

## THE PREVALENCE OF AVIAN PATHOGENIC *Escherichia coli* (APEC) IN PENINSULAR MALAYSIA

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**ABSTRACT.** Avian colibacillosis is considered a major bacterial disease in the poultry industry world-wide and is also one of the most common avian diseases that is communicable to humans. A prevalence study on APEC was carried out in Peninsular Malaysia by Veterinary Research Institute for a period of 3 years (2007-2009). Organs, intestines, cloacal swabs and cultures received (predominantly from Perak poultry and duck farms) were cultured onto blood agar and MacConkey agar for the isolation and identification of *Escherichia coli* (*E.coli*), followed by serotyping to detect the pathogenic strains. This study was done to establish the incidence of positive infections in different type of chickens with the hope to identify the pathogenic strains potentially infecting consumers. APEC isolates commonly belong to certain serogroups such as 01, 02 and 078. A total of 3768 samples from 471 cases were screened, of which 178 isolates were APEC. A total of 141 (79%) of the isolates were *E. coli* 01:K1, 16 (9%) were *E. coli* 02:K1 and another 21 (12%) were *E. coli* 078:K80. Of the 141 *E. coli* 01:K1 isolates, 125 (89%) were from chickens and 16 (11%) from ducks. As for the *E. coli* 02:K1 isolates, 12 (75%) were from

chickens and 4 (25%) from ducks. For the *E. coli* 078:K80 isolates 18 (86%) were from chickens and 3 (14%) were from ducks. The use of a wider range of *E. coli* antisera in the laboratory is necessary to identify new pathogenic strains. Further to this, the use of DNA probes and PCR would be more reliable as another tool for identification of its virulence factors.

*Keywords:* poultry, colibacillosis, avian pathogenic *Escherichia coli*

### INTRODUCTION

Avian colibacillosis is a major infectious disease in birds of all ages. This disease has an important economic impact on poultry production worldwide. The majority of economic losses result from mortality and decrease in productivity of the affected birds (Otaki, 1995). *E. coli* is a gram-negative, non-acid-fast, uniform staining, non-spore-forming bacillus that grows both aerobically and anaerobically and may be variable in size and shape. *E. coli* is considered a member of the normal microflora of the poultry intestine, but certain strains, such as those designated as avian pathogenic *E. coli* (APEC), spread into various internal organs and cause

colibacillosis characterized by systemic fatal disease (Barnes *et al.*, 1997; La Ragione and Woodward 2002). *E. coli* isolates pathogenic for poultry commonly belong to certain serogroups, particularly the serogroups O78, O1, and O2, and to some extent O15 and O55 (Gross, 1994; Chart *et al.*, 2000). In domestic poultry, avian colibacillosis is frequently associated with *E. coli* strains of serotypes O78:K80, O1:K1 and O2:K1. Avian colibacillosis was found widely prevalent in all age groups of chickens (9.52 to 36.73%) with especially high prevalence rate in adult layer birds (36.73%) (Rahman, 2004).

APEC affect poultry either as a primary or secondary pathogen. It causes a variety of disease manifestations in poultry including yolk sac infection, omphalitis, respiratory tract infection, swollen head syndrome, septicemia, polyserositis, coligranuloma, enteritis, cellulitis and salpingitis. (Calnek *et al.*, 1997). Infectious viral and bacterial agents such as Infectious bursal disease,

Newcastle disease, Infectious bronchitis and mycoplasma together with nutritional deficiencies predispose birds to this disease. (Wray *et al.*, 2001).

**MATERIALS & METHODS**

A total of 471 cases from poultry and ducks comprising organs, intestines, cloacal swabs and cultures were sent to bacteriology unit of Veterinary Research Institute for isolation and identification of *E. coli* infections. Standard bacteriological procedures based on O.I.E. for processing and culturing were carried out to isolate *E. coli*. Specimens were cultured individually onto blood agar and MacConkey agar. Plates were then incubated aerobically at 37°C for 18-24 hours. Suspected colonies were subcultured to get pure colonies. Colonies should be greyish white on blood agar and lactose fermenters (rarely non-lactose fermenters) on MacConkey agar. Further identification of the suspected colonies is based on biochemical reactions

**TABLE 1.** APEC (2007-2009)

Serotype	01:K1					02:K1					078:K80			
	Breeder		Broiler		Pet	Breeder		Broiler		Layer	Breeder		Broiler	
	Chicken	Duck	Chicken	Duck	Bird	Chicken	Duck	Chicken	Duck	Chicken	Chicken	Duck	Chicken	Duck
2007	81	0	16	16	1	1	0	1	4	0	8	0	0	2
2008	12	0	1	0	0	4	0	1	0	1	5	0	0	1
2009	13	0	1	0	0	2	0	1	0	1	5	0	0	0
Sub total	106	0	18	16	1	7	0	3	4	2	18	0	0	3
Total	106		34		1	7		7		2	18		3	
Grand total	141					16					21			

(indol production, fermentation of glucose with gas production, presence of  $\beta$ -galactosidase, absence of hydrogen sulphite production and urease, and the inability to use citrate as a carbon source (Dho-Moulin and Fairbrother, 1999 ).

Upon confirmation as *E. coli*, 0-serotyping was used as the typing method to identify pathogenic strains. The *E. coli* isolate was cultured onto agar slant and incubated at 37°C for 18-24 hours. The next day 1 ml of saline was added to emulsify the bacteria on the surface of the blood agar. A drop of the *E. coli* suspension was placed onto a clean slide, followed by adding a drop of antiserum eg. *E. coli* 01:K1 to the suspension. The slide was rocked and observed for agglutination. If agglutination occurs then that strain is *E. coli* 01:K1. If no agglutination occurs, then the suspension will be further tested against other pathogenic strains such as *E. coli* 02:K1 and *E. coli* 078:K80. The pathogenic strains from avian are predominantly 01:K1, 02:K1, 078:K80.

## RESULTS

Of the 471 cases (3768 samples) received, 178 isolates were identified as APEC. APEC isolated were mainly from 3 main serotypes, that is, *E. coli* 01:K1, 02:K1 and 078:K80. From table 1, the most number of isolates were from *E. coli* 01:K1(141), followed by *E. coli* 078:K80 (21) and the least from *E. coli* 02:K1(16). During this 3 year period (2007-2009), all the 3 pathogenic strains were predominantly

from breeder chickens (106 were *E. coli* 01:K1, 18 were *E. coli* 078:K80 and 7 were *E. coli* 02:K1) as compared to broilers (chickens = 18, ducks = 16) that is, 34 isolates of *E. coli* 01:K1, 7 chicken isolates of *E. coli* 02:K1 and 3 duck isolates of *E. coli* 078:K80. The least number of isolates were from layer chickens having only 2 isolates as *E. coli* 02:K1

## DISCUSSION & CONCLUSION

The data above showed that APEC were frequently isolated from breeders, followed by broilers and least from layers. In breeder farms, infection of eggs occur during laying or during its formation in the oviduct, thus leading to embryo and early chick mortality (Dziva *et al.*, 2008). *E. coli* found in contaminated faeces is able to penetrate the egg shell and may also give rise to yolk sac infection (Lutful Kabir, 2010). To prevent egg contamination, eggs should be fumigated within two hours of lay. Cracked and soiled eggs should be removed.

In broiler farms, competitive exclusion, that is, by inoculating day-old chicks with normal flora from adult healthy chickens or a monoculture such as *Bacillus subtilis* (La Ragione *et al.*, 2001) should be used to control environmental APEC contamination. It is advisable to have a close house system whereby environmental parameters such as humidity, ventilation, temperature, dust can be controlled for optimal growth and well being of the poultry. Excess of ammonia or dust renders

the respiratory system more susceptible to APEC infection through deciliation of the upper respiratory tract (Barnes *et al.*, 1997). Good animal husbandry and avoiding overcrowding are very important in reducing the risk of colibacillosis. Disease introduction can be greatly reduced by having a suitable housing infrastructure and the correct use of transition zone (for changing clothes, shoes and washing hands) together with pest control.

For layer farms, cost benefit analysis should be carried out to determine optimal hen density in cages for maximal economic returns and minimal risk of disease outbreak.

Of public health importance, it had been shown that APEC strains can be easily transmitted to humans (Linton *et al.*, 1977). There is clonal relationship for 02:K1 isolates from human and chicken (Achtman *et al.*, 1986). It had been found that APEC share identical serotypes and specific virulence genes with human pathogen (Ewers *et al.*, 2004), their zoonotic potential is considered. (Manges *et al.*, 2007) demonstrated that antimicrobial resistant, urinary tract infection (UTI) causing by *E. coli*, could have been from a food reservoir, possibly originating from poultry meat or pork. APEC have the ability to spread to humans, to act as human uropathogenic *E. coli* (UPEC) or as a reservoir of virulence genes for UPEC (Rodriguez-Siek *et al.*, 2005). Being extraintestinal pathogenic *E. coli*, APEC are able to live extraintestinal life which may increase the human risk of infection from consumption of poultry

meat. Epidemiology reports suggest that poultry meat is still the primary cause of human food poisoning (Yashoda *et al.*, 2001), APEC is an emergent food pathogen with increasing zoonotic potential.

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