Title: Development of real-time PCR test to quantify infectious bronchitis virus to tissues of chickens

Authors: Juliet Roberts and Kapil Chousalkar

Byline

This study was conducted to develop and evaluate the use of reverse transcriptase real time polymerase chain reaction (RT-PCR) to detect and quantify the viral copy number of infectious bronchitis virus (IBV) from infected tissues of vaccinated and unvaccinated laying hens. The real-time PCR test designed during this project is highly sensitive and can detect as low as 10 viral copy numbers. Along with virulent 'T' and vaccine strains A3 and Vic S, the test will also be able to detect at least seven other Australian IBV strains.

Summary

Infectious bronchitis virus (IBV) has always been considered a threat to the Australian layer industry as it can cause drops in egg production and quality a great concern to egg producers than hen mortality. Despite the risks posed by IBV very little research has been done on the direct interaction of IBV with the oviduct of the laying hen.

While the effects of IBV on the mature oviduct of laying hens in full lay were studied in the US in 1957, in Australia little effort has been made prior to the current study to investigate the effects of Australian strains of IBV on the mature oviduct of laying hens and to establish its direct relationship with production drops. Also, there is considerable confusion in the industry regarding revaccination during lay for IBV and the factors affecting egg and egg shell quality.

Traditional virus isolation tests are laborious and time-consuming clearly pointing to a need for rapid detection of Australian strains of infectious bronchitis virus (IBV) directly from clinical samples in order to detect and quantify the viral load.

This project was conducted to design a real time PCR test to detect and quantitate the IBV viral copy number from clinical samples. The process is complete for the T, A3 and VicS strains of IBV but the quantitative test for the N1/88 strain requires further optimisation. The present test was able to detect both the pathogenic (T) and vaccine (A3, VicS) strains of IBV present at as low as 10 viral copy numbers from clinical samples. The test is highly sensitive when compared to the traditional virus isolation techniques.

Although only a small number of samples were processed, it is evident that, based on the viral copy number data, rearing phase vaccination offered protection for the mature oviduct of laying hens. Viral copy number was generally higher for T strain than for the two vaccine strains, suggesting that it may be possible to distinguish between vaccine and wild strains of IBV in tissues, based on quantitative levels. However, the analysis of further samples is required to establish this relationship.

The test was also able to detect the pathogenic strains of virus from faecal samples and vaccination reduced the virus shedding in the faeces.

A non-quantitative PCR has been developed for the strain N1/88 and the RT-PCR product has been successfully purified, cloned and sequenced. However, the real time PCR test for N1/88 requires further optimisation.