

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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

R.A. Jafari¹, A. Fazlara² and M. Govahi

¹Department of Clinical Science, ²Department of Food Hygiene,
Faculty of Veterinary Medicine, Shahid Chamran University, P.O.Box: 61355-145, Ahvaz, Iran

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 7.6%. 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 (6-8 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 tetrathionate (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, 1996) 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 65 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 10^6 , 10^5 , 10^4 , 10^3 , 10^2 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			Total No.
	Superficial canal	Stream	Well	
<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, Calvinia, 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 (1980) 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). Tadjbakhch *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.

Acknowledgements

The authors are grateful to the research vice-chancellor of Shahid Chamran University for the facilities provided to do the work, and to Miss M. Taslimi for typing the manuscript.

References

- Amaral, L.A., 2004. Drinking water as a risk factor to poultry health. *Braz. J. Poult. Sci.*, 6: 191-199.
- Amaral, L.A., A. Nader Filho, O.D. Rossi Jr. and L.H.C. Penha, 1995. Características microbiológicas da água utilizada no processo de obtenção do leite. *Pesq. Vet. Bras.*, 15: 85-88.
- Amaral, L.A., O.D. Rossi Junior and V. Cardoso, 1999. Qualidade higiênico-sanitária da água de bebedouros pendular e nipple utilizados na criação de frangos de corte. *Rev. Bras. Cienc. Avic.*, 1: 145-148.
- Amaral, L.A., A. Nader Filho, H. Isa and L.S.S. Barros, 2001. Qualidade higiênico-sanitária e demanda de cloro da água de dessedentação de galinhas de postura coletadas em bebedouros tipo nipple e taca. *Rev. Bras. Cienc. Avic.*, 3: 193-198.
- Barros, L.S.S., L.A. Amaral and O.D. Rossi Jr., 2001. Aspecto microbiológicos e demanda de cloro de amostras de água de dessedentação de frangos de corte coletadas em bebedouros pendulares. *Rev. Bras. Cienc. Avic.*, 3: 193-198.
- Carter, T.A. and R.E. Sneed, 1996. Drinking water quality for poultry. North Carolina Cooperative Extension Service, Available at: <http://www.bae.ncsu.edu/programs/extension/evans/PS&T-42.html>.
- Douglas Waltman, W., R.K. Gast and E.T. Mallinson, 1998. Salmonellosis. In: Swayne, D.E., Glisson, J.R., Jackwood, M.W., Pearson, J.E., Reed, W.M (Eds.), *A Laboratory Manual for the Isolation and Identification of Avian Pathogens*. The American Association of Avian Pathologists, Pennsylvania, pp: 4-13.
- Furlani, R.J., J.M. Watt and M. Corriveau, 1991. *Salmonella nienstedten* outbreak – Ontario. *Can. Dis. Wkly Rep.*, 17: 59-60.
- Geldreich, E.E., 1998. The bacteriology of water. In: Colier, L., Balows, A. and Sussman, M. (Eds.), *Microbiology and Microbial Infections*. 9th ed. Arnold Publication, London.
- Goan, H.C., T.N. Burcham, P.H. Denton and F.A. Draughon, 1992. Quality of well water on Tennessee poultry farms. *Poult. Sci.*, 71: 103.
- Guerin, M.T., S. Swayne Martin, G.A. Darlington and A. Rajic, 2005. A temporal study of *Salmonella* serotypes in animals in Alberta between 1990 and 2001. *Can. J. Vet. Res.*, 69: 88-99.
- International Standard (ISO 9308), 1990. Water quality detection and enumeration of coliform thermotolerant coliform and presumptive *Escherichia coli*.
- Kapperud, G., E. Skjerve, L. Vik, K. Hauge, A. Lysaker, I. Aalmen, S.M. Ostroff and M. Potter, 1993. Epidemiological investigation of risk factors for *Campylobacter* colonization in Norwegian broiler flocks. *Epidemiol. Infect.*, 111: 245-255.
- Kirk, J., R. Atwill, C. Holmberg, M. Arana, C. Collar, D. Ghirardelli, G. Higginbotham, G. Markagaard, D. Mullinax and A. Wubishet, 2002. Prevalence of and risk factors for *Salmonella* in water offered to weaned dairy calves in California, USA. Available at: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd>.
- Morgan-Jones, R.J., 1980. The occurrence of salmonellae during the rearing of broiler chicks. *Br. Poult. Sci.*, 21: 463-470.
- Poppe, C., R.J. Irwin, S. Messier, G.G. Finley and J. Oggel, 1991. The prevalence of *Salmonella enteritidis* and other *Salmonella* spp. among Canadian registered commercial broiler chicken flocks. *Epidemiol. Infect.*, 107: 201-211.
- Public Health Laboratory Service (PHLS), 1994. *Salmonella* in humans. *Communicable Disease Report (CDR)*, 4: 143-146.
- Public Health Laboratory Service (PHLS), 1999. Outbreaks of food-borne illness in human. *Communicable Disease Report (CDR)*, 9: 217-218.
- Reddy, M.R., M.V.L.N. Raju, M.M. Chawak and S.V. Rama Rao, 1995. Importance of water in poultry health. *Poult. Adv.*, 28: 31-37.
- Renwick, A.S., R.J. Irwin, R.C. Clarke, W.B. McNab and C. Poppe, 1992. Epidemiological association between characteristics of registered broiler chicken flocks in Canada and the *Salmonella* culture status of floor litter and drinking water. *Can. Vet. J.*, 33: 449-458.
- Simmon, P.C., 1976. Isolation of a rare serotype of *Salmonella* (*S. nienstedten*). *Can. Vet. J.*, 17: 323.
- Tadjbakhch, H., G.H. Baba-Ali and M. Haghigat, 1994. Incidence of *Salmonella* carriers among sheep and dromedaries in Iran. *Arch. Fac. Vet. Med., Univ. Tehran*, 6: 455-468.
- Walt, M.L., F.W. Huchzermeyer and H.C. Steyn, 1997. *Salmonella* isolated from crocodiles and other reptiles during the period 1985-1994 in South Africa. *Onderstepoort J. Vet. Res.*, 64: 277-283.
- Zimmer, M., H. Barnhart, U. Idris and M.D. Lee, 2003. Detection of *Campylobacter jejuni* in the waterlines of a commercial broiler and their relationship to the strains that colonized the chickens. *Avian Dis.*, 47: 101-107.