An Investigation into *Salmonella* and Fecal Coliform Contamination of Drinking Water in Broiler Farms in Iran

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**Abstract:** In view of the effect of water quality on poultry performance, from May to July 2005, the drinking water (source water, pipe water and drinker water) from 40 broiler farms in the rural area of Ahvaz (a city in the southwestern Iran) was sampled to determine the extent of *Salmonella* & coliform contamination. For *Salmonella* isolation, 25 ml of water was cultured in 225 ml of lactose broth at 37°C. After 24 hr, the samples were at first subcultured in TT & SC broths (1:9), and then plated on both BG & SS agars. After purified on blood agar, the suspected colonies were confirmed by usual biochemical & serological tests. Coliform count was carried out as per MPN method. The positive samples were examined for fecal coliform. Coliform counts in 20 farms (50%) were over the max. acceptable level (50 CFU/ml) for poultry water. Fecal coliform was detected in the water of all farms. From the drinker water of 5 farms, *Salmonella* serotypes Nienstedten, Tinda, Calvinia, Oysterbeds and Sterrenbos were isolated. This is the first report of the isolated serotypes in poultry in Iran.

**Key words:** *Salmonella*, coliform, broiler, water

**Introduction**
Water, in addition to being a vital nutrient, is involved in many aspects of poultry metabolism. It plays important roles in the digestion and absorption of food, the transport of nutrients in the body, and the elimination of waste products via the urine. It also has a high specific heat and heat of vaporization which means that large amounts of heat can be transported in the blood and dissipated through respiration. In the poultry industry, the use of water with adequate physical, chemical and microbiological qualities is of fundamental importance. Since many birds have access to the same water source, quality problems will affect a great number of animals. The drinking water plays an important role in the transmission of many pathogenic agents among poultry. Diseases that can be transmitted to the bird flock through the drinking water may originate from water contamination by feces and secretions of sick birds in the same flock or from the utilization of water already contaminated by pathogenic organisms originating from other animal species and the man. There have been many reports about water contamination with the main poultry pathogens such as *Salmonella* spp., *Campylobacter* spp. and *Escherichia coli*. Amaral et al. (1999) and Amaral et al. (2001) reported that the samples from the water sources and reservoirs were contaminated by *E. coli* in 10 broiler and laying hen farms evidencing fecal pollution of the samples. Goan et al. (1992) assessed water samples from 105 wells of 65 flocks in the United States, and reported that fecal coliforms were present in 43% of the samples, whereas *Salmonellae* were present in 7.9%. Also *Salmonellae* were isolated from 21.6% of the broiler farms, and from 12.3% of the water samples examined in Canada (Poppe et al., 1991). Kapperud et al. (1993) reported a risk 3.5 higher of birds being infected by *Campylobacters*, when the drinking water was not disinfected with chloride. Furthermore, *Campylobacter jejuni* was isolated from the biofilm present in the nipple supplying pipes when the birds were infected, but no micro-organism was isolated when the birds were not colonized (Zimmer et al., 2003). Therefore, the present work was aimed to determine the extent of *Salmonella* and coliform contamination in the drinking water in some broiler farms.

**Materials and Methods**
From May to July 2005, water samples from 40 broiler farms in the rural area of Ahvaz (a city in the southwestern Iran) were investigated for *Salmonella* and coliform contamination. In each farm, the samples were collected from the water source, pipe water and drinker water. The drinker water was assessed only for *Salmonella* contamination. The water sources included superficial canals (29), streams (3) and wells (8). The wells were shallow (5-8 meters deep) and placed at a distance of 10-40 meters from the streams adjacent to the farms. The pipe water was sampled after being allowed to run for several minutes, and after flaming the outlet. The drinking water was not treated with any disinfectants or antibiotics. The samples were placed in sterilized flasks, shipped to the laboratory and tested within a maximum of 8 hours. For *Salmonella* isolation, 25 ml of water was cultured in 225 ml of lactose broth (Hi-Media, India) at 37°C. After 24 hr, the samples were at first subcultured in tetraphionate (Merck, Germany) and...
selenite cysteine (Oxoid, England) broths (1:9 ratio), and then plated on both brilliant green (Hi - Media, India) and salmonella - shigella (Merck, Germany) agars. To inhibit the growth of Proteus species, novobiocin (Hi - Media, India) was added to BG and SS agars, and to TT broth at a rate of 20 μg/ml and 40 μg/ml respectively. After purified on blood agar, the suspected colonies were confirmed by biochemical and serological tests as recommended by Douglas Waltman et al. (1998). Coliform count was carried out by using five different dilutions, each with 3 replicates, as per MPN method [International standard (ISO, 9308), 1990]. Then the positive samples were examined for fecal coliform contamination.

Results and Discussion

Schwartz (1984) and Waggoner et al. (1984) (cited by Carter and Sneed, 1986) and Reddy et al. (1995) considered that the number of microorganisms in the drinking water of birds should be 100 CFU/ml for total bacteria and 50 CFU/ml for coliforms. The results (Table 1) showed that the coliform counts in 20 farms (50%) were over the maximum acceptable level. The numbers were at a range of 50-500 CFU/ml in 9 cases, and over 500 CFU/ml in the rest. There was not a remarkable difference in coliform count between the pipe water and the source water. Fecal coliform was also detected in all samples from the water sources, indicating the occurrence of fecal pollution that could be due to free access of wild and domestic animals to the superficial water sources, disposal of animal excreta and dead carcasses, and even the drainage of human's sewage from the rural villages. Although the superficial water sources are more subjected to fecal contamination, the underground water is also susceptible to this type of pollution (Amaral, 2004). In the present study, all of the wells were contaminated, with a less severity, with coliforms. This problem could be due to the drainage from the contaminated streams adjacent to the farms, since the wells were shallow and didn’t placed at a proper distance from the streams. Amaral et al. (1995) assessed the water sources in rural areas and showed that 90% of the water samples from wells and 100% of the samples originated from springs had bacteria indicative of fecal pollution. As well, Goan et al. (1992) examined water samples collected from 105 wells of 85 flocks in the United States, and reported that fecal coliforms were present in 43% of the samples. Although we couldn’t isolate any Salmonellae from the samples tested, one of the main consequences of fecal pollution of drinking water is to increase the risk of infection to enteric pathogens. Geldreich (1998) verified that when the levels of fecal coliforms in the water were $10^1$, $10^2$, $10^3$, $10^4$, and 10, the rates of Salmonella isolation were 100%, 99%, 66%, 33%, 21% and 11% respectively. In the present study, the drinker water samples from five farms (12.5%) were only found to be contaminated with

| Table 1: Coliform count status of different water sources in broiler farms |
|-----------------|-----------------|-----------------|-----------------|
| Coliform count (CFU/ml) | Water sources | Coliform count | Water sources |
| (Superficial canal) | (Stream) | (Well) | (Total No.) |
| <50 | 14 | - | 6 | 20 |
| 50-500 | 7 | 1 | 1 | 9 |
| >500 | 8 | 2 | 1 | 11 |
| Total | 29 | 3 | 8 | 40 |

Salmonellae. The isolates were identified as serotypes Nienstedten, Tinda, California, Oysterbeds and Sterrenbos, each from one farm. The type of drinker is an important factor that interferes in the quality of the water that is provided to the birds. In all farms examined, the drinkers were of bell type. So, the isolation of the Salmonellae from the drinkers could be due to chicks’ feces contamination. Renwick et al. (1992) suggested that the risk of contamination with Salmonellae was 6-7 times higher, when the water given to birds was exposed to the environment. Besides, Morgan-Jones (1960) found that more water samples were positive to Salmonellae in a broiler facility, when water was provided in troughs. Salmonellae were also isolated from 21.6% of the broiler farms, and from 12.3% of the water samples examined in Canada by Poppe et al. (1991). The use of open drinkers in the majority of the farms was favorable to contamination, and the presence of Salmonellae in the litter was considered an important contamination route of the water provided to the birds. Furthermore, Kirk et al. (2002) isolated S. serotypes Typhimurium and Meleagridis from the water of troughs used by weaned dairy calves in California. Whereas, Barros et al. (2001) analyzed 72 drinking water samples of broiler chickens (18 from the entrance & 54 from the bell-shaped drinkers), and reported a high bacteriological contamination on the first week without any isolation of Salmonella strain, probably because of no existence of infected poultry in the band. The isolated Salmonella serotypes have been rarely reported from poultry and human. Salmonella serotype Nienstedten was obtained from a turkey in 1975 (Simmon, 1976), from the feces of an apparently healthy pet, Iguana mexicana, in a public aquarium in British Columbia (Simmon, 1976), and from chickens in Alberta between 1990 and 2001 (Guerin et al., 2005). Furlani et al. (1991) reported S. serotype Nienstedten in a human outbreak in Ontario. It was also recorded in the Salmonella data set (PHLS, 1994; PHLS, 1999) among the rare serotypes that caused food-borne illness in human in England and Wales. Walt et al. (1997) identified 92 different Salmonella serotypes amongst 173 isolates obtained from crocodiles and other reptiles in South Africa between 1985 and 1994, and reported that S. serotype Tinda was one of the most frequent isolates (6). Tadjbakch et al. (1994) examined lymph node, bile and fecal samples collected from 500 sheep and 113 dromedaries in Iran, and could isolate S. serotype Sterrenbos from the feces of only one.
dromedary. But the isolated serotypes have not been yet reported in poultry in Iran.

The results showed a high bacteriological contamination of the drinking water. So, the usage of more hygienic drinkers and or continuous water chlorination along with weekly monitoring of chlorine residue are recommended, aiming to obtain a satisfactory potable water.

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References


