Supplemental Material

* Tet(C) gene transfer between Chlamydia suis strains occurs by homologous recombination after co-infection: Implications for spread of tetracycline-resistance among Chlamydiaceae

Hanna Marti*a, Hoyon Kim*a, Sandeep J. Josephb,c, Stacey Dojiria, Timothy D. Readb,c, Deborah Deanad

Center for Immunobiology and Vaccine Development, UCSF Benioff Children's Hospital Oakland Research Institute, Oakland, California, USAa; Department of Medicine, Division of Infectious Diseasesb and Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia, USAc; Joint Graduate Program in Bioengineering, University of California, San Francisco, California, USA, and University of California, Berkeley, Berkeley, California, USAd.
1. Co-infection Protocols:

a. Protocol 1

Immediate tetracycline challenge (2μg/ml) or after 1 passage

Plaque Assay (passage no. 2 or 3) and identification of putative recombinants by PCR and ompA genotyping

Inf. with recipient (MOI 4)  
Inf. with donor (MOI 1)

24 h  48 h  36-72 h

b. Protocol 2

Simultaneous infection with donor

Recipient grown in shell vials

1-2 passages (to 100% infection)

Inf. with recipient

Immediate tetracycline challenge (0.25 μg/ml) or after 1 passage

48-72 h  36-72 h

As Protocol 1

2. Co-infection Tetracycline Conditions A, B and C (tetracycline added at time of co-infection):

Co-infection (recipient, donor)

Condition A: No tetracycline

Condition B: Subinhibitory tetracycline MIC<sub>Tp</sub> of recipient

Condition C: Inhibitory tetracycline 2x MIC of recipient

Culture for 48-72 h according to Co-infection Protocol 1 or 2

Immediate tetracycline challenge or after 1 passage

FIG S1. Co-infection Protocols and Conditions. 1) Shown are the two co-infection protocols, which consisted of a) staggered infection of the donor (strains R19, R27 and Rogers132) 24 hours post infection with the recipient strain S45 (Protocol 1) and b) simultaneous co-infection of recipient and donor after the recipient was first grown to 100% infection in shell vials (Protocol 2). 2) Shown are the culturing conditions at the time of co-infection without tetracycline (Condition A), subinhibitory concentrations of tetracycline (Condition B) and inhibitory concentration of tetracycline (Condition C) for the recipient strain.
Figure S2. The tetracycline repressor gene tetR(C) is highly conserved across genera. Shown is the unrooted Maximum Likelihood (ML) phylogenetic tree of tetR(C) for a number of tetracycline resistant bacteria (NCBI, BLASTN search, identity cover: 98%) and ten C. suis strains, which correspond to two separate clades (Clade 1: red; Clade 2: green) and H7 (blue).
Figure S3. Phylogeny of invasin gene of *C. suis* and *C. caviae*. Shown is the unrooted Bayesian phylogenetic tree of the chlamydial invasin gene that is found only in *C. suis* and *C. caviae*. 