

Molecular Epidemiology of Avian Pathogenic Escherichia coli

Shaiful Islam[†], Kelly A. Tivendale, Marc S. Marenda, Philip F. Markham, Glenn F. Browning Asia-Pacific Centre for Animal Health, School of Veterinary Science, University of Melbourne, Parkville, Victoria 3010, Australia

Introduction

Avian pathogenic *Escherichia coli* (APEC) are responsible for respiratory tract disease and colisepticaemia in poultry, resulting in considerable economic losses

Intestinal tract is believed to be the main source of APEC, whereas faeces and dust preserve the bacteria for further spread (1)

Inhalation of contaminated dust is the most important route of entry, and hence the environment is a potential source of infection leading to outbreaks (2)

Air sampling and colony blot hybridisation were developed as a rapid diagnostic tool for detecting *E. coli* in the air of poultry sheds

Shed 2Shed 3Shed 4Shed 5Concentration of
total E. coli
(CFU/1000 L of air) $\int_{0}^{0} \int_{0}^{0} \int_$

Results

*Objectives

 \rightarrow to determine the concentration of total *E. coli* and of *E. coli* containing the APEC-specific virulence genes, *iroB*, *iucA* and *hlyF*, at different stages of broiler production and

 \rightarrow to compare the genotypes of *E. coli* isolated from the air of poultry sheds with that of *E. coli* isolated from lesions in birds

Materials and Methods

The M Air T air sampler (Milipore) was used to collect 750 L of air from each of four individual commercial broiler sheds (Little River, Victoria) housing approximately 42,000 to 49,000 chickens per shed at three different time points

Ten recently deceased birds were collected from each shed at each time point, examined at post-mortem and swabs were taken from the liver of each bird

The cultures grown from the air samples and liver swabs were screened by colony and array blot hybridisations using digoxigenin (DIG) labeled DNA probes for the presence of *iroB*, *iucA* and *hlyF*Southern blot hybridisation to *Eco*RV and *Bgl*I digests of genomic DNA of selected *E. coli* was performed to compare the genotypes of the *E. coli* populations recovered from the air and livers of dead birds



General linear model of ANOVA using Tukey's simultaneous test was conducted to analyse the data in Minitab 16 for Windows



M Air T air sampler (Milipore)



Study design in sheds 2 and 3



Study design in sheds 4 and 5



Lactose fermenting *E. coli* grown from the air samples



Chemiluminescent detection of *E. coli* isolates containing the *iroB* gene

Chemiluminescent detection of an array of *E. coli* isolates containing the *iroB* gene

Age of the birds (days)Age of the birds (days)

Distribution of *E. coli* genotypes



A total of 25 different genotypes were observed amongst 96 *E. coli* isolates from the air and from lesions of birds, with 7 common genotypes

С

air isolates

lesions isolates

Conclusions



Genotypic characterisation of *E. coli* isolates using RFLP and Southern blot hybridisation

✤The total concentration of *E. coli* (CFU/1000 L) increased significantly from days 2 and 3 to days 14 and 15 and decreased significantly at days 26 and 27 of age (*P*<0.05)</p>

In most cases, the concentration of *E. coli* positive for either the *iroB*, *iucA* or *hlyF* probes remained higher up to days 14 and 15 of age, and decreased by days 26 and 27

✤The proportion of *E. coli* positive for the *iroB* and *hlyF* genes remained high at days 2 and 3, whereas the proportion of *E. coli* positive for *iucA* or all three genes remained high either at days 2 and 3 or days 26 and 27

Southern blot hybridisation revealed that similar genotypes of *E. coli* were present in the air and the liver lesions in birds

Detection of higher concentration of airborne pathogenic *E. coli* early in the production cycle in broiler sheds could be used to guide the introduction of preventive measures to control colibacillosis in chickens

Acknowledgements & References

The project was partially funded by an International Postgraduate Research Scholarship (IPRS) and a Melbourne International Research Scholarships (MIRS)

^I Email: psaifuldvm@yahoo.com Phone: +61-433638043

(1) Timothy, S. *et al.* (2008), Avian Pathol **37**, 375-378
(2) Gross, W.G., 1994, Diseases due to Escherichia coli in poultry, In: Gyles, C.L. (Ed.) *Escherichia coli in domestic animals and humans.* Wallingford: CAB International, United Kingdom, pp. 237-259.