

# **Tick populations from endemic and non-endemic areas in Germany show differential susceptibility to TBEV**

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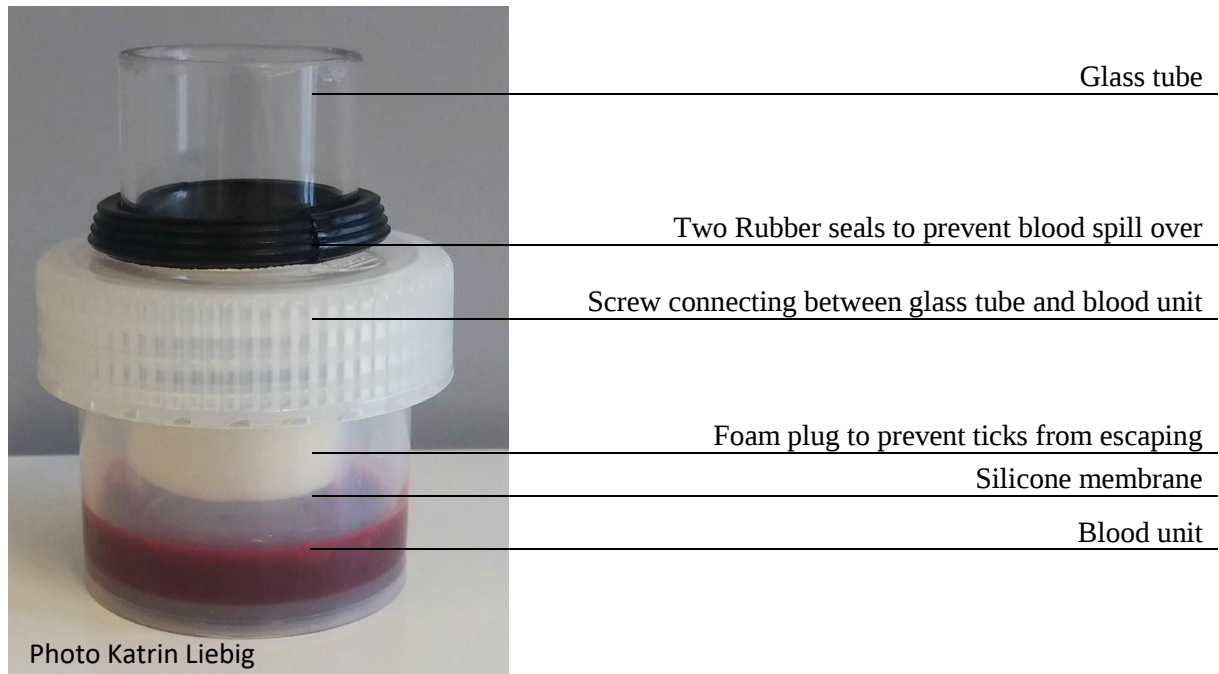
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**Supplementary Table 1. Feeding rates of *I. ricinus* nymphs after *in-vitro* feeding and infection with TBEV 2018 and 2019.**

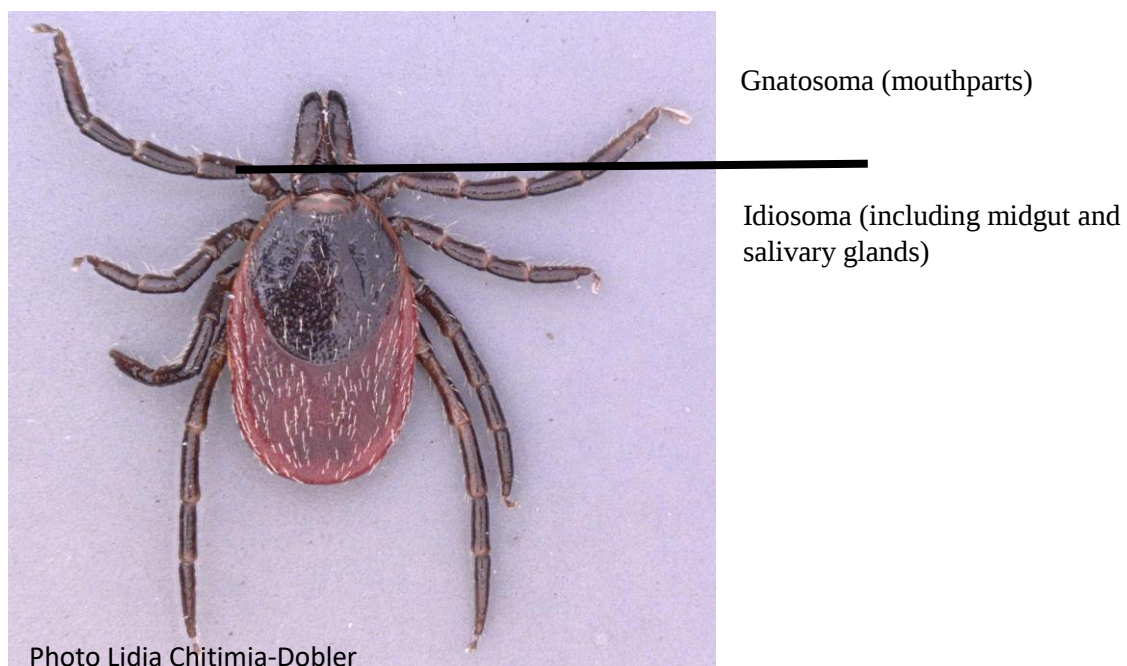
|             |  | 2018             |                         |                  |                         |                  |         |                  |         |
|-------------|--|------------------|-------------------------|------------------|-------------------------|------------------|---------|------------------|---------|
|             |  | April            |                         | May              |                         | June             |         | July             |         |
| Tick origin |  | Feeding rate (%) | p value                 | Feeding rate (%) | p value                 | Feeding rate (%) | p value | Feeding rate (%) | p value |
| Haselmühl   |  | 5.33             | p< 0.001                | 43.81            | 1.84 x 10 <sup>-6</sup> | 58.24            | 0.9967  | 20.00            | 0.1209  |
| Hanover     |  | 28.00            |                         | 19.44            |                         | 43.01            |         | 38.57            |         |
|             |  | 2018             |                         |                  |                         |                  |         |                  |         |
|             |  | August           |                         | October          |                         |                  |         |                  |         |
| Tick origin |  | Feeding rate (%) | p value                 | Feeding rate (%) | p value                 |                  |         |                  |         |
| Haselmühl   |  | 33.13            | p< 0.0001               | 68.04            | 0.0830                  |                  |         |                  |         |
| Hanover     |  | 78.08            |                         | 51.11            |                         |                  |         |                  |         |
|             |  | 2019             |                         |                  |                         |                  |         |                  |         |
|             |  | April            |                         | May              |                         | June             |         | July             |         |
| Tick origin |  | Feeding rate (%) | p value                 | Feeding rate (%) | p value                 | Feeding rate (%) | p value | Feeding rate (%) | p value |
| Haselmühl   |  | 82.66            | 5.20 x 10 <sup>-8</sup> | 47.33            | 4 x 10 <sup>-15</sup>   | 8.00             | 0.1176  | 15.83            | 0.6841  |
| Hanover     |  | 53.33            |                         | 0                |                         | 2.00             |         | 26.00            |         |

# Supplementary Figure 1: Tick *In vitro* feeding system adapted to BSL3 conditions and tick dissection

a



b



**Supplementary Table 2: Mean temperatures and precipitation at the collection sites Hanover and Haselmühl/Amberg in 2018 and 2019 stratified by month and site**

| Month   | Year | Mean temperature<br>(deviation from mean) | Mean temperature<br>(deviation from mean) | Higher feeding/infection rate | Mean precipitation<br>(% of annual mean) | Mean precipitation<br>(% of annual mean) |
|---------|------|-------------------------------------------|-------------------------------------------|-------------------------------|------------------------------------------|------------------------------------------|
|         |      | <b>Hanover</b>                            | <b>Amberg</b>                             |                               | <b>Hanover</b>                           | <b>Amberg</b>                            |
| April   | 2018 | 12.6 +3.7                                 | 12.9 +4.6                                 | Hanover/ Hanover              | 26.8 (67%)                               | 14.1 (37%)                               |
|         | 2019 | 9.9 +1.0                                  | 10.0 +1.7                                 | Amberg/ Amberg                | 26.2 (66%)                               | 19.5 (51%)                               |
| May     | 2018 | 16.9 +3.5                                 | 16.9 +3.6                                 | Amberg/ Amberg                | 10.0 (18%)                               | 68.2 (108%)                              |
|         | 2019 | 11.5 -1.9                                 | 11.3 -2.0                                 | Amberg/                       | 28.7 (51%)                               | 45.2 (72%)                               |
| June    | 2018 | 18.1 +2.1                                 | 18.6 +2.4                                 | Amberg/ Amberg                | 21.2 (36%)                               | 32.2 (45%)                               |
|         | 2019 | 20.3 +4.3                                 | 21.0 +4.8                                 | Amberg/ Amberg                | 42.8 (73%)                               | 37.5 (53%)                               |
| July    | 2018 | 20.7 +2.3                                 | 20.8 +2.5                                 | Hanover/ Amberg               | 81.8 (134%)                              | 32.0 (41%)                               |
|         | 2019 | 19.0 +0.6                                 | 20.4 +2.1                                 | Hanover/                      | 23.4 (38%)                               | 62.9 (80%)                               |
| August  | 2018 | 20.0 +2.1                                 | 20.8 +3.3                                 | Hanover/ Amberg               | 38.6 (56%)                               | 45.4 (66%)                               |
| October | 2018 | 11.5 +1.6                                 | 9.6 +1.1                                  | Amberg/ Amberg                | 18.4 (35%)                               | 20.2 (37%)                               |

**Supplementary Table 3: TBEV detection rates ticks from Hanover and Haselmühl**

Ticks were grouped into 5 nymphs per feeding chamber, fed with virus-free blood for 5 days and surviving engorged nymphs were homogenized and tested for TBEV infection using the RT-qPCR assay by Schwaiger *et al.*<sup>1</sup>.

| <b>Cohort</b>          | <b>No. feeding chamber<br/>(number of ticks)</b> | <b>Ticks blood fed<br/>(feeding rate)</b> | <b>TBEV detected</b> |
|------------------------|--------------------------------------------------|-------------------------------------------|----------------------|
| Haselmühl              | 1 (5)                                            | 2 (40)                                    | -                    |
|                        | 2 (5)                                            | 3 (60)                                    | -                    |
|                        | 3 (5)                                            | 2 (40)                                    | -                    |
|                        | 4 (5)                                            | 2 (40)                                    | -                    |
|                        | 5 (5)                                            | 1 (20)                                    | -                    |
|                        | 6 (5)                                            | 4 (80)                                    | -                    |
|                        | 7 (5)                                            | 4 (80)                                    | -                    |
|                        | 8 (5)                                            | 0 (0)                                     | -                    |
| <b>Total Haselmühl</b> | <b>40</b>                                        | <b>18 (45%)</b>                           | <b>0</b>             |
| Hanover                | 9 (5)                                            | 2 (40)                                    | -                    |
|                        | 10 (5)                                           | 0 (0)                                     | -                    |
|                        | 11 (5)                                           | 4 (80)                                    | -                    |
|                        | 12 (5)                                           | 0 (0)                                     | -                    |
|                        | 13 (5)                                           | 5 (100)                                   | -                    |
|                        | 14 (5)                                           | 1 (20)                                    | -                    |
|                        | 15 (5)                                           | 2 (40)                                    | -                    |
|                        | 16 (5)                                           | 1 (20)                                    | -                    |
| <b>Total Hanover</b>   | <b>40</b>                                        | <b>15 (37,5)</b>                          | <b>0</b>             |

**Supplementary Table 4: TBEV viral particles in blood incubated at room temperature for 15h-24h**

| Sample                 | TBEV Titre (virus particles) |
|------------------------|------------------------------|
| TBEV spiked blood 0h   | $1.61 \times 10^5$           |
| TBEV spiked blood 15 h | $1.61 \times 10^3$           |
| TBEV spiked blood 24h  | $8.18 \times 10^0$           |

## References

- 1 Schwaiger, M. & Cassinotti, P. Development of a quantitative real-time RT-PCR assay with internal control for the laboratory detection of tick borne encephalitis virus (TBEV) RNA. *J Clin Virol* **27**, 136-145, doi:10.1016/s1386-6532(02)00168-3 (2003).