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**Molecular identification of hookworm isolates from stray dogs, humans and selected wildlife from South Africa**

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**Supplementary Table S1.** Expected DNA fragment sizes for different hookworm species, presence/absence of cleavage sites for two restriction enzymes and predicted fragment sizes after digestion with EcoRII and BsuRI (Liu *et al*. (2015).



**Supplementary Table S2.** Species identification of hookworm isolates from faecal samples obtained from selected SPCAs and primary schools in KwaZulu-Natal and in game reserves from Mpumalanga province, South Africa using PCR-RFLP with EcoRII and BsuRI restriction endonucleases.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Origin** | **Sample** | **EcoRII** | **BsuRI** | **Hookworm species** |
| Amanzimtoti | A1 | 404 bp | 404 bp | *A. caninum* |
| A2 | 404 bp | 404 bp | *A. caninum* |
| A3 | 404 bp | 404 bp | *A. caninum* |
| A5 | 404 bp | 404 bp | *A. caninum* |
| A6 | 404 bp | 404 bp | *A. caninum* |
| Kloof | B1 | 404 bp | 404 bp | *A. caninum* |
| B2 | 404 bp | 404 bp | *A. caninum* |
| B3 | 404, 328, 210, 122, 72 bp | 404 bp | *A. caninum, A. ceylanicum, A. braziliense* |
| B4 | 404 bp | 404 bp | *A. caninum* |
| B5 | 404 bp | 404 bp | *A. caninum* |
| Springfield | C1 | 404 bp | 404 bp | *A. caninum* |
| C2 | 404 bp | 404 bp | *A. caninum* |
| C3 | 404, 328, 210, 122, 72 bp | 404 bp | *A. caninum, A. ceylanicum A. braziliense* |
| C4 | 404, 328, 212, 72 bp | 404 bp | *A. caninum, A. ceylanicum* |
| C5 | 404 bp | 404 bp | *A. caninum* |
| C6 | 404, 328, 212 bp | 404 bp | *A. caninum,* Unknown |
| C7 | 404, 328, 210, 122, 72 bp | 404 bp | *A. caninum, A. ceylanicum, A. braziliense* |
| C8 | 404 bp | 404 bp | *A. caninum* |
| C9 | 404 bp | 404 bp | *A. caninum* |
| C10 | 404 bp | 404 bp | *A. caninum* |
| C11 | 404 bp | 404 bp | *A. caninum* |
| C12 | 404 bp | 404 bp | *A. caninum* |
| C13 | 404, 328, 210, 72 bp | 404 bp | *A. caninum, A. ceylanicum* |
| C14 | 404 bp | 404 bp | *A. caninum* |
| C15 | 404, 328, 210, 122, 72 bp | 404 bp | *A. caninum, A. ceylanicum, A. braziliense* |
| C16 | 404 bp | 404 bp | *A. caninum* |
| C17 | 404, 328, 210, 122, 72 bp | 404 bp | *A. caninum, A. ceylanicum, A. brazliense* |
| C18 | 404 bp | 404 bp | *A. caninum* |
| C19 | 404, 328, 212, 72 bp | 404 bp | *A. caninum, A. ceylanicum* |
| C20 | 404, 328, 210, 122, 72 bp | 404 bp | *A. caninum, A. ceylanicum, A. braziliense* |
| C21 | 404 bp | 404 bp | *A. caninum* |
| C22 | 404 bp | 404 bp | *A. caninum* |
| C23 | 404 bp | 404 bp | *A. caninum* |
| C24 | 404, 122, 50 bp | 404 bp | *A. caninum,* Unknown |
| C25 | 404 bp | 404 bp | *A. caninum* |
| C26 | 404, 328, 210, 122, 72 bp | 404 bp | *A. caninum, A. ceylanicum A. braziliense* |
| C27 | 404 bp | 404 bp | *A. caninum* |
| C28 | 404 bp | 404 bp | *A. caninum* |
| C29 | 404 bp | 404 bp | *A. caninum* |
| C30 | 404 bp | 404 bp | *A. caninum* |
| C31 | 404 bp | 404 bp | *A. caninum* |
| C32 | 404 bp | 404 bp | *A. caninum* |
| C33 | 404, 328, 122, 76 bp | 404 bp | *A. caninum, A. ceylanicum* |
| C34 | 404, 210 bp | 404 bp | *A. caninum,* Unkown |
| C35 | 404 bp | 404 bp | *A. caninum* |
| C36 | 404 bp | 404 bp | *A. caninum* |
| C37 | 404 bp | 404 bp | *A. caninum* |
| C38 | 404 bp | 404 bp | *A. caninum* |
| Ballito | D1 | 404 bp | 404 bp | *A. caninum* |
| Westville Veterinary Clinic | E1 | 404 bp | 404 bp | *A. caninum* |
| Pongola | F1 | 404 bp | 404 bp | *A. caninum* |
| F2 | 404 bp | 404 bp | *A. caninum* |
| F3 | 404 bp | 404 bp | *A. caninum* |
| Siyathuthuka | G1 | 404 bp | 404 bp | *A. caninum* |
| Timbavati | H1 | 404, 328, 212 bp | 404 bp | *A. caninum,* Unknown |

**Supplementary Table S3**. p-distance matrix between hookworm species haplotypes as shown in figure 6.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 21  |
| 1 | Hap 1 | - |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 2 | Hap 2 | 0.007 | - |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 3 | Hap 3 | 0.047 | 0.054 | - |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 4 | Hap 4 | 0.027 | 0.020 | 0.074 | - |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 5 | Hap 5 | 0.013 | 0.020 | 0.060 | 0.013 | - |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 6 | Hap 6 | 0.020 | 0.013 | 0.067 | 0.007 | 0.007 | - |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 7 | Hap 7 | 0.081 | 0.087 | 0.034 | 0.107 | 0.094 | 0.101 | - |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 8 | Hap 8 | 0.114 | 0.121 | 0.081 | 0.141 | 0.128 | 0.134 | 0.101 | - |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 9 | Hap 9 | 0.013 | 0.007 | 0.060 | 0.013 | 0.027 | 0.020 | 0.094 | 0.128 | - |  |  |  |  |  |  |  |  |  |  |  |  |
| 10 | Hap 10 | 0.054 | 0.060 | 0.007 | 0.067 | 0.054 | 0.060 | 0.040 | 0.087 | 0.067 | - |  |  |  |  |  |  |  |  |  |  |  |
| 11 | Hap 11 | 0.008 | 0.013 | 0.054 | 0.034 | 0.020 | 0.027 | 0.087 | 0.121 | 0.020 | 0.060 | - |  |  |  |  |  |  |  |  |  |  |
| 12 | Hap 12 | 0.013 | 0.020 | 0.047 | 0.040 | 0.027 | 0.034 | 0.081 | 0.114 | 0.027 | 0.054 | 0.007 | - |  |  |  |  |  |  |  |  |  |
| 13 | Hap 13 | 0.020 | 0.027 | 0.040 | 0.047 | 0.034 | 0.040 | 0.074 | 0.107 | 0.034 | 0.047 | 0.013 | 0.007 | - |  |  |  |  |  |  |  |  |
| 14 | Hap 14 | 0.027  | 0.034 | 0.034 | 0.054 | 0.040 | 0.047 | 0.067 | 0.101 | 0.040 | 0.040 | 0.020 | 0.013 | 0.007 | - |  |  |  |  |  |  |  |
| 15 | Hap 15 | 0.040 | 0.047 | 0.020 | 0.067 | 0.054 | 0.060 | 0.054 | 0.101 | 0.054 | 0.027 | 0.047 | 0.040 | 0.034 | 0.040 | - |  |  |  |  |  |  |
| 16 | Hap 16 | 0.034 | 0.040 | 0.027 | 0.060 | 0.047 | 0.054 | 0.060 | 0.107 | 0.047 | 0.034 | 0.040 | 0.034 | 0.040 | 0.047 | 0.007 | - |  |  |  |  |  |
| 17 | Hap 17 | 0.054 | 0.060 | 0.007 | 0.081 | 0.067 | 0.074 | 0.040 | 0.087 | 0.067 | 0.013 | 0.060 | 0.054 | 0.047 | 0.040 | 0.013 | 0.020 | - |  |  |  |  |
| 18 | Hap 18 | 0.027 | 0.034 | 0.047 | 0.054 | 0.040 | 0.047 | 0.081 | 0.114 | 0.040 | 0.054 | 0.020 | 0.027 | 0.020 | 0.027 | 0.054 | 0.060 | 0.054 | - |  |  |  |
| 19 | Hap 19 | 0.020 | 0.027 | 0.040 | 0.047 | 0.034 | 0.040 | 0.074 | 0.107 | 0.034 | 0.047 | 0.027 | 0.034 | 0.027 | 0.034 | 0.047 | 0.054 | 0.047 | 0.007 | - |  |  |
| 20 | Hap 20 | 0.020 | 0.027 | 0.040 | 0.047  | 0.034 | 0.040 | 0.074 | 0.107 | 0.034 | 0.047 | 0.013 | 0.020 | 0.013 | 0.020 | 0.034 | 0.040 | 0.047 | 0.020 | 0.027 | - |  |
| 21 | Hap 21 | 0.013 | 0.020 | 0.034 | 0.040 | 0.027 | 0.034 | 0.067 | 0.101 | 0.027 | 0.040 | 0.020 | 0.027  | 0.020 | 0.027 | 0.027 | 0.034 | 0.040 | 0.027 | 0.020 | 0.007 | - |
| 22 | Hap 22 | 0.912 | 0.913 | 0.919 | 0.933 | 0.926 | 0.926 | 0.933 | 0.926 | 0.919 | 0.926 | 0.913 | 0.919 | 0.913  | 0.906 | 0.919 | 0.926 | 0.913 | 0.899 | 0.899 | 0.913 | 0.913 |