

Supplementary Table 1. PCR assays used on isolates from working farm dogs and their home-killed raw meat diet in Manawatu, New Zealand.

PCR assay	Primer sequences	Target gene (product size in base pairs)	Amplification conditions*	Master mix content†
<i>Campylobacter</i> spp. ⁴	C412F: 5'-GGATGACACTTTCGGAGC-3' C1228R‡: 5'-CATTGTAGCACGTGTGTC-3'	16S rDNA (816)	40 cycles annealing at 56°C	200 mM Tris-HCl and 1,000 mM KCl (pH 8.3; 10× buffer), 100 µM each dNTP, 2.5 mM MgCl ₂ , 4 pmol of each primer, Platinum Taq (1 unit), ~40 ng sample DNA, and distilled H ₂ O (dH ₂ O) to 20 µL of total volume
<i>C. jejuni</i> ⁶ / <i>C. coli</i> ¹	CJF: 5'-ACTTCTTTATTGCTTGCTGC-3' CJR: 5'-GCCACAACAAGTAAAGAAGC-3' COL3: 5'-AATTGAAAATTGCTCCAATG-3' MDCOL2: 5'-TGATTATTATTGTAGCAGCG-3'	<i>hipO</i> (323) <i>ceuE</i> (462)	40 cycles annealing at 55°C	10× buffer, 100 µM each dNTP, 1.5 mM MgCl ₂ , 4 pmol of each primer, Platinum Taq (1 unit), ~40 ng of sample DNA, and distilled H ₂ O to 20 µL of total volume
<i>C. upsaliensis</i> / <i>C. helveticus</i> ⁴	CHCU146F: 5'-GGGACAACACTTAGAAATGAG-3' CU1024R: 5'-CACTCCGTATCTCTACAGA-3' CH1371R: 5'-CCGTGACATGGCTGATTCAC-3'	16S rDNA (878/1,225 or 1,375)	35 cycles annealing at 55°C	10× buffer, 200 µM each dNTP, 1.5 mM MgCl ₂ , 4 pmol of each primer, Platinum Taq (1 unit), ~40 ng of sample DNA, distilled H ₂ O to 20 µL of total volume
<i>C. fetus</i> / <i>C. hyointestinalis</i> ⁴	CFCH57F: 5'-GCAAGTCGAACGGAGTATTA-3' CF1054R: 5'-GCAGCACCTGCTCTCACT-3' CH1344R: 5'-GCGATTCCGGCTTCATGCTC-3'	16S rDNA (997/1,287)	35 cycles annealing at 55°C	10× buffer, 200 µM each dNTP, 1.5 mM MgCl ₂ , 4 pmol of each primer, Platinum Taq (1 unit), ~40 ng of sample DNA, and distilled H ₂ O to 20 µL of total volume
<i>C. lari</i> ⁴	CL594F: 5'-CAAGTCTTGTGAAATCCAAC-3' CL1155R: 5'-ATTAGAGTGCTCACCCGAAG-3'	16S rDNA (561)	35 cycles annealing at 55°C	10× buffer, 200 µM each dNTP, 1.5 mM MgCl ₂ , 4 pmol of each primer, Platinum Taq (1 unit), ~40 ng of sample DNA, and distilled H ₂ O to 20 µL of total volume
<i>Arcobacter</i> <i>butzleri</i> ²	AB959F: 5'-CCTGGACTTGACATAGTAAGAATGA-3' AB1338R: 5'-CGTATTACCGTAGCATAGC-3'	16S rDNA (401)	35 cycles annealing at 61°C	10× buffer, 200 µM each dNTP, 1.5 mM MgCl ₂ , 4 pmol of each primer, Platinum Taq (1 unit), ~40 ng of sample DNA, and distilled H ₂ O to 20 µL of total volume

16S rDNA ⁵	27F: 5'-AGAGTTGATCMTGGCTCAG-3' 1492R: 5'-GGTTACCTTGTACGACTT-3'	16S rDNA (1,465)	30 cycles annealing at 58.5°C	10× buffer, 100 μM each dNTP, 1.5 mM MgCl ₂ , 4 pmol of each primer, Platinum Taq (1 unit), ~40 ng of sample DNA, and distilled H ₂ O to 20 μL of total volume
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* All PCR assays had initial denaturation at 96°C and final extension at 72°C for 2 min each. All amplification cycle steps were 30 s, and all elongation and extension steps were at 96°C and 72°C, respectively. Some of the assay parameters (annealing temperature and concentrations of dNTP, primers, and magnesium chloride) have been modified from the original publications as a result of in-house optimization procedures that involved validation with one target and one non-target strain.

† All master mix components and 1 Kb Plus marker ladder sourced from Invitrogen (Carlsbad, CA) except for in-house prepared distilled water. All PCR assays included distilled H₂O as a negative control and a sample DNA of target bacterial species (*C. jejuni* NCTC 11168, *C. coli* NCTC 11366, *C. upsaliensis* NCTC 11541, *C. helveticus* CCUG 30682, *C. fetus* NCTC 10842, *C. hyoilealis* NCTC 11608, *C. lari* NCTC 11352, and *A. butzleri* NCTC 12481 as appropriate for the PCR assay) as a positive control.

‡ Inglis and Kalischuk³ reported a typographical error in the paper by Linton et al. (1996)⁴: primer C1288R should read C1228R.

References

- Denis M, et al. Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. Lett Appl Microbiol 1999;29:406–410.
- Houf K, et al. Development of a multiplex PCR assay for the simultaneous detection and identification of *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*. FEMS Microbiol Lett 2000;193:89–94.

3. Inglis GD, Kalischuk LD. Use of PCR for direct detection of *Campylobacter* species in bovine feces. *Appl Environ Microbiol* 2003;69:3435–3447.
4. Linton D, et al. Rapid identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals. *Res Microbiol* 1996;147:707–718.
5. Suzuki MT, Giovannoni SJ. Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Appl Environ Microbiol* 1996;62:625–630.
6. Wang G, et al. Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus*. *J Clin Microbiol* 2002;40:4744–4747.