

DRINKING WATER QUALITY BY THE USE OF CONGO RED MEDIUM TO DIFFERENTIATE BETWEEN PATHOGENIC AND NON PATHOGENIC *E. coli* AT POULTRY FARMS

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ABSTRACT

Keeping in view the importance of drinking water and its effect on poultry performance, 86 water samples from 55 poultry farms were collected to determine the presence and pathogenicity of *Escherichia coli*. All samples were cultured on MacConkey's agar plates. The positive samples were examined for fecal coliform. Identification of *E. coli* was carried out on the basis of microscopic, cultural and biochemical characteristics. *In vitro* pathogenicity test for *E. coli* was conducted by using Congo red binding activity. Out of 86 water samples processed for coliform, 73 produced growth on MacConkey's agar. Indole test and biochemical reactions identified 26 of these as *E. coli*. Out of 26 isolates of *E. coli*, 20 resulted in the growth of brick red colored colonies indicative of pathogenic nature where as the remaining 6 produced grayish white colonies after 96 hours of growth and were considered as non pathogenic.

Key words: *Escherichia coli*, drinking water, coliform, Congo Red, Pathogenicity

INTRODUCTION

Water a vital nutrient in poultry metabolism which plays an important role in the digestion, absorption of food, transportation of nutrients in the body and elimination of waste products via urine. Physical, Chemical and Microbiological qualities of drinking water have a fundamental importance in poultry industry (Jafari *et al.*, 2006).

The role of water in spreading communicable diseases is much evident due to combined source of water i.e. drinkers. Contaminated water with faecal coliform severely affects the performance of birds. The sources of water contamination are birds by faeces and secretions of sick birds, animals and the man (Desmarais *et al.*, 2002). *Salmonellae*, *Compylobacter* spp. and *Escherichia coli* are the main poultry pathogens responsible for water contamination (He *et al.*, 2007). Water quality used for poultry production and health is one of the most significant segments in health management

E. coli is a gram-negative bacterium of the family Enterobacteriaceae and is a normal inhabitant of intestinal tract of birds (Singleton and Sainburg, 1981). It is one of the opportunist pathogen responsible for number of disease conditions such as yolk sac infection, air sac disease, perihepatitis, enteritis, omphalitis, coligranuloma, colibacillosis etc. (Rosenberger *et al.*, 1985).

The present study was conducted to check the presence and pathogenicity of *E. coli* in drinking water at various poultry farms.

MATERIALS AND METHODS

A total of 86 water samples from 55 poultry farms were investigated for the presence of *E. coli*. Out of these, 51 samples collected directly from the water source (wells) and 35 from the drinkers placed in poultry shed. The drinking water was not treated with any disinfectants or antibiotics. A water sample of 20 ml was collected directly in sterile screw capped tubes after running the tap for about half a minute.

Each sample was mixed and an aliquot of 200 µl was cultured on MacConkey's agar (Oxoid, England). Plates were incubated at 37°C under aerobic condition for 24 hours. Numbers of colony forming units produced by lactose fermenting organisms were counted. Further identification of *E. coli* was carried out on the basis of Gram's staining reaction, indole test and biochemical reactions on triple sugar iron medium (Ewing, 1986). Positive samples were further investigated by *in vitro* pathogenicity test using Congo red binding activity as described by Berkhoff and Vinal (1985). Results of Congo red binding were recorded after 24 hours of incubation at 37° C and then after 48 hours of incubation at room temperature. Organisms, which failed to bind the dye after 96 hours of incubation at room temperature and produced white colored colonies, were recorded as non-pathogenic *E. coli* (Hofstra and Veld., 1988).

RESULTS AND DISCUSSION

Out of 86 water samples processed for Coliform count, 73 produced growth on MacConkey's agar. Out of these, 27 samples were identified as Coliform (Table-I). However, one of these failed to fulfill the criteria used for identification of *E. coli*.

Table-I Grouping of Samples on the basis of viable Counts

Coliform count (CFU/ml)	No. of total samples	No. of lactose fermenting Colonies
5-50	28	10
51-100	13	3
101-250	12	6
> 250	20	8
Total	73	27

On the basis of Indole test and biochemical reaction, 26 isolates were identified as *E. coli*. All the 26 isolates produced luxuriant growth on Congo red medium. Out of 26 isolates 20 resulted in the growth of brick red colored colonies while the remaining 6 produced grayish white colonies after 96 hours of growth.

The waste material such as animal faecal material, vegetable matter, and sewage water are main sources of *E. coli* and other Infections. The introduction of pathogenic Coliform in poultry farms has been attributed to various factors. Present study was aimed at the isolation of *E. coli* from water sources and poultry drinkers on different poultry farms.

Sri Purnomo *et al.* (1992) reported different strains of *E. coli* in different samples e.g. diseased bird, feed, drinking water and litter. Feed is often contaminated with pathogenic Coliform, but hot pelleting processes can destroy these. Rodent droppings frequently contain pathogenic Coliform. Pathogenic serotypes can also be introduced into poultry flocks through contaminated well water (He *et al.*, 2007) and its presence in drinking water is considered indicative of fecal contamination. These bacteria persist for long periods, particularly under dry conditions. During rain, these substances get mixed with water and by flooding; these substances are discharged into shallow water wells (Ortega *et al.*, 2009).

The maximum acceptable level for microorganism in drinking water of birds should be 100 CFU/ml for total bacteria and 50 CFU/ml for coliform (Jafari *et al.* 2006). The results showed in Table-1 revealed that 31.40% of the total 86 water samples were contaminated with coliform. Out of these 26 (30.24%) were identified as *E. coli*. Although it is not so towering count as compared to the findings of He *et al.* (2007), who assessed the water sources in rural areas and showed that 90% of the water samples from wells and 100% of the samples originating from springs had bacteria

indicative of faecal pollution. Goan *et al.* (1992) examined water samples collected from 105 wells of 65 flocks in the United States, and reported that faecal coliform were present in 43% of the samples. Jafari *et al.* (2006) reported the faecal coliform in water samples from 20 farms (50%) were over the maximum acceptable level.

Avian Pathogenic *Escherichia coli* isolated from poultry is pathogenic only for birds and represent a low risk of disease for people or other animals (Caya *et al.* 1999). However, chickens are susceptible to colonization with *E. coli* O157:H7, an important Shiga-producing, enterohemorrhagic pathogen of humans, and a low occurrence of natural infection has found in both chickens and turkeys in different geographic areas (Guo *et al.* 1998 and Pilipcinec *et al.* 1999).

During the course of work, the incidence of other bacteria such as Salmonella, Edwardsiella spp., Citrobacter and Aeromonas was also reported. Since the present study was focusing only on the prevalence of *E. coli*, therefore characterization of other coliform was ignored. Mukherjee *et al.* (1994) also found concurrent infection with *E. coli*, Newcastle disease virus and infectious bursal disease virus in 24 broiler flocks.

Out of 26 samples 77% bind the Congo red dye and were grouped as pathogenic *E. coli*. It is in accordance with the findings of many scientists who advocated the use of Congo red dye with the objective of distinguishing between pathogenic and non-pathogenic microorganisms (Berkhoff and Vinal., 1985; Stebbins *et al.* 1992). In contrast to these finding many other scientist reported that the pathogenic and nonpathogenic isolates of *E. coli* are similar in biochemical characteristics and drug sensitivities (Cloud *et al.* 1985; Spears *et al.* 1992)

It is concluded that the presence of faecal coliform has great significance in farm hygiene and management. Greater the faecal pollution higher is the chance of pathogenic *E. coli*. Moreover, isolation of other Enterobacteriaceae is of less significance as they are considered to be introduced mainly from local soil and vegetation (Elmir *et al.*, 2007). It was further observed that a low-level infection of pathogenic *E. coli* is present in environment.

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