**SUPPLEMENTARY FILES**

**Influence of breed and parasite challenge on the immune response to naturally acquired intestinal nematode infection in sheep**

Ana Cláudia A. Albuquerque\* 1,2; Fabiana Alves de Almeida 2; Cesar Cristiano Bassetto 2; José Gabriel Gonçalves Lins1,2; Alessandro F. T. Amarante 2

1 UNESP – São Paulo State University, School of Veterinary Medicine and Animal Science, Rua Professor Doutor Walter Mauricio Correa s/n, 18618-681, Botucatu, SP, Brazil.

2UNESP – São Paulo State University, Institute of Biosciences, Rua Professor Dr. Antônio Celso Wagner Zanin, 250, 18618-689, Botucatu, SP, Brazil.

\* Corresponding author

E-mail: ac.alb@hotmail.com

**Table S1.** Anthelmintics administered to lambs under suppressive treatments.

|  |  |  |  |
| --- | --- | --- | --- |
| Experimental weeks | Treatments |  |  |
|  | Monepantel | Combination | Moxidectin |
| Arrival |  | x |  |
| 2 | x |  |  |
| 4 | x |  |  |
| 6 |  |  | x |
| 8 | x |  |  |
| 10 | x |  |  |
| 12 | x |  |  |
| 13 |  | x |  |
| 18 | x |  |  |
| 21 Slaughter |  |  |  |

Combination: Albendazole + Levamisole + Monepantel.

**Table S2.**Mean (minimum-maximum) values of *Cooperia curticei* in Santa Ines and Ile de France lambs under suppressive or targeted selective treatment (TST) programmes with anthelmintics.

|  |  |  |  |
| --- | --- | --- | --- |
| Development stages | Santa Ines  | Ile de France | Effects (P-value) |
| Suppressive (n=9) | TST (n=10) | Suppressive (n=10) | TST (n=9) | Breed | Treatment programme | Breed x Treatment |
| Early L4 | 16 (0-80) | 296 (0-1060) | 42 (0-120) | 875 (240-2230) | 0.0080 | <0.0001 | ns |
|  | 0.64 (±0.26) | 1.61 (±0.39) | #1.16 (±0.27) | 2.84 (±0.11) |  |  |  |
| Female late L4 | 12 (0-70) | 111 (0-470) | 18 (0-70) | 253 (40-490) | 0.0106 | 0.0008 | ns |
|  | 0.50 (±0.25) | 1.05 (±0.37) | 0.84 (±0.24) | 2.29 (±0.13) |  |  |  |
| Male late L4 | 3 (0-10) | 23 (0-110) | 4 (0-20) | 149 (10-400) | 0.0137 | <0.0001 | 0.0061 |
|  | 0.35 (±0.17) a\* | 0.87 (±0.25) a | 0.34 (±0.17) a | 2.01 (±0.15) b |  |  |  |
| Female L5 | 0 | 28 (0-120) | 7 (0-50) | 232 (0-490) | 0.0194 | <0.0001 | ns |
|  | 0  | 0.84 (±0.29) | 0.30 (±0.20) | 1.90 (±0.36) |  |  |  |
| Male L5 | 0 | 31 (0-140) | 2 (0-20) | 194 (0-440) | ns | <0.0001 | ns |
|  | 0  | 0.95 (±0.27) | 0.13 (±0.13) | 1.83 (±0.35) |  |  |  |
| Adult female | 1 (1-10) | 517 (0-1410) | 1 (0-10) | 979 (0-3350) | ns | <0.0001 | ns |
|  | 0.12 (±0.12) | 1.92 (±0.43) | 0.10 (±0.10) | 2.40 (±0.38) |  |  |  |
| Adult male | 1 (1-10) | 368 (0-1140) | 0 | 660 (0-2510) | ns | <0.0001 | ns |
|  | 0.12 (±0.12)  | 1.92 (±0.41)  | 0 | 2.20 (±0.36) |  |  |  |
| Total worm burden | 33 (0-110) | 1374 (0-3360) | 74 (0-190) | 3339 (600-8400) | 0.0292 | <0.0001 | ns |
| 0.93 (±0.31) | 2.58 (±0.37) | 1.58 (±0.22) | 3.38 (±0.13) |  |  |  |

L4: fourth stage larvae; L5: fifth stage juveniles.

\* Means (± standard error) of log transformed values followed by different letter in the same line differ from each other by Tukey’s test (P<0.05).

ns = not significant (P>0.05).

**Table S3.** Mean (minimum-maximum) values of*Trichostrongylus colubriformis* in Santa Ines and Ile de France lambs under suppressive or targeted selective treatment (TST) programmes with anthelmintics.

|  |  |  |  |
| --- | --- | --- | --- |
| Development stages | Santa Ines  | Ile de France | Effects (P-value) |
| Suppressive (n=9) | TST (n=10) | Suppressive (n=10) | TST (n=9) | Breed | Treatment programme | Breed x Treatment |
| Early L4 | 0 | 24 (0-60) | 0 | 156 (20-570) | 0.0052 | <0.0001 | 0.0016 |
|  | 0 a \* | 0.83 (±0.28) b | 0 a | 1.97 (±0.16) c |  |  |  |
| Female late L4 | 2 (0-10) | 56 (0-300) | 1 (0-10) | 428 (20-1060) | 0.0145  | <0.0001 | 0.0008 |
|  | 0.23 (±0.15) a | 1.16 (±0.29) b | 0.10 (±0.10) a | 2.46 (±0.16) c |  |  |  |
| Male late L4 | 1 (0-10) | 90 (0-290) | 4 (0-30) | 487 (60-1450) | 0.0095 | <0.0001 | 0.0163 |
|  | 0.12 (±0.12) a | 1.44 (±0.29) b | 0.25 (±0.17) a | 2.56 (±0.13) c |  |  |  |
| Female L5 | 2 (0-10) | 136 (0-400) | 2 (0-20) | 969 (440-2040) | 0.0197 | <0.0001 | 0.0012 |
|  | 0.23 (±0.15) a | 1.67 (±0.30) b | 0.13 (±0.13) a | 2.93 (±0.08) c |  |  |  |
| Male L5 | 0 | 166 (0-660) | 1 (0-10) | 1772 (640-3060) | 0.0006 | <0.0001 | .0005 |
|  | 0 a | 1.55 (±0.35) b | 0.10 (±0.10) a | 3.19 (±0.08) c |  |  |  |
| Adult female | 1 (0-10) | 2806 (0-6690) | 0 | 4748 (1780-18030) | ns | <0.0001 | 0.0412 |
|  | 0.26 (±0.17) a | 2.61 (±0.05) b | 0 a | 3.55 (±0.10) b |  |  |  |
| Adult male | 1 (0-10) | 2806 (0-6690) | 5 (0-30) | 2744 (190-14810) | ns | <0.0001 | ns |
|  | 0.12 (±0.12) | 2.52 (±0.49) | 0.36 (±0.19) | 3.07 (±0.19) |  |  |  |
| Total worm burden | 10 (0-30) | 6997 (0-16190) | 13 (0-50) | 11253 (4670-35150) | ns | <0.0001 | ns |
| 0.77 (±0.20) | 2.93 (±0.53) | 0.67 (±0.23) | 3.98 (±0.08) |  |  |  |

L4: fourth stage larvae; L5: fifth stage juveniles.

\*Means (± standard error) of log transformed values followed by different letter in the same line differ from each other by Tukey’s test (P<0.05).

ns = not significant (P>0.05).

**Table S4.** Mean (minimum-maximum) values of*Strongyloides papillosus* in Santa Ines and Ile de France lambs under suppressive or targeted selective treatment (TST) programmes with anthelmintics.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Stage of development | Santa Ines |  | Ile de France |  | Effects (P-value) |
| Suppressive (n=9) | TST (n=10) | Suppressive (n=10) | TST (n=9) | Breed | Treatment programme' | Breed x Treatment |
| Early L4 | 0 a | 0 | 0 | 39 (0-180) | 0.0259 | 0.0193 | 0.0193 |
|  | 0 a \* | 0 a | 0 a | 0.74 (±0.32) b |  |  |  |
| Late L4 | 0 | 15 (0-50) | 10 (0-60) | 62 (0-310 | 0.0136 | 0.0002 | ns |
|  | 0 | 0.72 (±0.25) | 0.44 (±0.23) | 1.4 (±0.23) |  |  |  |
| L5 | 4 (0-20) | 19 (0-80) | 3 (0-20) | 110 (0-790) | ns | 0.0034 | ns |
|  | 0.37 (±0.19) | 0.77 (±0.26) | 0.23 (±0.16) | 1.29 (±0.30) |  |  |  |
| Adult female | 107 (10-460) | 5179 (80-36370) | 431 (0-2490) | 20244 (760-78870) | ns | <0.0001 | ns |
|  | 1.71 (±0.18) | 3.01 (±0.25) | 1.90 (±0.32) | 4.00 (±0.20) |  |  |  |
| Total worm burden | 113 (10-470) | 5244 (80-36460) | 447 (0-2520) | 20526 (790-79260) | ns | <0.0001 | ns |
| 1.77 (±0.18) | 3.05 (±0.25) | 1.91 (±0.32) | 4.00 (±0.20) |  |  |  |

L4: fourth stage larvae; L5: fifth stage juveniles.

\* Means (± standard error) of log transformed values followed by different letter in the same line differ from each other by Tukey’s test (P<0.05).

ns = not significant (P>0.05).



**Figure S1.** Percentages of optical density of IgG against *Trichostrongylus colubriformis* L3 (A), IgA anti-L3 (B) and anti-adult (C) of *T. colubriformis* in Santa Ines lambs under Target Selective Treatment (TST), presenting low and high worm burden. The ends of the box are the upper and lower quartiles; the median is marked by a vertical line inside the box; and the two lines outside the box extend to the highest and lowest observations.



**Figure S2.** Number of eosinophils (A), mast cells (B) and globule leukocytes (C) in the intestinal mucosa of Santa Ines lambs under Target Selective Treatment (TST), presenting low and high *Trichostrongylus colubriformis*burden. The ends of the box are the upper and lower quartiles; the median is marked by a vertical line inside the box; and the two lines outside the box extend to the highest and lowest observations.