Materials and Methods

Bacteria strains and growth conditions. 230 *S. enterica* strains were isolated *post mortem* from deceased harbour porpoises between 1992 and 2010 (Table S2). Isolates were stored on Dorset Egg slopes at ambient temperature since their isolation. Bacterial cultures were grown at 37˚C overnight in Tryptone Soya broth (CM0129, Oxoid Ltd., Basingstoke, UK), which was supplemented with 35.6 mM K2HPO4, 13.2 mM KH_2PO_4, 0.8 mM $MgSO_4$, 1.7 mM $C_6H_5Na_3O_7{\cdot}2H_2O$, 6.8 mM $(NH_4)_2SO_4$ and 4.4% (v/v) glycerol to allow freezing immediately after cultivation.

The properties of 21 porpoise isolates plus 27 additional strains of related serovars that were tested by MLST are listed in Table S3.

Pulsed-field gel electrophoresis. PFGE typing was performed as described (4,5). TIFF images were analysed with Bionumerics Version 5 (Applied Maths, Belgium) with optimization set at 1% and band tolerance at 1.3%. Relationships were determined by Dice correlation and UPGMA clustering. Only restriction fragments of >30 kb were included in the analysis.

Multilocus sequence typing. DNA was extracted using an X-tractor Gene fluidics robot (Corbett, Australia) as described by the manufacturer except that the lysis step was performed with the JETFLEX Genomic DNA Purification Kit (Genomed, Germany). The *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA* and *thrA* gene fragments were amplified and sequenced from at least two independent PCR products in forward and reversed orientation as described (2), except that the PCR and sequencing primers used were those listed on the *Salmonella* MLST website (http://mlst.ucc.ie/dbs/Senterica). All results are publicly available at the MLST website.

PCR amplification and sequencing of *fliC* and *fljB*. PCR amplification of *fliC* and *fljB* was carried out as described (1). When PCR amplification of *fljB* failed with the external PCR primers, internal sequencing primers specific for phase 2 antigen groups "e,n" and "1" were used instead (Table S3). 10 µl PCR products were digested with 1 µl of ExoSAPIT (Affymetrix, USA) for 15 minutes at 37ºC, followed by 15 minutes inactivation at 80ºC. The cleaned PCR product was diluted 1:10 with H_2O and 3 µl of diluted PCR product were used as template in a total volume of 10 µl sequencing reaction containing sequencing primers (Table S4) at a final concentration of 4 pmol. The sequencing reaction consisted of an initial denaturation at 96ºC for 2 minutes, followed by 30 cycles of denaturation for 10s at 96ºC, annealing for 5s at 50ºC (*fliC*) or 47º (*fljB*) and elongation for 2 minutes at 60ºC. The sequencing products were purified by ethanol precipitation and sequenced at the sequencing facility at the Department of Zoology at Oxford University, UK. Traces were assembled and analysed with Bionumerics Version 6 (Applied Maths, Belgium). The sequences, trimmed to positions 37-1463 of *fliC* and 165-1357 of *fljB* of strain LT2, have been submitted to the EMBL-EBI nucleotide database under the accession numbers HE801371 to HE801515. Nonsynonymous (dN) and synonymous (dS) substitution rates were calculated using the Nei-Gojobori model implemented in Mega Version 5.05 (3,6).

Reference List

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