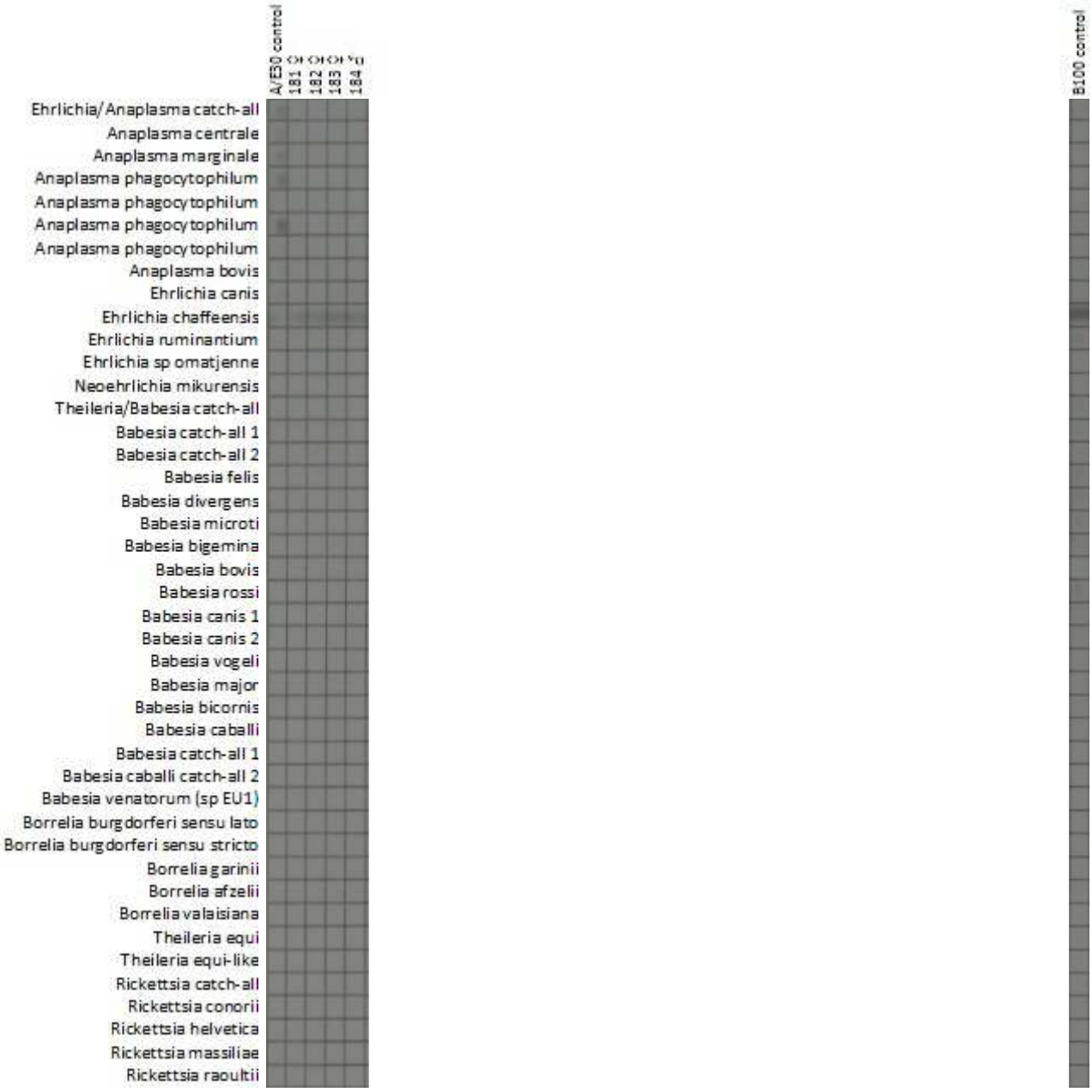
RLB 10-11-2011 Panne



RLB 15-11-2011 Panne



RLB 18-11-2011 Panne



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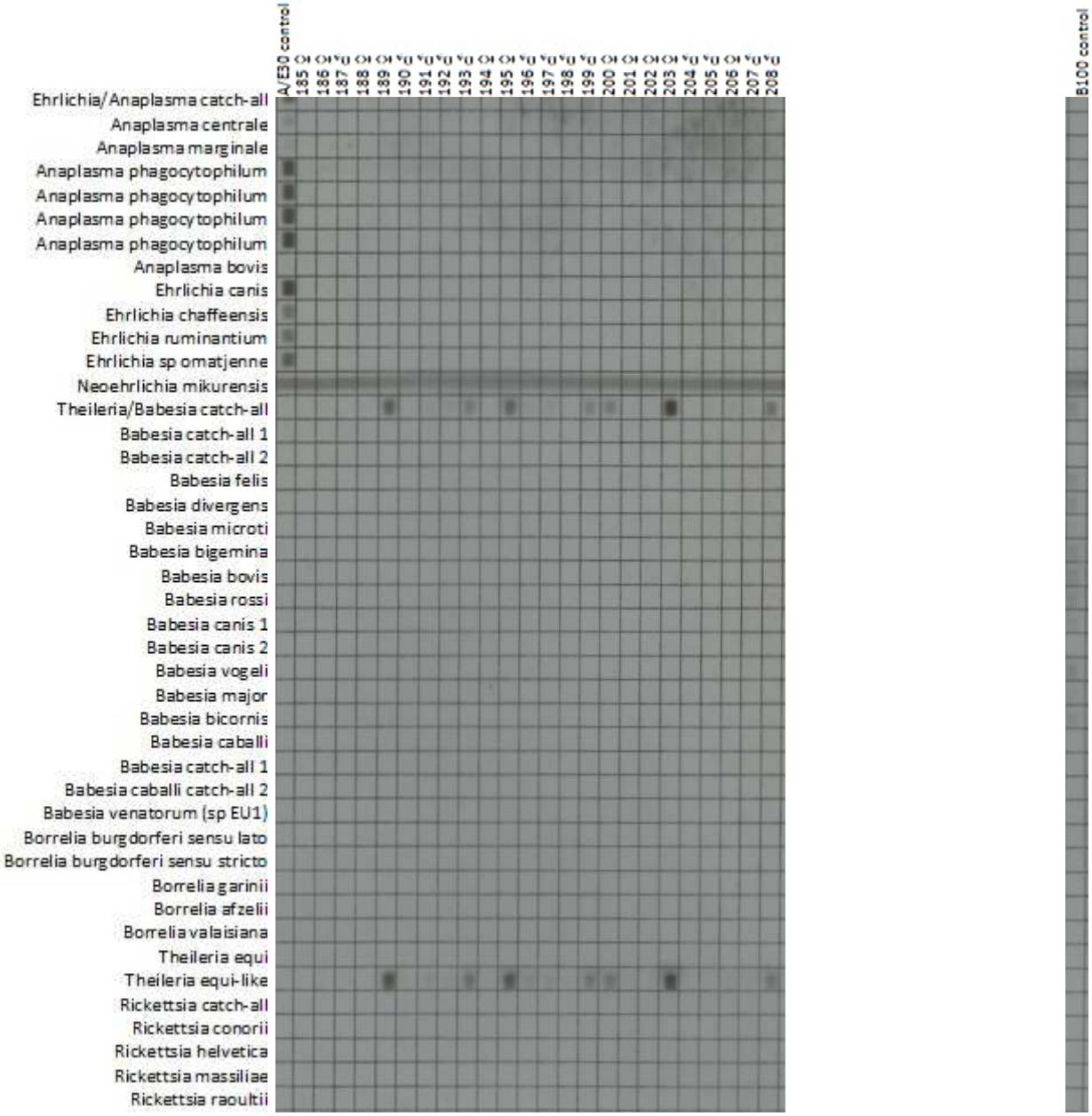
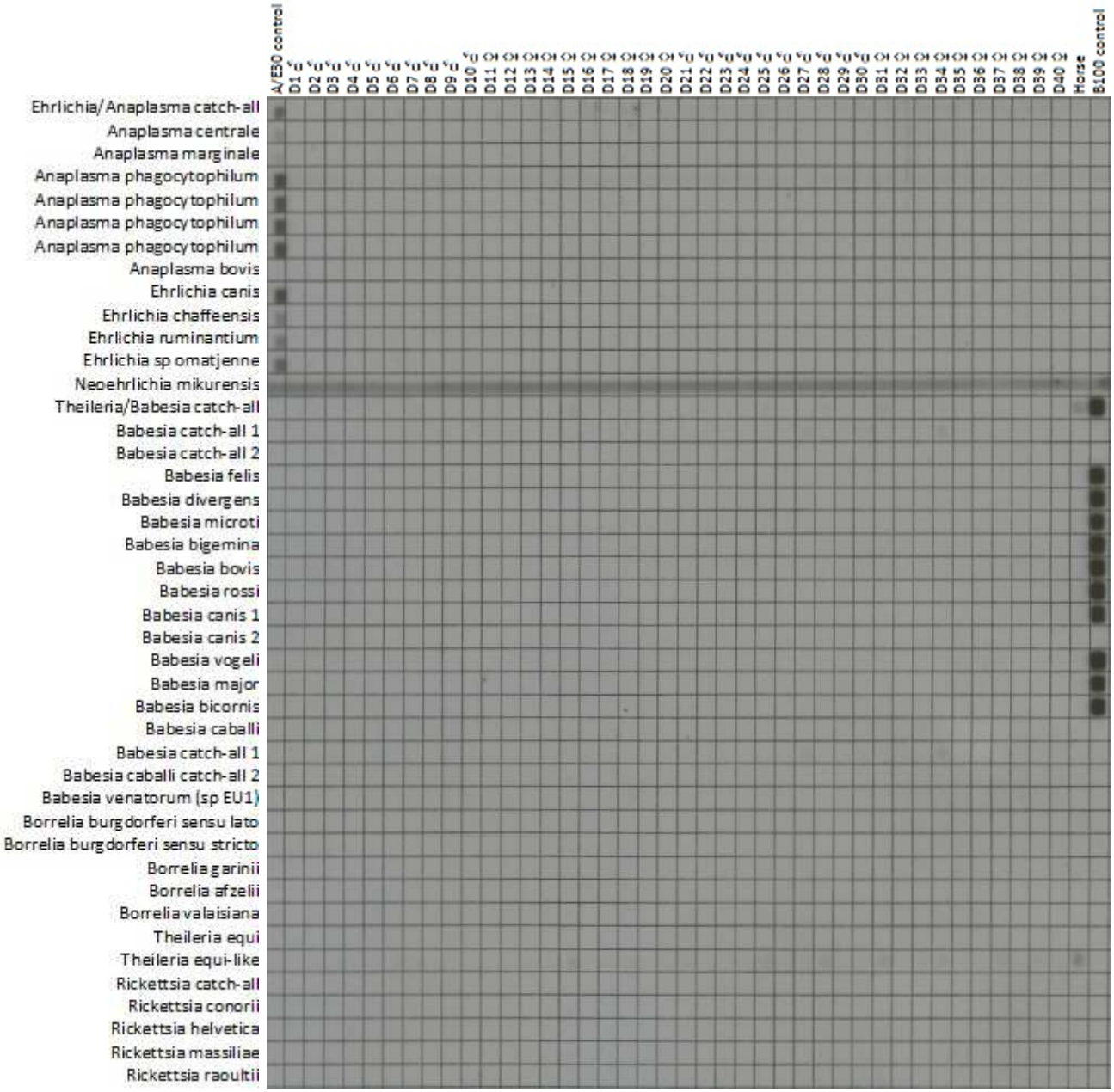
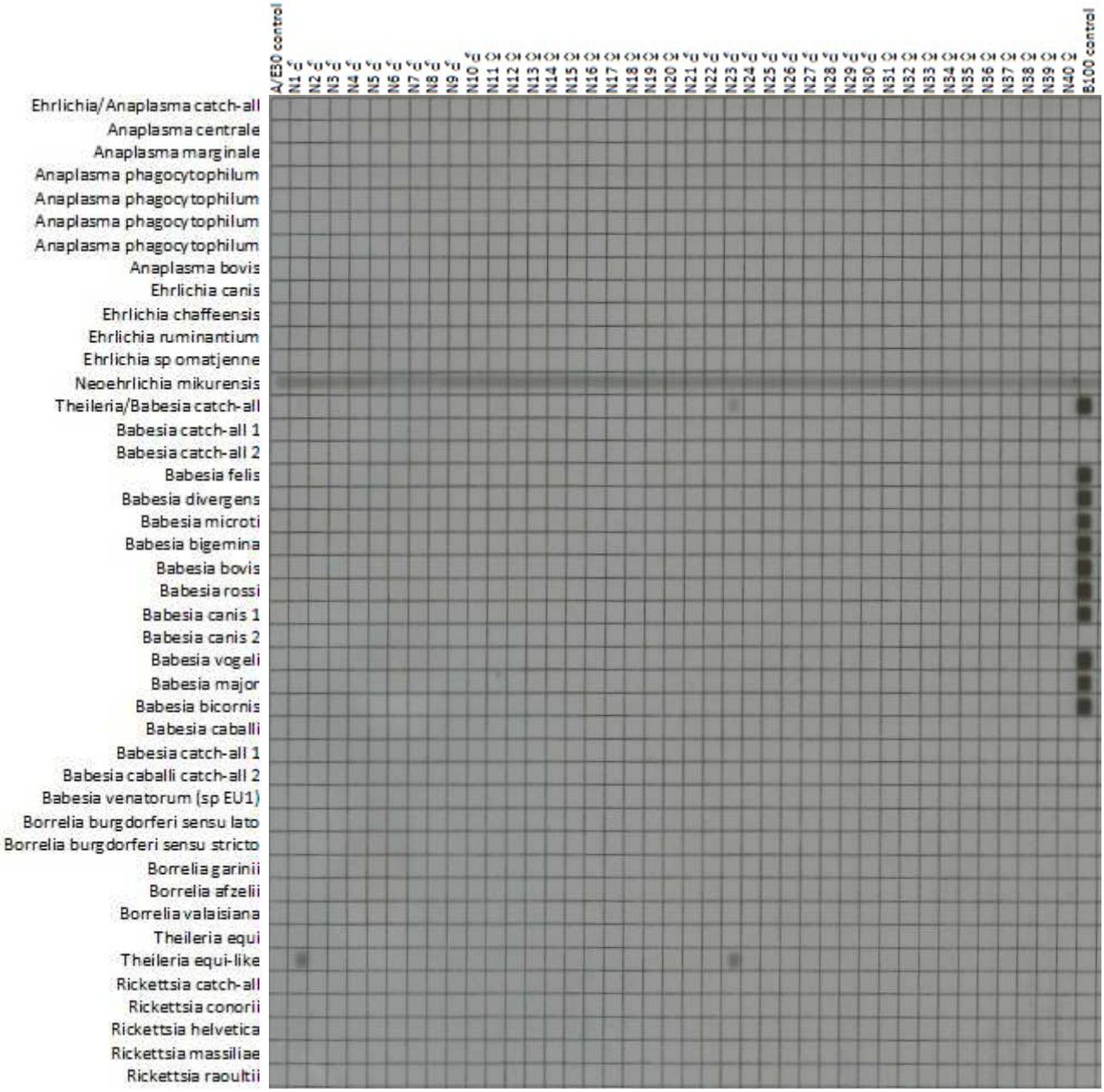


RLB 6-12-2011 Dintelse Gorzen and blood horse



RLB 21-12-2011 Cattle of farm from the Netherlands



RLB 40 *D. reticulatus* ticks from the vegetation of Rozenburg