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Bactofection of mammalian cells by an attenuated derivatives of *Pasteurella multocida* B:2

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A feature of haemorrhagic septicaemia (HS), caused by *Pasteurella multocida* B:2 strains, is the rapid spread of infecting bacteria from the respiratory tract to the blood and lymph to cause a fatal septicaemia. To pass into the blood stream, the bacteria must migrate through the pulmonary and vascular tissues. A *Pasteurella multocida* B:2 strain from a case of bovine haemorrhagic septicaemia (HS) and two derivatives, JRMT12 and GDH7 that were attenuated by a deletion in the aroA gene and gdhA gene respectively, were shown to adhere to, invade and survive within cultured embryonic bovine lung (EBL) cells. Transmission electron microscopy (TEM) showed that, after entry into the mammalian cells, the B:2 strain resided in a vacuolar compartment. However, only a low percentage of mammalian cells appeared to contain one or more *P. multocida* B:2, suggesting that only certain EBL cells in the population were capable of being invaded by, or of taking up, the bacteria. A reporter plasmid pSRG has been developed which expresses red fluorescent protein (RFP) from a constitutive prokaryotic promoter within *Pasteurella multocida* B:2 and green fluorescent protein (GFP) from a constitutive eukaryotic promoter within mammalian cells. This construct has been used to determine the location and viability of the bacteria when moving from the extracellular environment into the intracellular compartment of mammalian cells. Invasion assays with embryonic bovine lung (EBL) cells and JRMT12 strain harbouring the plasmid pSRG, showed that RFP-expressing bacteria could be detected intracellularly at 3 h post-invasion. At this stage, some EBL cells harbouring RFP-expressing bacteria were observed to express GFP simultaneously, indicating release of the plasmid into the intracellular environment. At 5 h post-invasion, more EBL cells were expressing GFP, while still harbouring RFP-expressing bacteria. Concurrently, some EBL cells were shown to express only GFP, indicating loss of viable bacteria within these cells. These experiments proved the functionality of the pSRG dual reporter system and the potential of *P. multocida* B:2 JRMT12 for bactofection and delivery of a DNA vaccine.

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