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Selection of protective antigens in *Lawsonia intracellularis* by reverse vaccinology

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Lawsonia intracellularis is a bacterial pathogen that infects intestinal epithelial cells in pigs. This causes proliferative enteropathy, which is characterized by diarrhea and reduced growth, and *L. intracellularis* infection is one of the main reasons for antibiotic treatment of production pigs in Denmark.

Experimental challenge studies previously performed at DTU-Vet show that a primary infection results in complete protection against reinfection due to induction of immunological memory. We aim to develop a subunit vaccine that mimics the induction of the immune response and hence causes protection against *L. intracellularis*.

To this end, a reverse vaccinology approach was applied: the entire *L. intracellularis* genome encoding 1340 proteins was screened *in silico* using bioinformatics tools to identify potential protein antigens. Advanced software algorithms predicted 150 secreted and outer membrane proteins, and these were analyzed and given a score for presence of B and T cell epitopes. Using another *in silico* technology platform, which identifies novel B cell antigens eliciting a highly protective immune response, we obtained a second list of potential vaccine candidates. Six proteins were present in top 30 of both lists, and a combined rank was calculated. The two highest ranking proteins were initially selected for production.

Synthetic genes have been designed, and the proteins are currently being produced in recombinant forms in bacterial expression systems. They will be analyzed by ELISA and Western blot to determine if they are recognized by serum antibodies from protected pigs. If so, the proteins are promising vaccine candidates that can be investigated in future immunization experiments.