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Title: In vitro adherence and invasion of primary chicken oviduct epithelial cells by *Gallibacterium anatis*

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### Highlight

1. In order to better understand the mechanism of colonization and infection for *G.anatis* in natural host , in this study, we study adherence and invasion of *G.anatis* with two *G. anatis* strains of different virulence (Yu-PDS-RZ-1-SLG strain, highly virulent and F149T strain, nonvirulent) via infection of the primary chicken oviduct epithelial cells (PCOECs), and the levels of cytokines was detected.
2. Our data demonstrate that highly virulent, Yu-PDS-RZ-1-SLG strain was able to attach at higher levels compared to F149<sup>T</sup> strain, but no invasion was observed. However, cell debris and cell apoptosis were observed after being exposed to *G. anatis* Yu-PDS-RZ-1-SLG for 90 min, while *G. anatis* F149<sup>T</sup> strain (nonvirulent) did not cause cell damage, and adherence was prevented by trypsin treatment of the bacterial cells.
3. Transmission electron microscopy (TEM) and an immunohistochemical assay were also used to support tissue invasion experiment, TEM studies revealed that both *G. anatis* Yu-PDS-RZ-1-SLG strain and F149T strain were not detected intracellularly. The control PCOECs with no infection had clear subcellular structures and integrated microvilli, while the cells infected with Yu-PDS-RZ-1-SLG strain displayed a broken, fuzzy cellular structure, microvilli shedding and increased intracellular vacuoles. However, the cells infected with F149T strain did not undergo the morphological changes described above. Immunohistochemical assay results showed that strain Yu-PDS-RZ-1-SLG strain was also not detected intracellularly after infection. However, tight junctions between cells began to degrade as early as 30 min post

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