## **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* 

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## 1. Co-infection Protocols:



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



**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.



100 *A. salmonicida* AY043298.1

**Figure S2. The tetracycline repressor gene** *tet***R**(**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 Baysian phylogenetic tree of the chlamydial invasin gene that is found only in *C. suis* and *C. caviae*.