Ledger, M.L., Grimshaw, E., Fairey, M., Whelton, H.L., Bull, I.D., Ballantyne, R., Knight, M. & Mitchell, P.D. Intestinal Parasites at the Late Bronze Age settlement of Must Farm, in the Fens of East Anglia, UK (9th century B.C.E.). *Parasitology*

**SUPPLEMENTARY MATERIAL**

*Faecal Lipid Biomarker Methodology*

Approximately, 0.5 g of the coprolite was crushed using a mortar and pestle then passed through a 2 mm sieve. Suitable amounts of two internal standards (hyocholic acid and preg-5-en-3β-ol; 50 μL, 0.1 mg mL-1 solution) were added to the powdered samples. The powdered coprolites were then microwave extracted (10 min ramp to 70 °C (1000 W), 10 min hold at 70 °C (1000 W), and 20 min cool down) using 10 mL of 2:1 DCM/MeOH (*v/v*). The total lipid extract (TLE) obtained was subsequently hydrolysed and the sterol and bile acid fractions were isolated as outlined in Bull *et al.* (1999) and Elhmmali *et al.* (1997), respectively. For the methylation of the bile acids BF3-MeOH was used as an alternative methylation reagent. The fractions containing the target biomarkers were then analysed by gas chromatography (GC) and GC-mass spectrometry (GC-MS).

Analyses of biomarker isolates were performed using an HP 5890 Series II gas chromatograph. Trimethylsilylated sterols and methylated/trimethylsilylated bile acids were introduced (1.0 μL) *via* an on-column injector. The analytical column was a 50 m × 0.32 mm fused silica capillary column coated with a 100% dimethylpolysiloxane HP1 non-polar stationary phase (0.17 μm; Agilent). For the analyses of sterols the GC temperature programme was set to hold at 50°C for 2 min, followed by a gradient increase to 200°C at 10 °C min−1, and then to 300°C at 3°C min−1 before a final isothermal at 300°C for 20 min. For the analyses of bile acids the GC temperature programme was set to hold at 40°C for 1 min, followed by a gradient increase to 230°C at 20°C min−1, and then to 300°C at 2°C min−1 before a final isothermal at 300°C for 20 min. Helium was used as the carrier gas set to constant flow of 2.0 mL min−1 and the FID used to monitor column effluent was kept at a constant temperature of 300 °C. Data was acquired using DataApex Clarity (version 2.6.2.226) and eluting peaks were identified by comparison of retention times with those of external standards.

GC-MS analyses were performed using a ThermoScientific Trace 1300 gas chromatograph couple to an ISQ single quadrupole mass spectrometer. Samples were introduced *via* a PTV injector set to splitless mode, with a splitless time of 2 min, onto a 50 m × 0.32 mm fused silica capillary column coated with a 100 % dimethylpolysiloxane HP1 non-polar stationary phase (0.17 μm; Agilent). The GC temperature programmes employed were the same as for the corresponding GC analyses. Helium was used as the carrier gas, set to a constant flow of 2 mL min−1. The MS was operated in electron ionisation (EI) mode with an electron energy of 70 eV, a GC transfer line temperature of 300 °C and a source temperature of 300 °C. The emission current was set to 150 μA and the MS was set to acquire in the range of *m/z* 50-650 at 0.2 scans s−1 in full-scan mode. The data acquisition and processing were carried out using XCalibur software (version 3.0).

*Lipid Biomarker Results*

Table S1: Summary of the faecal biomarker analyses.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **Ratio 2** | **Ratio 3** | **LCA** | **DCA** | **CDCA** | **CA** | **UDCA** | **HDCA** | **Species** |
| SF4029 | 2.78 | 2.35 | 55.82 | 10.37 | 0.00 | 4.82 | 0.00 | 5.68 | 9.68 | 8.50 | 0.28 | 0.59 | ✓ | ✓ | nd | nd | nd | nd | canine |
| SF3080 | 4.35 | 1.29 | 28.36 | 11.93 | 0.00 | 13.13 | 0.00 | 10.44 | 22.62 | 7.88 | 0.25 | 0.27 | trace | trace | nd | nd | nd | nd | n/a |
| SF3082 | 14.91 | 2.50 | 39.68 | 2.91 | 2.47 | 0.87 | 11.38 | 6.29 | 11.76 | 7.24 | 0.83 | 1.05 | ✓ | ✓ | nd | ✓ | nd | nd | human |
| SF3127 | 19.05 | 10.44 | 25.40 | 10.08 | 0.00 | 0.00 | 5.43 | 5.40 | 12.10 | 12.10 | 0.75 | 3.21 | ✓ | ✓ | nd | nd | nd | nd | human |
| SF3177 | 2.77 | 1.57 | 63.11 | 9.55 | 0.00 | 0.00 | 4.17 | 2.94 | 7.54 | 8.36 | 0.27 | 0.67 | ✓ | ✓ | nd | ✓ | nd | nd | canine |
| SF3178 | 2.26 | 1.19 | 79.22 | 5.58 | 0.00 | 0.00 | 2.10 | 1.71 | 3.81 | 4.13 | 0.26 | 0.78 | ✓ | ✓ | nd | nd | nd | nd | canine |
| SF4034 | 0.95 | 1.04 | 68.80 | 6.60 | 0.00 | 2.57 | 0.00 | 2.23 | 12.25 | 5.56 | 0.21 | 0.67 | nd | trace | nd | nd | nd | nd | n/a |
| SF3677 | 5.38 | 2.12 | 52.85 | 6.30 | 0.00 | 6.09 | 0.00 | 3.53 | 14.63 | 9.10 | 0.59 | 2.23 | ✓ | ✓ | ✓ | ✓ | ✓ | nd | canine |
| SF3642 | 22.88 | 17.45 | 21.19 | 12.67 | 0.00 | 0.00 | 12.22 | 3.66 | 3.81 | 6.13 | 0.75 | 2.54 | ✓ | ✓ | nd | ✓ | nd | nd | human |
| SF3631 | 18.07 | 0.54 | 9.82 | 2.69 | 1.13 | 0.00 | 12.13 | 5.35 | 35.30 | 14.96 | 0.83 | 1.07 | ✓ | ✓ | ✓ | ✓ | nd | nd | human |
| SF3365 | 10.00 | 4.71 | 19.36 | 11.36 | 0.00 | 0.00 | 20.55 | 8.17 | 17.22 | 8.63 | 0.47 | 0.57 | trace | trace | nd | nd | nd | nd | n/a |
| SF3175 | 2.49 | 1.06 | 79.01 | 7.21 | 0.00 | 2.71 | 0.00 | 0.00 | 3.21 | 4.31 | 0.32 | 2.34 | ✓ | ✓ | nd | nd | nd | nd | canine |
| SF3176 | 0.84 | 0.45 | 82.89 | 7.16 | 0.00 | 2.03 | 0.00 | 0.52 | 4.09 | 2.01 | 0.12 | 1.47 | trace | trace | nd | nd | nd | nd | n/a |
| SF3492 | 7.51 | 2.69 | 50.46 | 5.19 | 1.05 | 0.00 | 7.94 | 4.28 | 12.12 | 8.77 | 0.62 | 0.95 | ✓ | ✓ | nd | nd | ✓ | nd | canine |
| SF3955 | 3.09 | 1.01 | 60.73 | 6.36 | 0.28 | 5.04 | 0.00 | 3.36 | 14.55 | 5.59 | 0.37 | 1.08 | ✓ | ✓ | nd | nd | ✓ | nd | canine |

Percentages of faecal sterols where: 1. coprostanol, 2. epicoprostanol, 3. cholesterol, 4. 5α-cholestanol, 5. 5β-campestanol, 6. campesterol, 7. 5β-stigmastanol, 8. epi-5β-stigmastanol, 9. sitosterol, 10. 5α-stigmastanol. For the ratios of faecal sterols, values of Ratio 2 >0.7 indicate faeces and values of Ratio 3 >1 indicate omnivore faeces following the ratios presented in Bull *et al.*, 2002. Bile acids identities are as follows: LCA is lithocholic acid, DCA is deoxycholic acid, CDCA is chenodeoxycholilc acid, CA is cholic acid, UDCA is ursodeoxycholic acid, HDCA is hyodeoxycholic acid and nd denotes none detected. Coprolites which contain trace levels of bile acids confirm that they do not contain notable quantities of faecal material.

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