Title: Development of Molecular Tests for Serovar-Specific Identification and Typing of *Haemophilus paragallinarum*
Project No: DAW-226A
Authors: P. J. Blackall, M. I. Khan, X. Chen, C. Song and R. Bowles

Summary

Haemophilus paragallinarum is the causative agent of infectious coryza, an upper respiratory tract disease of chickens characterised by relatively mild clinical signs. The economic impact of the disease is attributed to the increase in unhealthy chickens and significant reductions in egg production.

There have been few studies on methods to type isolates of *H. paragallinarum*. Most typing of *H. paragallinarum* has involved the use of two of the three recognised serotyping schemes - the Page scheme and the modified Kume scheme.

In this project, two different types of rapid, DNA based typing methods were evaluated for their ability to recognise subtypes within the species *Haemophilus paragallinarum*. The two techniques were based on a molecular biology technique known as the polymerase chain reaction (PCR). One of the techniques was based on the use of random DNA sequences and is termed RAPD (Random Amplification Polymorphic DNA). The other technique (ERIC-PCR) is based on DNA sequences that were originally discovered in bacteria that belong to the family *Enterobacteriaceae*.

The two techniques were examined from two view points:-

A) Ability to produce patterns that were serovar specific ie an ability to replace conventional serotyping
B) Ability to subtype below species and serovar level - allowing fingerprinting that is useful for disease outbreak investigations

Despite extensive efforts, the RAPD technique could not be established to a stage where reproducible patterns could be obtained. The technique was thus abandoned. ERIC-PCR did not give serovar specific patterns and thus cannot replace conventional serotyping.

However, the technique was shown to be as effective as a typing tool for disease outbreak investigations. ERIC-PCR was shown to have the capacity to clearly distinguish unrelated isolates. The technique was also shown to be as good as some alternative molecular techniques for typing reference strains of *H. paragallinarum* and field isolates of *H. paragallinarum* from outbreaks of infectious coryza in China.

The very limited genetic diversity of Australian isolates of *H. paragallinarum* was confirmed with ERIC-PCR. This project has validated ERIC-PCR as a molecular typing tool for investigating outbreaks of infectious coryza. The technique, however, is of limited application in Australia because of the unique nature of the limited genetic diversity of Australian isolates of *H. paragallinarum.*