Water as Source of Campylobacter jejuni and E. coli O157 contamination in broiler farms in Jordan

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Abstract:

The poultry industry in Jordan plays a crucial role in food security and economic stability. However, water-borne disease transmission poses significant challenges within the 'One Health' framework, particularly as climate change intensifies these risks. This study aimed to find out the prevalence of Campylobacter jejuni and E. coli O157 contamination in broiler farm in Jordan as well as the sources of this contamination.

This study involved collecting and examining samples from 10 broiler farms located in five Jordanian Governorates; Amman, Irbid, Karak, Zarqa and Madaba. This study was covered three rearing cycles of chickens in each farm. A total of (90) broiler drinking water samples and (150) broiler samples were examined for the presence of Campylobacter jejuni and E. coli O157 contamination.

Analysis done using culture and biochemical tests as well as PCR. The prevalence of C. jejuni was (76.6%) 69 as waterborne bacterial indicators in (90) broiler drinking water samples. And the prevalence of E. coli O157:H7 was (24.4%)22. However, the highest prevalence was of C. jejuni (47.3%)71 identified in (150) broilers samples and the least percentage E. coli O157 which was (14%)21.

Prevalence of Campylobacter jejuni is much higher than E. coli O157, although both are still high. Recommendation to decrease this contamination include establish drinking water guidelines and biosecurity standards to decrease waterborne bacterial disease is recommended to improve the poultry industry sanitation and population health.

Keywords: Campylobacter jejuni; E. coli O157; contamination; prevalence; broiler water; broiler; one health; Jordan.

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INTRODUCTION

The effects of global climate change are known to have important, especially water security in arid regions. We consider Jordan as a case in point; Jordan is the second most water scarce country in the world. Jordan's annual renewable water resources are less than 100 m3 per person, significantly below the threshold of 500 m3 per person which defines severe water scarcity (Rajsekhar and Gorelick, 2017).

In Jordan poultry industry sector has a high priority on agriculture investments due to high demand for animal protein sources due to increase number of population. This is translated into 1337 broiler farms rearing about 29.2 million birds and producing about 156 million of birds for meat (MOA, 2023). This large number of farms demands the adoption of stringent regulatory acts to protect this important strategic sector of economy. However, poultry production in Jordan faces many problems and obstacles such as; competition between local production and high quality, subsidized European imported products. Moreover, technical and health problems that increase the mortality rate in poultry farms, furthermore, Jordan is a semi-arid country and facing water scarcity, water resources and quality due to climate change are critical factors that receive intensive attention (Abu-Iteleh, et al., 2008, FAO, 2009).

Worldwide, over 50% of poultry meat is contaminated with Campylobacter (Suzuki, et al.,2009). However, no effective measures to limit Campylobacter infections in primary broiler chicken production exist to date (Hermans et al, 2011). Once a chicken is infected, the pathogen rapidly spreads infecting almost 100% of the flock within a week (Stern et al, 2001). E. coli is a gram-negative bacterium of the family Enterobacteriacae and is a normal inhabitant of intestinal tract of birds (Singleton and Sainburg, 1981). E. coli 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). Poultry and poultry meat is considered to be one of the major sources for human campylobacteriosis (Humphrey et.al, 2007). Beside poultry and raw poultry meat other sources for *C. jejuni* have been described such as livestock, including sheep and pigs, but also cats and dogs, water, humans and vehicles, raw milk, rodents and insects are known as possible vectors (Corry and Atabay, 2001). These different sources are not only involved in the horizontal transmission of *Campylobacter* to humans but also to poultry flocks. 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 broiler chicken. 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 O157 that belongs to enterohemorrhagic E. coli (EHEC) was first reported in the United States in 1983 during an outbreak of severe bloody diarrhea due to contaminated hamburgers (Rangel, et al., 2005). It was estimated that 73,000 illnesses occur each year due to E. coli O157 infection in the United States leading to 2,000 hospitalizations and 60 deaths (Rangel, et al., 2005). Waterborne transmission of VTEC, in particular E. coli O157, from zoonotic reservoirs is well documented, primarily in developed countries. Private and municipal drinking-water supplies have been implicated as sources of outbreaks and causes of sporadic illness (Bopp, et al., 2003). The presence of low numbers of target organisms in water makes microbiological confirmation difficult (Rangel, et al., 2005). Therefore, epidemiological evidence has been essential in outbreak investigations. Indeed, due to the low infective dose of E. coli O157, a significant risk of infection may arise in waters that only just meet standards for index organisms (Lejeune, et al., 2001c). Contaminated water troughs with sediments provide an environment for survival, proliferation, and horizontal spread of E. coli O157 (Lejeune, et al., 2001b). The ability of this organism to colonize chicken cecae indicates that chickens might serve as hosts and possibly as reservoirs for E. coli O157 (Beery, et al., 1985). Doyle and Schoeni (1987) detected the presence of E. coli O157 on 1.5% of poultry carcasses from the state of Wisconsin in the US.

In Jordan, where water scarcity is exacerbated by climate change, ensuring hygienic water in poultry farming is crucial. Hygienic drinking water is important for disease prevention, food safety, and mitigating of antibiotic use (AMR) risks, and reducing microbial contamination of water will lead to improvement one health (OH) (Brown, et al., 2024). Aim The present study in Jordan was conducted to evaluate the prevalence of Campylobacter and E. Coli O 157 in broiler farms and to highlight its mechanisms of transmission and to recommend the preventive measure to reduce this prevalence and cut this transmission.

1. MATERIALS AND METHODS

This study was conducted on the broiler chicken farms selected from some Governorates in Jordan. . The geographical regions of the north, center, and south were covered, and the governorates with the highest number of broiler chicken farms were selected. The Governorates selected were Amman, Irbid, Madaba, Zarqa and Karak (fjgure 1).

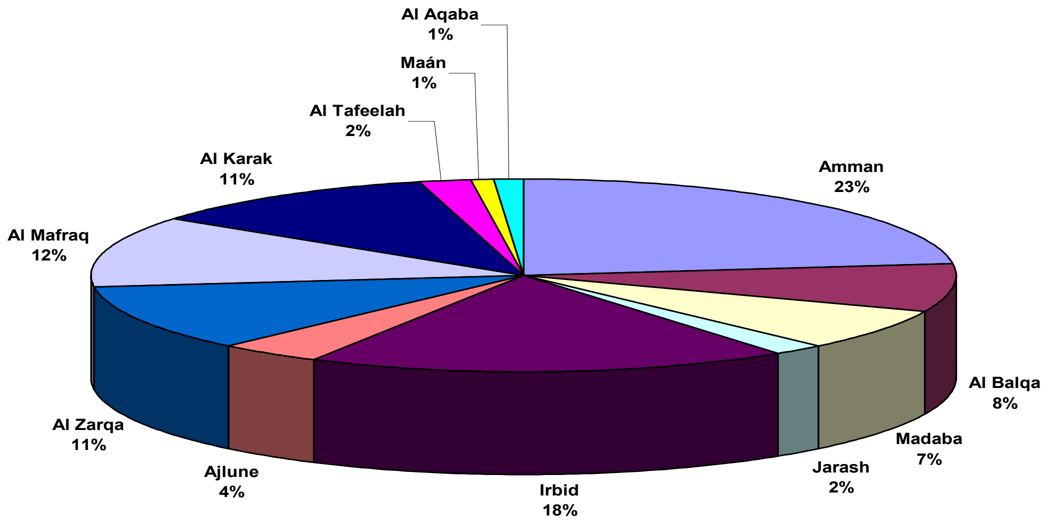

Figure 1. Distribution of broiler farms in Jordan

Water samples were taken from the main farm tank, broiler house tank, and different types of drinkers, provided for the flocks inside the farming house. This study was qualitative analysis, broiler cloacal swabs from live broiler chickens were collected randomly from each farm during the age of chickens between 3rd to 8th weeks of broiler life during each cycle. Water Sample Collection Protocol; physical analysis included determination of the temperature (oC), sampling and transportation of broiler drinking water samples for microbiological examination were performed according to American Public Health Association (APHA, 2017):

1. Sample containers: Sterile 500ml glass bottles with sodium thiosulfate

2. Sampling technique:

- Main tank: Collected from outlet tap after 2-minute flushing

- Drinker water: Collected directly from nipple/trough

- Preservation: Maintained at 4°C, processed within 4 hours

3. Transport: Cold chain maintained using insulated boxes with ice packs

4. Documentation: GPS coordinates, water source type, collection time recorded

Quality Control Measures included, sterile sampling techniques verified through blank controls, positive controls: C. jejuni ATCC 33291 and ATCC 29428, Negative controls: Sterile water processed alongside samples.

Moreover, PCR validation: All positive cultures confirmed by PCR, Inter-laboratory validation: 10% of samples analyzed at reference lab, Finally, contamination prevention by separated processing of samples by governorate.

Detailed survey regarding hygienic conditions, water resources, and other related indicators of the investigated farms was performed using a questionnaire. The questionnaire was modified to include the broiler farms name, address, farm location, flock age, flock size, number of houses in 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.

1.1. Detection and Identification of *Campylobacter jejuni*

The reference bacterial strains used in this study as positive control were C. jejuni ATCC 33291 and ATCC 29428 (Microbiologics® Inc., USA). The C. jejuni ATCC strains were activated and cultured according to the manufacture instructions, by using Preston Campylobacter culture media (Oxoid Ltd. UK). The presence of C. jejuni was detected by using Preston method (Bolton and Robertson, 1982; Scates, et al., 2003). It was specifically formulated to be suitable for isolation of Campylobacter species from all types of specimens (human, animal, avian and environmental) (Baylls, et al., 2000). Membrane filtration technique was used to detect C. jejuni cells in water samples according to method described by APHA, (2017). Birds were sampled by cloacal swab. Sterile cotton swabs were used to sample live broiler chickens.

PCR assay was applied to confirm the identify of the isolates C. jejuni. All isolates that were identified as C. jejuni by biochemical test were subjected to PCR using a pair of primers (Forward 5'- CAA ATA AAG TTA GAG GTA GAA TGT3' and Reverse 5'- GGA TAA GCA CTA GCT AGC TGA T-3' primers) to amplify 160 base pair (bp) DNA fragment that target the oxidoreductase gene sequence which is specific for C. jejuni (Burnett, et al., 2002; Nayak, et al., 2005).

1.2. Detection and Identification of *E. coli* O157

100-ml of water samples were inoculated into 50-ml triple-strength of (LTB) medium and incubated at 35°C for 24 h. Cloacal cotton swabs were collected then the swab broken off into selective broth, for isolation of E. coli O157. Samples were collected under aseptic conditions (WOAH, 2021). The biochemical examinations of E. coli O157 such as; oxidase test and *E. coli* O157 latex agglutination test were tested. The

PCR for identification of eaeA gene for E.coli O157 was done according to Gannon, et al., 1993 and Fratamico, et al., 1995.

2. Statistical analysis

The results were analyzed with the Statistical Analysis System (version 9.2) (SAS, 2009) package. All data were presented as means ± standard error of mean (SEM). One-way analysis of variance (ANOVA) and Chi-Square Test for independent samples were used to analyze the differences between the sample means. The differences were considered significant at $P < 0.05$. Pearson's correlation coefficient was estimated for interaction between different parameters.

3. Results

3.1. *C. jejuni and E. coli* O157 isolated from Broiler farms drinking water

The C. jejuni were found all broiler farms drinking water in all tested farms and its average prevalence was 76.6% (69/90). The highest prevalence of all samples was in Irbid 94.4% (17/18) and the lowest prevalence was in Amman 50.0% (9/18) as seen in the table (1).

The E. coli O157 24.4% (22/90)was also present in all farms but it was significantly less prevalent than C. jejuni 76.6% (69/90). The overall average of E. coli O157 was 24.4% (22/90). The highest percentage was in Zarqa and Irbid which were 38.3% (7/18) and 24.4% (22/90) respectively. And the lowest was in Madaba and Karak which were 11.1% (2/18) and 11.1% (2/18) respectively as shown in table (1).

Table 1. The prevalence, the P value and the percentage (number) of C. jejuni and E. coli O157 isolated and identifeid by PCR among broiler farms drinking water contamination in the five Governorates.

Governorate*	% (No.) of *C. jejuni*	% (No.) of *E. coli* O157
Amman	50.0% (9/18)	22.2% (4/18)
Madaba	77% (14/18)	11.1% (2/18)
Karak	88% (16/18)	11.1% (2/18)
Zarqa	72% (13/18)	38.3% (7/18)
Irbid	94.4% (17/18)	38.3% (7/18)
Total	76.6% (69/90)	24.4% (22/90)

*Significantly different according to farms location ($P < 0.05$).

3.2. *C. jejuni* and *E. coli* O157 isolated from boiler chicken in broiler farms:

Regarding contamination in broiler farms we find that all broiler farms are contaminated with C. jejuni and E. coli O157. However, the prevalence was significantly lower than in drinking water. Also, we found that C. jejuni more prevalent than E. coli O157 (Table 2).

C. jejuni was present in all broiler farms with highest prevalence in Irbid and Zarqa which were 70% (21/30) and 63% (19/30). While it was the least in Amman 23% (7/30). E. coli O157 14% (21/150) was isolated from broiler chicken samples but it was significantly less prevalent than C. jejuni 47.3% (71/150) in broiler samples. The overall average of E. coli O157 was 14% (21/150). The highest percentage was in Zarqa 13.3% (4/30) while the lowest was in the Madaba 3% (1/30).

The highest percentage of isolates in all broiler chickens samples were C. jejuni (47.3%)71 out of 150 and the least E. coli O157 were 21 out of 150 (14.4%) (Table 2).

Table 2. The percentage (number) of C. jejuni and E. coli O157 isolated and identifeid by PCR from boiler chicken in broiler farms contamination in the five Governorates.

Governorate*	% (No.) of *C. jejuni*	% (No.) of *E. coli* O157
Amman	23% (7/30)	10% (3/30)
Madaba	33% (10/30)	3% (1/30)
Karak	46% (14/30)	20% (6/30)
Zarqa	63% (19/30)	23.3% (7/30)
Irbid	70% (21/30)	13.3% (4/30)
Total	47.3% (71/150)	14% (21/150)

*Significantly different according to farms location ($P < 0.05$).

The typical water temperature for broilers is 21.0ºC as recommended by Fairchild and Ritz, (2009). During the 1st cycle, the mean ± (SEM) 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± (SEM) and range of water temperature in the 2nd cycle was 22.3 ± 1.39°C, (16.0° to 25.0°C). Whereas in the 3rd rearing cycle the means ± SEM and ranges for temperature of the main water source was 14.1 ± 0.52 °C (12.0 to 16.0°C). the results statistically significant at ($P < 0.05$) of variables.

4. Discussion

The poultry farming industry is scattered all over Jordan in this study, five different Governorates were selected where intensive poultry farming is observed (figure 1). 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 (Anonymous, 2024).

The percentage of presence of C. jejuni in this study was 47.3% (71/150) in broiler chickens cloacal swabs. This comes in agreement with many studies of C. jejuni that contaminated broiler the percentage of C. jejuni was 47% in the province of Ontario, Canada, but 60% in Quebec broiler farms (Arsenault, et al., 2007). In France, C. jejuni was detected in (42.7%), Denmark (42.5%), Germany (41.0%), Japan (45.0%) and Italy (80.0%) in broilers (Humphrey, et al., 2007). Nevertheless, these results were higher than the percentage of C. jejuni in broilers at Norway (18.0%), Sweden (27.0%), Chile (19.7%) and Taiwan (24.1%) (Newell and Fearnley, 2003). However, Al-Akhras (2010) found that percentage of C. jejuni in broiler chickens was (40%) in Amman slaughterhouse.

The high percentage 76.6% of C. jejuni were recorded in drinking water. However, percentage of C. jejuni in broiler chickens was (47.3%), which correlates with water contamination. The presence of *C. jejuni in broiler drinking water* might serve as a reservoir for Campylobacter spp. infection of poultry flocks (Zimmer, et al., 2003).

The current research indicated significant differences ($P < 0.05$) between the five Governorates on the presence of *C. jejuni* in drinking water and broiler according to the locations of farms and farming practices. Moreover, *C. jejuni* isolated from broiler at the third rearing cycle (56%) (during winter season) was slightly higher than the first and the second cycles (46%) and (40 %) (during summer and autumn seasons), respectively. This may be related to better ventilation during summer and autumn time or probable more frequent flocking together which could indicate some seasonal effect (Newell and Fearnley, 2003). Corry and Atabay, (2001), Stern, *et al.*, (2002) concluded that water was the principal source for the spread of such organism in a commercial broiler farms.

The *Campylobacter* spp. is ubiquitous in the environment and could be readily carried into the house by a number of vehicles, including human activity associated with routine flock management (Ogden *et al.,2007)*. However, it seems likely that infection in broiler farms could introduced sporadically to chicks from the external site environment, perhaps by poultry farm workers or wildlife vectors as concluded from results of the questionnaire. Which, showed that the percentage of broiler farms that had mice were (90%), rats (36%) and resident insects (25%) and other livestock which definitely increased the risk of contamination (Tablante, *et al.,* 2002; Newell and Fearnley, 2003).

The literature suggested that standard biosecurity procedures were inadequate for the maintenance of broiler negativity (Jeffrey, 2001; Kapperud, *et al.,* 1993; Wedderkopp, *et al.,* 2000). Nevertheless, stringent biosecurity might either delay positivity or reduce the number of flocks that become positive (Zimmer *et al.,* 2003). However, it was generally considered that adequate biosecurity procedures were difficult to sustain in the farm environment. Well-designed and well-located farms, the development of appropriate standard operating procedures to minimize risk factors, staff education, and incentives to maintain biosecurity at the highest level would all contribute to the reduction of flock positivity (Jeffrey, 2001).

The effective hygiene barriers such as housing birds in buildings in good state of repair, appropriate use of disinfectant boot dips outside broiler houses, a high standard of cleansing and disinfection of drinking water supply equipment could protect broilers from contamination by *Campylobacter* spp. (Arsenault, *et al.,* 2007, Amaral, 2004). All these factors had effected the one health (OH) which, is defined as a unifying approach aiming to sustainably balance and optimize the health of people, animals and the ecosystem and each influencing the others by direct and indirect methods (Brown, *et al.,* 2024).

The mechanisms by which *E. coli* O157 was able to persist in water were not well investigated. The role of environmental sources in the ecology and epidemiology of *E. coli* O157 in broiler is still unclear. However, it was very probable that environmental sources were involved in the persistence, multiplication, and/or transmission of this organism both within and between broiler farms (La Ragione, *et al.,* 2005; Brown, *et al.,* 2002).

From the (90) water samples studied in this investigation, it appeared that there was a substantial variability in persistence of *E. coli* O157 (24.4%) in all different sources of water samples. The percentage of contaminated drinking water samples was (10%) in the main water source, (16.6%) in broiler house tanks and (46.6%) in drinkers water. Water sources for broiler were frequently contaminated with relatively high numbers of *E. coli* O157. Water sources close to broiler farming systems would represent a potential reservoir for enteric pathogens, allowing cycles of broiler re-infection and increasing the potential for the organism to spread (Ahmed, *et al.,* 2009).

The drinkers with higher generic *E. coli* counts due to fecal contamination were also the ones most likely to test positive for *E. coli* O157 (Stern, *et al.*, 2002). Similarly, fecally contaminated drinkers water appeared to strong support the persistence of *E. coli* O157 (Lejeune, *et al.,* 2001a). Fecal isolate of *E. coli* O157 were for 245 days in the sediments of troughs McGee, *et al.*, (2002) and similar observations were reported by Trachoo, *et al.*, (2002) and Avery, *et al.*, (2008).

These results are consistent with previous studies, which suggest that, *E. coli* O157 might persist in drinkers' water for months or years, facilitating re-infection of animals (Lejeune, *et al.,* 2001b). The longevity displayed by *E. coli* O157 corroborated the findings of other investigations into the survival of the organism in aquatic environments (Brown, *et al.,* 2002; Avery, *et al.*, 2008).

Poultry meat is widely consumed because of its flavor and nutritious characteristics. The contamination of poultry meat by foodborne pathogens such as *E. coli* O157 can occur along the food chain and but may harbor significant *E. coli O157* (Schouten, *et al.*, 2005, Dursun and Kaya, 2010).

In the present study the percentage of *E. coli* O157 was (14%) in our examined broilers. These findings indicated that poultry could also be a source of *E. coli* O157 infections for humans (Pilipcinec, *et al.*, 1999). Because of this *E. coli* O157 were associated with both foodborne and waterborne contamination of drinking water (Hakkinen and Schneitz, 1996; LeJeune, *et al.*, 2001c). The presence of pigeons in (44%) of broiler farms may represent a natural reservoir for the organism and that it could potentially represent as a vector to transmit this pathogen into broiler. Further studies on pigeons and others flies are required to determine if they are involved in the ecology of *E. coli* O157 in broiler farms (Dziva and Stevens, 2008).

The maximum acceptable level of broiler drinking water temperature is 21°C as recommended by Fairchild and Ritz, (2009). In the current research, the average temperature of water drinkers was 25.6°C in first cycle (during summer season) (table 3), it slightly exceeded the maximum acceptable level (Fairchild and Ritz, 2009). Water requirement increases by approximately 6.5% per degree centigrade over 21°C since the water consumed by birds will help to dissipate the body heat temperature. In the second and third cycle, the average temperature and the ranges of drinkers water were 20.5°C (17 to 25°C) and 18.5°C (17 to 20°C), respectively and that was approximately about the acceptable level 21°C (Carter and Sneed, 2007; Fairchild and Ritz, 2009). Several studies examined the effects of providing cool water to birds during hot weather; they indicated that water temperature improved the performance of broilers (Fairchild and Ritz, 2009). Therefore developing methods of cooling water temperature in hot weather should be investigated further. This might involve running the drinker supply pipes through a cool pad reservoir or even across the face of the cool pad airflow as reported by Zimmerman, *et al.,* (1993). Positioning the water tanks and supply pipes underground might also help to protect the water from the ambient air temperature, keeping it cool as recommended by Scott and Ahern, (2009).

Biosecurity in poultry farms were essential to reduce financial losses, which result from disease outbreaks. It had been reported that the financial loss in only one farm in Pennsylvania, USA, in 1997, due to the outbreak of avian influenza exceeded $344,000 and more recently great loss occurred in Thailand due to the same disease (Davison, *et al.*, 1999; Leung, *et al.*, 2007; Sims, 2007).

For the reason of low bio-security level in the current study, life threatening bacteria such as *E. coli* O157, *Campylobacter* and other pathogens were routinely detected on chicken collected at the broiler farm level. There were numerous studies suggested that measures should be implemented at farm level to prevent birds contamination (Guerin, *et al.*, 2007).

Runoff water, especially after heavy rains, could the reason for contaminated wells and other community water systems. This contamination might be resulted from entry of *Escherichia coli* O157 and *Campylobacter* spp. from neighboring farms into the water supply (Clark, *et al.,* 2003). These bacterial populations could be spread into the environment through manure and runoff water (Lefebvre, *et al.,* 2006).

There is a need to upgrade and comprehend the biosecurity programs in Jordan, and it is important to follow all regulations recommended and implement and monitor them carefully in the farm.

In order to reduce infectious agents in the environment, dead birds should be removed daily and disposed correctly and avoiding their contact with insects, rodents or other animals that might be present on the farm (Santaniello, *et al*., 2007). It was also likely that manure from animals raised with high levels of biosecurity present a lower risk of food and water contamination. Disposal of immense quantities of manure generated by poultry operations had been a major challenge in many watersheds.

The high densities of chickens relative to the broiler houses area were a common problem seen in most broiler units in Jordan, particularly under small farm operations. This might influence the infection rate within the flock and could consequently be associated with increased incidence of pathogens such as *C. jejuni* and *E. coli* O157. It is of paramount importance to limit the unnecessary usage of antibiotics in broiler feed and drinking water, so that the spread of drug-resistant to many bacterial species can be decreased (Altekruse, *et al.,* 2006).

5. Conclusions and Future works

The use of drinking water at broiler farms with acceptable physicochemical and microbiological quality was of a fundamental importance to decrease the waterborne related bacteria and improve broiler health. Annual testing for compliance to the bacteriological standard mentioned should be performed to ensure that the drinker water supply conforms to this standard. This test might be performed at the point the water source enters the farm and immediately after treatment. The highest percentage of isolates in the broiler drinking water was *C. jejuni* (76.6%), *E. coli* O157 (24.4%). While, the highest percentage of isolates in broiler chickens was *C. jejuni* (46.6%), and the least one was *E. coli* O157 (14.4%) were exceeding the recommended levels that reported in the USA guidelines and Canadian standard. These results indicated the poor microbial water quality and a potential for presence of pathogens.

Recommendations

The significant presence of *Campylobacter jejuni* and *E. coli* O157 in Jordanian broiler farms underscores the urgent need for improved water management programme, biosecurity measures, and public health monitoring strategy to decrease waterborne related diseases and improve broiler performance. Monitoring of water disinfectant procedure and sanitation can be controlled to improve microbial quality of broiler drinking water. These efforts are essential to safeguard food safety and public health, especially as climate change amplifies environmental risks associated with pathogen transmission.

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