Appendix S1

SUPPLEMENTARY MATERIAL AND METHODS

Laboratory methods

Specimens were collected in universal transport medium (UTM, Copan, Brescia, Italy) tubes and DNA was extracted using BT Chelex 100 Resin (Bio-Rad Laboratories Inc., CA, USA).The samples were tested for the presence of *T. pallidum* using two real-time diagnostic PCR assays with internal amplification controls, amplifying segments of the flaA (S1) and polA (S2) genes of *T. pallidum* and the bacterial loads were determined from these PCR assays using 10-fold dilutions of purified T. pallidum DNA. The bacterial load was used for comparison between typeable and non-typeable specimens. Subsequently, the specimens were stored at –20ºC until they were subjected to strain typing. PCR was used to determine the number of 60-bp repeats within the arp gene as previously described (6), but with primer modifications as described by Katz et al. (14). In brief, 5 µl of DNA was amplified in a 100 µl reaction, using 2 U Taq DNA polymerase (Platinum Taq, Invitrogen, Paisley, UK), 125 µM each dATP, dGTP, and dCTP; 250 µM dUTP, 200 nM primers, 1.5 mM MgCl2 and 1X PCR reaction buffer (Invitrogen). We used the primers ARP N1 (forward) and ARP N2 (reverse) (14). Cycling conditions, including a touchdown step, consisted of an initial cycle of 94ºC for 2 min, followed by 10 cycles of 94ºC for 30 s; 74ºC for 45 s decreasing 1ºC per cycle, and 72ºC for 90 s. The next step consisted of 35 cycles of 94ºC for 30 s; 64ºC for 45 s, and 72ºC for 90 s. Final extension was achieved at 72ºC for 10 min. The amplification yielded different amplicon sizes depending on the different number of 60-bp repeats within the arp gene. The number of 60-bp repeats was estimated using plasmids with a known number of repeats as reference, kindly provided by Bess Charmie Godornes from the University of Washington, Seattle, USA. Further, specimens were compared to the Nichols strain of *T. pallidum* with the known amplicon size of 1155 bp, corresponding to 14 repeats.

A nested PCR was used to analyze tpr. In brief, 10 µl of DNA was amplified in a 100 µl reaction, using 2 U Taq DNA polymerase (Platinum Taq, Invitrogen), 125 µM each dATP, dGTP, dTTP, and dCTP; 400 nM primers, 1.5 mM MgCl2 and 1X PCR reaction buffer (Invitrogen). A first-round was performed using primers 5'CAGGTTTTGCCGTTAAGC3' (forward) and 5'AATCAAGGGAGAATACCGTC3' (reverse) as previously described (7). We used the following cycling conditions, including a touchdown step: an initial cycle of 95ºC for 2 min was followed by 10 cycles of 95ºC for 15 s; 70ºC for 15 s decreasing 1ºC per cycle, and 72ºC for 2 min. The next step consisted of 40 cycles of 95ºC for 15 s; 60ºC for 15 s, and 72ºC for 2 min. Final extension was achieved at 72ºC for 7 min. A second-round was performed using primers 5'CTGTTATGGGGCCTA CC3' (forward) and 5' CTCTATGAGACTGGCTGAAA3' (reverse). One microliter template was amplified in a 50 µl reaction, using 1 U Immolase, 125 µM each dATP, dGTP, dTTP, and dCTP; 400 nM primers, 2.5 mM MgCl2 and 1X Immolase buffer. The following cycling conditions were used for the second round: an initial cycle of 95ºC for 10 min was followed by 15 cycles of 94ºC for 30 s; 50ºC for 15 s, and 72ºC for 90 s. Amplicons from the second PCR were digested with the restriction endonuclease MseI (New England Biolabs Inc., MA, USA). The digestion products were separated by gel electrophoresis and the patterns were compared with published data (6, 22).

Sequence analysis of an 84-bp region of the tp0548 gene was performed as described previously by Marra et al. (7) with some modifications. In brief, 5 µl of DNA was amplified in a 100 µl reaction, using 2 U Taq DNA polymerase (Platinum Taq, Invitrogen), 125 µM each dATP, dGTP, dTTP, and dCTP; 400 nM primers, 3.5 mM MgCl2 and 1X PCR PCR reaction buffer (Invitrogen). We used the primers 5'GCGTGGTGGTGAGTT-CTTCT3' (forward) and 5'CGTTTCGGTGTGTGAGTCAT3' (reverse). An initial cycle of 94ºC for 2 min was followed by a touchdown step, consisting of 10 cycles of 94ºC for 15 s; 65 ºC for 15 s decreasing 1ºC per cycle, and 72ºC for 30 s. The next step consisted of 35 cycles of 94ºC for 15 s; 55ºC for 15 s, and 72ºC for 30 s. Final extension was achieved at 72ºC for 10 min. The PCR products were sequenced using standard methods. *T. pallidum* positive controls and negative (nuclease-free water) controls were used in all PCR assays. Specimens that initially gave negative results were repeated with the relevant assay.

REFERENCES

S1. Salazar JC, Rathi A, Michael NL, Radolf JD, Jagodzinski LL. Assessment of the kinetics of Treponema pallidum dissemination into blood and tissues in experimental syphilis by real-time quantitative PCR. Infect Immun 2007; 75: 2954–2958.

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