

***Borrelia burgdorferi* pilot study summary report**

1.1 Study design and layout

This study is a proof of concept study conducted on one group of ten dogs which were infested with *B. burgdorferi* infected field collected *Ixodes ricinus* ticks and monitored for *B. burgdorferi* s.l. transmission via SNAP 4DX and PCR on skin biopsy samples.

Ten healthy dogs (males and females) that were sero-negative for *B. burgdorferi*, based on SNAP 4DX in the acclimatisation period, were acclimatised to their cages for 11 days prior to the first tick infestation on Day 0. Veterinary examinations were performed on Day -7 and weekly after Day 0 until the end of study for each dog, general health observations (all days) and weighing of all dogs (Day -3) and again prior to skin biopsy procedures to obtain accurate body weights for sedation purposes were performed. A Veterinarian verified enrolment and inclusion of animals on Day -7. Additional body temperature monitoring is performed weekly after the last tick infestation.

Dogs were infested with 40 viable, naturally infected field collected female and 10 male *I. ricinus* ticks on Days 0, 7 and 14. In-situ counts were performed 5 days after each infestation and ticks were left on the dog to engorge fully till normal drop-off.

Blood collection for serum collection was done during the acclimatisation period and every two weeks starting from day 28. Monitoring for *B. burgdorferi* transmission was based on SNAP 4DX, from Day 56 and every two weeks. Skin biopsy samples were collected on Day 24 for *Borrelia* culture and PCR detection and will be collected again on the end of study for each dog for PCR analysis.

1.2 Results and current status of the pilot study

1.2.1 Tick counts

In situ tick counts five days after each infestation was conducted and an average tick attachment ranging from 23.0 to 30.3 female ticks were attached per dog over the three infestations.

A summary of in situ tick counts five days after each infestation is summarised in Table 1

Table 1 In situ counts 5 days after infestation

In situ counts 5 days after infestation (attached female ticks)				
Animal ID	Day 5	Day 12	Day 19	Average
902874	25	29	24	26.0
902605	19	33	30	27.3
902685	24	29	16	23.0
902407	29	25	21	25.0
902799	29	31	25	28.3
902634	22	36	33	30.3
902763	17	29	25	23.7
902657	17	29	31	25.7
901764	26	22	27	25.0
902624	27	30	14	23.7

1.2.2 Skin biopsy – Culturing and PCR results

Skin biopsies were collected on Day 24 and cultures were prepared and monitored weekly thereafter. No positive culture has been observed up to date (Day 98).

Biopsy samples collected on Day 24 was sent for PCR analysis and two dogs (902763 and 902657) were found to be positively infected with *B. burgdorferi* (see Table 2).

1.2.3 Serology – SNAP 4DX

All dogs were negative for *B. burgdorferi* on Day -7 prior to tick infestations and on Days 28 and 42 after tick infestations. Three dogs (902605, 902634 and 902763) were found positive on Days 70,84 and 98. One dog (902763) was positive on both PCR (skin biopsy) and SNAP 4DX (see Table 2).

Table 2 In situ counts 5 days after infestation

ID	Date of Birth	Age at the study start	skin biopsies	SNAP					
				Day -7 (prior to infestations)	Day 28	Day 42	Day 70	Day 84	Day 98
902874	9-Aug-2018	1 years 9 months	-	-	-	-	-	-	-
902605	26-Aug-2018	1 years 9 months	-	-	-	-	+	+	+
902685	19-Sep-2018	1 years 8 months	-	-	-	-	-	-	-
902407	19-Sep-2018	1 years 8 months	-	-	-	-	-	-	-
902799	9-Aug-2018	1 years 9 months	-	-	-	-	-	-	-
902634	28-Jan-2019	1 years 4 months	-	-	-	-	+	+	+
902763	9-Aug-2018	1 years 9 months	+	-	-	-	+	+	+
902657	19-Sep-2018	1 years 8 months	+	-	-	-	-	-	-
902795	9-Aug-2018	1 years 9 months	-	-	-	-	-	-	-
902624	28-Jan-2019	1 years 4 months	-	-	-	-	-	-	-

1.3 Information of ticks used during the pilot study

The batch of field collected ticks from the Netherlands used in the pilot study, had an infection ratio of 31.8% for *B. burgdorferi* s.l. and 7.8% for *B. burgdorferi* s.s..

A sample of 204 ticks (106 females and 98 males) were screened by following the assay from Courtney et al. (2004) for *B. burgdorferi* s.l. and an assay with primers developed by Clinomics for *B. burgdorferi* s.s. (see Table 3).

Table 3 Summary of PCR results

	<i>B. burgdorferi</i> s.l.	<i>B. burgdorferi</i> s.s.
Females sample 1	15/56	5/56
Females sample 2	21/50	6/50
Males sample 1	21/49	5/49
Males sample 2	8/49	0/49

1.4 Conclusion

A total of four dogs out of the included 10 were found to be positive for *B. burgdorferi* up to date (25 Sep 2020 - Day 104).