Jordanian Journal of Engineering and Chemical Industries
Print ISSN: 2616 - 9584 Online ISSN: 2617 - 720X

Water as Source of Salmonella Species Contamination in Jordanian Broiler Farms

Salam Alramini*[1)], Ziad Shraideh[2)], Muna Hindiyeh[3)], Hamzah Alqaderi[4)]

[1)] *Ministry of Agriculture, Manager of the training and farmer awareness department P. O. Box 2099, Amman 11181, Jordan.*
[2)] *Department of Biological Sciences, School of Science, The University of Jordan, Amman 11942, Jordan.*
[3)] *Department of Environmental and Water Engineering and its Management, German Jordanian University.*
[4)] *Department of Nutrition and Food Technology, School of Agriculture, The University of Jordan, Amman 11942, Jordan.*

Abstract

Broiler chickens are a major meat product worldwide. Maintaining the health of flocks is essential for poultry farming and it is critical to minimize exposure to waterborne pathogens. This study determined the extent of *Salmonella spp.* infections and identified sources of waterborne disease transmission at representative Jordanian broiler farms. We investigated 10 broiler farms located in five Jordanian Governorates; Amman, Irbid, Karak, Zarqa, and Madaba. Cloacal swabs were collected from chickens and water samples were collected from farm tanks, broiler house tanks, and drinking apparatus (drinkers) over three rearing cycles. Water was tested for pH and residual free chlorine. *Salmonella spp*. was isolated from the cloacal and water samples using biochemical methods and confirmed as *Salmonella enterica* serovar Enteritidis by PCR. *Salmonella enterica* serovar Enteritidis was detected at sampled farms at different percentages and the pathogen was found in 16.6% (15/90) of drinkers and 20% (30/150) of cloacal samples. The total and free residual chlorine concentrations in the water were lower than the recommended levels. The average pH was within the recommended values. This study concluded that *S*. Enteritidis is prevalent in broiler farms in Jordan. Thus, improved hygienic practices at broiler farms and the establishment of national water quality guidelines are necessary to decrease *S*. Enteritidis transmission in boiler farmers and thus enhance food safety in Jordan.

Paper type: Research paper

Keywords: *Salmonella enterica* serovar Enteritidis, water, contamination, broiler, Jordan.

Citation: Alramini, S., Shraideh Z., Hindiyeh M., and H., Alqaderi "Water as Source of Salmonella Species Contamination in Jordanian Broiler Farms", *Jordanian Journal of Engineering and Chemical Industries*, Vol. 5, No.2, pp: 24-31, (2022).

Introduction

The incidence of human illness associated with food contaminated with *Salmonella enterica* serovar Enteritidis had increased, which has been associated with pandemics in Europe, Canada and elsewhere (CDC, 2010; Vandeplas, *et al.*, 2010). Previous reports indicated that up to 3.7 million cases of salmonellosis in poultry farms occur in the United States of America (USA) every year, with economic losses to poultry farmers in the USA up to US$114 million annually (Omwandho and Kubota, 2010).

Salmonella enterica is the representative pathogen causing salmonellosis in humans and animals worldwide and is sub-classified into more than 2500 serovars. *Salmonella* serovars Enteritidis and Typhimurium are the most important agents of food-borne salmonellosis in humans (Popoff *et al*., 2003). According to Böhm, (2000) poultry drinking water should be free of *Salmonella spp.* and *Campylobacter* spp./100 ml, and free of *Escherichia coli*/10 ml. *Salmonella enterica* can also be transmitted in the flock through shared water supplies that are contaminated by faeces and secretions of sick birds (Jafari, *et al.*, 2006). The sources of *Salmonella enterica* may include human and animal faecal matter (Al-Gharabat, 2002). Water quality in poultry farm drinking water systems plays a significant role in the health and welfare of broiler chickens (Maes *et al*., 2019). The value of a clean, safe water supply is often ignored in poultry production. However, water is essential for poultry health (Zimmermann, *et al.*, 1993) and high-quality water is of fundamental importance to the poultry industry (Jafari, *et al.*, 2006; Barros, *et al.*, 2001). Broiler chickens consume 1.5 to 2 times as much water as feed, with a daily average of 18–23 litres/100 birds (Blake and Hess, 2001). It has been suggested that variation in water contents will influence broiler performance more than feed contents (Abbas, *et al.*, 2008). Additionally, the role of water in spreading pathogens is significant, as it is supplied via shared sources, i.e., drinkers.

* Corresponding author: E-mail: salamraminy@yahoo.com ORCID: https://orcid.org/0000-0002-6882-9814.
Received on December 25, 2021. Accepted on April 5, 2022.
Jordanian Journal of Engineering and Chemical Industries (JJECI), Vol.5, No.2, 2022, pp. 24-31. Revised: on April 1, 2022.

Contaminated water greatly affects the performance of broiler chickens and is a factor associated with body weight and feed conversion. Hence guidelines for poultry drinking water have been adopted in many countries, including USA and Canada (Carter and Sneed, 2007; Fairchild and Ritz, 2009). In Jordan, the poultry industry is the most developed animal production sector, with an estimated value of about one billion Jordanian Dinars (55.8 % of the total production of the livestock sector) (MOA, 2020). This has led to recent improvements in the sanitary environment of large-scale production farms. The drinking water supply on poultry farms is unregulated in Jordan, and this threatens both animal health, human health and industrial profits. This study aimed to determine the prevalence and distribution of *Salmonella* Enteritidis in drinking water in Jordan poultry farms. We tested 10 broiler farms in five governorates in Jordan for three production cycles. These results will lead to a better understanding of *S.* Enteritidis contamination in poultry farm water supplies and inform future efforts to improve water quality, limit disease transmission between birds and farm workers, improve the quality and quantity of poultry production, and decrease *S.* Enteritidis foodborne disease. This is the first study that focused on *S.* Enteritids contamination of poultry farm water supplies in Jordan.

1 Materials and Methods

1.1 Field study and sampling

This study was conducted on broiler chicken farms in five governorates in Jordan: Amman, Irbid, Madaba, Zarqa and Karak. Sampling was performed as follows. Two different representative broiler farms were included from each governorate. Each farm was sampled for three rearing cycles. The three rearing cycles were during the summer, autumn and winter seasons. Water samples were taken (1 sample per farm) from the main farm tank, broiler house tank, and drinkers that are provided for the flocks inside the farming house. Cloacal swabs from five live broiler chickens (3-8 weeks in age) were collected randomly from each farm during each of the three cycles. The bird was gently placed upright on the ground, and the swab was broken off into a bijou of sterile saline 0.85% NaCl (OIE, 2009). Swabs were collected and transported to the laboratory-using icebox and tested within two hours after collection.

1.2 Questionnaire and data collection

Detailed surveys regarding hygienic conditions, water resources, and other related indicators of the investigated farms were performed using the questionnaire. The questionnaire was modified to include the broiler farms name, address, farm location, flock age, flock size, number of houses on each farm and used bedding, type of drinkers (nipple, long trough or round trough), biosecurity used and other information related to sanitation and hygienic farm management. The total chlorine and free residual chlorine were measured in the field, using the DPD (N, N-diethyl-p-phenylene-diamine) method as recommended by American Public Health Association (APHA; 2005). Water samples were immediately transferred into an icebox to hold samples at 2–6 ºC, and were transported within a maximum of three hours to the laboratory (APHA, 2005). The pH value was measured using a pH meter based on the standard method 4500-HB (APHA, 2005).

1.3 Laboratory investigation

1.3.1 *Salmonella* detection in water and cloacal samples

Sampling and transportation of broiler drinking water samples for microbiological examination were performed according to American Public Health Association (APHA, 2005). The selective enrichment medium was Muller-Kauffmann tetrathionate novobiocin broth (MKTTn broth base) (Oxoid Ltd. UK) and Rappaport-Vassiliadis (RV) enrichment broth (Oxoid Ltd. UK) according to (ISO/DIS 19250) and (ISO 6579:2002) (ISO, 2007). The incubated MKTTn and RVS broths were subcultured into two selective solid media: xylose lysine deoxycholate (XLD) agar (Oxoid Ltd. UK) and brilliant green (BG) agar (Oxoid Ltd. UK). They were incubated at 37±1°C and examined after 24 ±3h. Combined use of triple sugar iron **(**TSI**)** agar (Oxoid Ltd. UK) and lysine iron agar (LI**)** (Oxoid Ltd. UK) were differential media for *Salmonella* spp. identification. To detect the presence of oxidase enzymes, ready-to-use oxidase strips were used (Oxoid Ltd. UK). All isolates were confirmed by 24E MICROBACT™ and latex polyclonal agglutination tests (Oxoid) which were used to confirm *Salmonella* spp. *identity* according to the manufacturer's specifications. Then, 4-5 colonies were resuspended in normal saline and tested by slide agglutination method with *Salmonella* O Polyvalent antisera (Group A–S; Remel, UK) and *Salmonella* H polyvalent Phases 1 and 2 antisera (Remel, UK). The presence of positive agglutination was observed by holding slides against the light.

1.3.2 Identification of *S. enterica* serovar Enteritidis by PCR

PCR was performed to confirm the identification of the *sefA* gene for *S. enterica* serovar Enteritidis of Salmonella spp. isolates according to (Pan and Lui, 2002). The detection of *sef*A gene of all isolated strain by using SEFA2 and SEFA4 primers to amplify a specific fragment of 310 bp on the DNA extracted. PCR for the *sef*A gene was performed on all isolated strains using SEFA2 (5'-GCAGCGGTTACTATTGCAGC-3') and SEFA4 (5'-TGTGACAGGGACATTTAGCG-3') primers (Alpha DNA, Montreal, Quebec) to amplify a specific 310 bp fragment from extracted DNA (Cooper, and Thorns, 1994; Ogunniyi**,** *et al*., 1997; Oliveira, *et al*., 2002; Madadgar, *et al*., 2008).

1.3.3 Detection of amplified PCR products by Electrophoresis

A 2% agarose TBE gel was prepared (100 ml), boiled, and 3 µl ethidium bromide added (10mg/ ml(, Promega, USA before polymerization following electrophoresis, the PCR products were visualized with a UV transilluminator and photographed with a gel documentation system (Gel Doc 2000, BIO-RAD, USA).

1.4 Statistical analysis

The results were analyzed statistically with Statistical Analysis System (version 9.2) (SAS, 2009) package. All data were presented as means (±) standard error of the mean (SEM). One-way analysis of variance (ANOVA) and student *t*-test for independent samples was used to analyze the differences between the means of the samples. The differences were considered significant at (P<0.05) was estimated for interaction between different parameters.

2 Results and Discussion

To determine the extent of Pearson's correlation coefficient contamination and infection in broiler farms, we obtained a total of 90 water samples (one sample each from the main water tank, broiler house tank, and drinker from each of 10 farms over 3 rearing cycles) and 150 samples from broiler chickens (5 birds from each of 10 farms over 3 rearing cycles).

2.1 Physicochemical quality of broiler drinking water
2.1.2Total and free residual chlorine

The total chlorine concentration in the main farm tank water sources (n=30) for all farms ranged from 0 to 0.5ppm (**Table 1**) which was lower than that of the recommendation level (2-4ppm) in broiler drinking water (WHO, 2008; Amaral, 2004). Nevertheless, the broiler drinking water in Amman and Irbid broiler farms had the highest level of total chlorine in the main water sources and ranged from 0.20 to 0.50ppm. The statistical analysis confirmed that the total and free residual chlorine in the main water sources were significantly different in the five Governorates (P<0.05) (**Table 1**). There were no significant differences (P> 0.05) in the three rearing cycles regarding the means of total chlorine concentration in the main farm tanks in all Governorates. However, there were significant differences (P<0.05) in the mean of free residual chlorine concentration in the three rearing cycles (**Table 2**). The mean ± SEM of the free residual chlorine of all main farm tank water samples were 0.12±0.03 ppm and ranged from (0.0 to 0.3) ppm (Table 1). The means of free residual chlorine showed a lower concentration than that of recommended level (0.2 to 2.0ppm).

2.2 pH

In Table 2, the results showed that the mean pH in all drinking water samples from the main farm tanks was within the recommended range (6.0-8.0). The means ± SEM of pH of main water sources samples in the three cycles ranged from 7.60±0.11 (6.10 to 8.63) (Table 2).

2.3 Temperature

The typical water temperature for broilers is 21.0ºC as recommended by Fairchild and Ritz, (2009). During the summer 1st cycle, the mean and range of drinking water temperature of the main water source was 27.1±0.55°C (25.0 to 30.0°C). The mean and range of water temperature in the autumn 2nd cycle was 22.3±1.39°C, (16.0° to 25.0°C). Whereas in the winter 3rd rearing cycle

the means ± SEM and ranges for the temperature of the main farm tanks were 14.1±0.52 ºC (12.0 to 16.0°C) were significantly different (P<0.05) (Table 2).

Table 1. Main farm tank chlorine concentrations and pH (by Governorates).

Governorates (number of samples)	Total chlorine (ppm) mean ± SEM (range)	The free residual chlorine (ppm) mean ± SEM (range)	pH
Amman(6)	0.33±0.06 (0.20-0.50) [a]	0.19±0.04 (0.05-0.30) [a]	7.72±0.19 (6.86-8.26)
Madaba(6)	0.18±0.02 (0.10-0.20) [bc]	0.09±0.01 (0.05-0.10) [bc]	7.85±0.10 (7.50-8.12)
Karak(6)	0.06±0.05 (0.00-0.30) [c]	0.04±0.03 (0.00-0.20) [c]	7.30±0.37 (6.08-8.08)
Zarqa(6)	0.18±0.07 (0.00-0.50) [bc]	0.08±0.04 (0.00-0.30) [bc]	7.64±0.16 (7.05-7.95)
Irbid(6)	0.28±0.03 (0.20-0.40) [ab]	0.13±0.02 (0.00-0.20) [ab]	7.72±0.28 (6.84-8.63)

[a,b,c]Different superscript within the same column for a given effect indicates a statistical difference

Table 2. Water chlorine concentrations, temperature, and pH (by rearing cycle).

Cycle (no. of water samples)	The free	Free residual chlorine (ppm)	pH	Temperature (ºC)
1st cycle (10)	0.27±4.37 (0.10-0.50) [a]	0.16±3.32 (0.05-0.30) [a]	7.90± 0.10 (7.20- 8.20) [a]	27.1±0.55 (25.0-30.0) [a]
2nd cycle (10)	0.16±3.7 (0.00-0.30) [b]	0.05±1.57 (0.00–0.10) [c]	7.10±0.2 (6.10-8.00) [bc]	22.3±1.39 (16.0-25.0) [c]
3rd cycle (10)	0.20±5.38 (0.00-0.50) [ab]	0.11±3.15 (0.00–0.30) [ab]	7.90±0.10 (7.50-8.60) [a]	14.1±0.52 (12.0-16.0) [f]

[a,b,c] Different superscript within same column for given effect indicates a statistical difference (P < 0.05)

* Statistically significant at (P<0.05) of variables

2.4 *S. enterica* serovar Enteritidis in the broiler drinking water and broiler chicken

The prevalence of *S.* Enteritidis in drinking water was 10% (3/30) in the main farm tank samples, 13% (4/30) in broiler house tanks, and 27% (8/30) in drinkers (**Table 3**). In total, 20% (30/150) of cloacal samples were positive for *S.* Enteritidis, though there was a significant difference (P<0.05) in the percentage of positive broilers among the 5 governorates, where Irbid had the highest positivity rate (30%) and Madaba the lowest (3%) (**Table 4**).

Table 3. The percentage (number) of samples positive for *S. enterica* serovar Enteritidis by biochemical Conventional method compared with PCR confirmation in Jordan.

Samples	Salmonella spp. detection by Conventional method	S. enterica serovar Enteritidis by PCR technique
Main water source	17% (5/30)	10% (3/30)
Broiler house water tanks	33% (10/30)	13% (4/30)
Drinker water	53% (16/30)	27% (8/30)
All water samples	34% (31/90)	16.6% (15/90)
Broiler chickens	28% (43/150)	20% (30/150)

The highest percentage was detected in farms located at Al Zarqa farms (38%) followed by Irbid (33%), while *S.* Enteritidis was not detected in Amman and Madaba broiler farms. However, *S.* Enteritidis was statistically different among drinking water samples collected from different five Governorates (One-way ANOVA; P>0.05), the highest percentage was in Zarqa broiler farms water 7 (38%) followed by Irbid broiler farms 6 (33%) Table (4). The presence of *S. enterica* serovar Enteritidis in the broiler drinking water was significantly different among the periods of rearing cycles (P<0.05) (**Table 5**).

Table 4. The number (percentage) of *S. enterica* serovar Enteritidis among drinking water and broiler chicken samples in five Governorates.

Governorate*	Main water source	Broiler house water tanks	Drinker water	All Water samples	Broiler chicken
Amman	0(0/6)	0% (0/6)	0% (0/6)	0% (0/18)	6.0% (4/30)
Madaba	0(0/6)	0% (0/6)	0% (0/6)	0% (0/18)	3% (1/30)
Karak	0(0/6)	16.6% (1/6)	16.6% (1/6)	11% (2/18)	20% (6/30)
Zarqa	33.3% (2/6)	16.6% (1/6)	66.6% (4/6)	38% (7/18)	23% (7/30)
Irbid	16.6% (1/6)	33.3% (2/6)	50% (3/6)	33% (6/18)	30% (12/30)
Total (30)	10% (3/30)	13% (4/30)	27% (8/30)	16.6% (15/90)	20% (30/150)

* Difference was significant according to farms location in Governorates (P <0.05)

Table 5. The percentage (number) of *S. enterica* serovar Enteritidis among drinking water in the three rearing cycles

The* (%) NO. of *S.* enterica *serovar Enteritidis				
	Samples (No. of Samples)			
Cycle*	Main water source (10)	Broiler house water tanks (10)	Drinkers water (10)	All water Samples (30)
1st Cycle	(20%) 2	(20%)2	(40%)4	(27%) 8
2nd Cycle	(10%) 1	(10%)1	(20%)2	(13.3%) 4
3rd Cycle	(0.0) % 0	(10%)1	(20%)2	(10.0%) 3

* There was a significant difference in water samples according to the rearing cycle (P<0.05).

Next, is the examination of whether there is an association between water supply contamination with chicken infection rates. The presence of *S.* Enteritidis in drinker water showed a high correlation (P<0.05) with the detection of *S.* Enteritidis in the broiler chicken cloacal samples. *S.* Enteritidis was not detected in water samples examined at Amman and Madaba, while it was detected in 11% of Karak water samples (2/18), 38% of Zarqa samples (7/18) and 33% of Irbid samples (6/18). Likewise, broiler chickens in Amman and Madaba showed low rates of PCR-confirmed *S.* Enteritidis infection, with 6% in Amman (4/30) and 3% in Madaba (1/30). Significantly higher rates of infection were found in other regions, with 20% (6/30) of broiler chickens testing positive in Karak, 23% (7/30) in Zarqa and 30% (12/30) in Irbid.

This is the first report regarding the presence of *Salmonellae* Enteritidis in broiler drinking water and live chickens on broiler farms in Jordan. As populations increase around the world, there are greater demands for poultry production and thus an urgent need to improve broiler drinking water (Zimmermann, *et al.*, 1993). The current results revealed that the highest percentage of *S.* Enteritidis was found in drinkers (27%), possibly because they are open and therefore prone to faecal contamination. *S.* Enteritidis in these drinkers could contribute to the recycling of a pathogen directly back to the birds in the flock. We note an interesting trend, where the presence of *S.* Enteritidis increased from the main storage water sources (10%) to the broiler house tanks (13%) to the drinkers (27%). The main water sources are cleaner than the drinkers.

By understanding the distribution of microbial contamination, it will be possible to deploy resources and effective mitigation measures to improve poultry farms. The percentage of *S.* Enteritidis in broilers showed a significant difference (P < 0.05) among the broiler flocks on farms in different locations. The poultry farming industry is not distributed evenly across Jordan, and we investigated five different Governorates where intensive poultry farming is observed. Specifically, Amman represents 23% of broiler farms, Irbid 18% of broiler farms in the North, Zarqa represents 11% in the East, Karak represents 11% in the South and Madaba represents 7% in the West (MOA, 2020). Directing resources towards farms in regions with the highest rates of contamination will be most effective in mitigating infections. An opportunity for further study is to better understand the contributing factors that lead to differences in contamination rates in the various Governorates. The highest broiler positivity rates of *S. enterica* serovar Enteritidis were found in Zarqa (30%), Irbid (23%) and Karak (20%), while the lowest percentage was in Amman (6%) and Madaba (3%). These differences could reflect the differences in climate, topography, culture, inspection districts, biosafety/biosecurity protocols, feed companies, litter, hatcheries, and the interspecies transmission of *S.* enteritidis (directly or via contamination of open water supplies) or many other potentially confounding factors. It might be also explained by the hatchery effect of the geographical variation in the distribution of colonization flocks since flocks supplied by the same hatchery tend to be clustered within the same region. The results indicated that the highest percentage (26.6%) of *S. enterica* serovar Enteritidis in water samples was in the first cycle during the summer season, while the lowest (10%) was in the third cycle during winter.

The results of *S. enterica* serovar Enteritidis have been correlated with the weather, topology, hydrology, and other geographical characteristics of the growing site that may influence the magnitude and frequency of transfer of *Salmonella* spp. from environmental sources (WHO, 2011). This study showed that broiler farms had wild birds including pigeons (44%), sparrows (100%), rodents (36%), cats (31%), dogs (39%), and insects (25%) which might get infected by contact or ingestion of byproducts outside poultry houses. Since the increased concern, control and possible eradication programs should be functional. These might lead to contamination of effluents, surface waters, creeks, lakes, rivers, pastures, and soils to the colonization of birds as well as to contamination of animal feeds, or direct re-colonization of farm animals. The percentage of all these water samples was (16.6%) lower than that reported by Poppe, *et al.*, (1991) who has been isolated this pathogen from water samples (21.6%) of the Canadian broiler drinking water contaminated with *Salmonellae* spp. Renwick, *et al.*, (1992) suggested that the risk of contamination with *Salmonella* spp. was 6 to 7 times higher when the water given to birds was exposed to a contaminated environment. Poppe, *et al*., (1991) suggest that contamination is possible any time chickens dip their beaks, walk in, and defecate in drinking water. In addition, it may be that the pathogen arrives from feed given to poultry. Therefore, it should be recommended to change the drinkers to nipples instead of troughs or bell drinkers would likely result in a reduced level of ingestion of *Salmonella* spp. by birds from drinking water because they would be less likely to become contaminated.

Another possible source of microbial contamination is the farm personnel. Staff and workers on poultry farms may carry *Salmonella* spp. on contaminated footwear, clothing, and hands. In our study, we found that visitors were restricted to enter poultry units only in (50%) of broiler farms. To minimize the risk of contamination, farms should be located away from other poultry holding areas and visitors should park away from areas where poultry are present and follow biosecurity measures when entering the broiler houses.

The results indicate that contaminated water could be an important source of *Salmonella* spp. infection for the broiler chickens. There are several ways to minimize microbial contamination in water, including treatment with disinfectants such as chlorine, ozone, or sodium chlorate. However, these are most efficient and effective if the water system is free from organic matter, as the organic matter will neutralize the chlorine reaction (Renwick, *et al*., 1992; Jacobs-Reitsma, *et al.,* 1994). Another water treatment method is acidification of drinking water with organic acids, especially during feed withdrawal (Byrd, *et al.,* 2001). Water treatment is effective in reducing the degree of transmission and infections (Omwandho and Kubota, 2010). In fact, water treatment is more effective than other methods, including pest control, restrictions on visitors and improved clean-out methods (Arsenault, *et al.,* 2007; Rose, *et al.,* 1999).

Chlorinated water is especially effective in reducing microbial contamination in the drinking water systems of poultry farms (Poppe, *et al.,* 1986). Studies show that in farms where the level of free residual chlorine in water exceeds 0.2 ppm, *Salmonella* spp. is not detectable in our study, there was no free residual chlorine detected in broiler house water and drinkers. The prevalence of *Salmonella* in this study is similar to those reported in the European Union (23.7%) (EFSA, 2009) and in Chinese provinces detected *S. enteritidis* (22/95, 23.2%) Song, *et al.,* (2020). Our findings were more than the percentage of *Salmonella* spp. isolated from live broiler flocks in Saudi Arabia (4.87%) (Saád, *et al.,* 2009). Others have reported similarly high percentages of positive broiler cloacal swabs, such as the 13% found in Thailand (Sasipreeyajan, *et al.,* 1996). On the other hand, the current results are lower than the percentage reported in a survey conducted in England and Wales 48.3% (Poppe *et al.,* 1991); and in broiler houses in Canada in which 50% to 65% of *Salmonella* spp. was detected in flocks (Arsenault *et al.,* 2007). Studies have shown that in France *Salmonella* spp. was positive among 70% of broiler flocks (Rose, *et al.,* 1999) and 66.7% in Spain (Marin and Lainez, 2009). A previous study in Jordan found that the percentage of *S. enterica* serovar Enteritidis was 43% and showed that the isolates were more common in the broiler carcasses (Al-Matar, *et al.,* 2005). The variation in the percentages found among studies may be due to differences in sampling methods, flock characteristics, and local conditions. Despite this, the percentage of positive samples is likely to represent a surrogate variable for the quality of biosecurity measures implemented by producers (Altekruse, *et al.,* 2006). Rose, *et al.*, (1999) found that good hygienic barriers reduced the risk of *Salmonella* spp. colonization in breeder flocks. Proper cleaning and disinfection of equipment before restocking the house could be the key to preventing the hazards for the next flock (Amy, *et al.,* 2004). Litter, feed, dust and residual contamination of the drinkers can result in water contamination. Drinking water is a continuous risk for re-infection of *Salmonella* contaminated flocks mainly due to the presence of faecal material in the drinkers.

Conclusions

This study underscores the need for the development of Jordanian water quality guidelines for broiler farm drinking water and monitoring of water disinfectant procedures. Hygienic drinking water is important for disease prevention, food safety, and

reduction of antibiotic use, and reducing microbial contamination of water will lead to improvementAcknowledgemenarming across Jordan.

Acknowledgement

I would like to thank the team of JJECI for their assistance in revising the article and for their constructive suggestions and comments. Also, I would like to thank the deanship of scientific research at the University of Jordan for their support.

Nomenclature

C	=The degree Celsius	[-]
DPD	=N, N-diethyl-p-phenylene-diamine	[-]
EFSA	=European Food Safety Agency	[-]
ISO	=International Organization for Standardization	[-]
MKTTn	=Muller-Kauffmann tetrathionate novobiocin broth (broth base)	[-]
MOA	=Ministry of Agriculture	[-]
PCR	=Polymerase chain reaction	[-]
ppm	=part per million	[-]
RV	=Rappaport-Vassiliadis	[-]
WHO	=World Health Organization	[-]
XLD	=Xylose lysine deoxycholate	[-]

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