Microbiological, chemical and physical quality of drinking water for commercial turkeys: a cross-sectional study

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ABSTRACT Drinking water for poultry is not subject to particular microbiological, chemical and physical requirements, thereby representing a potential transmission route for pathogenic microorganisms and contaminants and/or becoming unsuitable for water-administered medications. This study assessed the microbiological, chemical and physical drinking water quality of 28 turkey farms in North-Eastern Italy: 14 supplied with tap water (TW) and 14 with well water (WW). Water salinity, hardness, pH, ammonia, sulphate, phosphate, nitrate, chromium, copper and iron levels were also assessed. Moreover, total bacterial count at 22°C, presence and enumeration of *Enterococcus* spp. and *E. coli*, presence of *Salmonella* spp. and *Campylobacter* spp. were quantified. A water sample was collected in winter and in summer at 3 sampling sites: the water source (A), the beginning (B) and the end (C) of the nipple line (168 samples in total). Chemical and physical quality of both TW and WW sources was mostly within the limits of TW for humans. However, high levels of hardness and iron were evidenced in both sources. In WW *vs.* TW, sulphate and salinity levels were significantly higher, whilst pH and nitrate levels were significantly lower. At site A, microbiological quality of WW and TW was mostly within the limit of TW for humans. However, both sources had a significantly lower microbiological quality at sites B and C. *Salmonella enterica* subsp. *enterica* serotype Kentucky was isolated only twice from WW. *Campylobacter* spp. were rarely isolated (3.6% of farms); however, *Campylobacter* spp. farm-level prevalence by real-time PCR was up to 43% for both water sources. Winter posed at higher risk than summer for *Campylobacter* spp. presence in water, whereas no significant associations were found with water source, site, recirculation system, and turkey age. Low salinity and high hardness were significant risk factors for *C. coli* and *C. jejuni* presence, respectively. These results show the need of improving sanitization of drinking water pipelines for commercial turkeys.

Key words: *Campylobacter*, chemical and physical quality, drinking water, microbiological quality, turkey

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INTRODUCTION

The quality of drinking water for livestock is a subject of utmost importance, as it can directly and indirectly affect animal health and productivity (Umar et al., 2014). Although general recommendations as well as specific guidelines for poultry water quality are available (Carter and Sneed, 1996; Amaral, 2004), farmers are often unaware of the importance of water quality (Umar et al., 2014). In the European Union (EU), this subject has received little attention in terms of EU legislation, as drinking water for poultry is not subject to particular microbiological, chemical and physical requirements. Regulation (EC) 852/2004 on the hygiene of foodstuffs establishes minimum requirements for livestock drinking water, but no qualitative parameters are listed (European Commission, 2004). Council Directive 98/58/EC on farmed animal welfare states that 'all animals must have access to a suitable water supply or be able to satisfy their fluid intake needs by other means', although no suitability thresholds are indicated (European Commission, 1998a). On the other hand, the Terrestrial Animal Health Code (Art. 6.4.5) of the OIE-World Organization for Animal Health states that 'the drinking water supply to poultry houses should be potable according to the WHO or to the relevant national standard' (OIE, 2016). This indication derives either from the possible transmission of pathogens, such as *Salmonella* and *Campylobacter* (Amaral, 2004), or from

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the possible infiltration of contaminants (Carter and Sneed, 1996).

Recently, the European Food Safety Authority (EFSA, 2011) indicated farm water as one of the sources of direct contamination with *Campylobacter* for livestock and humans. *Campylobacter* is more susceptible than *E. coli* to water chlorination (Lund, 1996), and it was reported a 3.5-fold risk of infection for broiler flocks supplied with unchlorinated *vs.* chlorinated water (Kapperud et al., 1993). Moreover, *C. jejuni* has been isolated from the biofilm of nipple drinking systems for poultry when the birds were also colonized (Zimmer et al., 2003), although there is limited evidence for such drinking systems to be the source of *Campylobacter* colonization. This may be due to failure to detect *Campylobacter* in water as a result of insufficient water volumes processed or of microorganisms in a viable, but not culturable, state (Sparks, 2009).

Data on the microbiological, chemical and physical quality of drinking water for livestock in Italy are scarce. Moreover, in the poultry sector, groundwater is frequently used, with no compulsory periodical water quality controls being requested for this source of water supply. Finally, turkeys are often treated with medicines, including antimicrobials, administered with drinking water, and some chemical and physical water properties like pH, hardness and iron levels may interfere with drug dissolution and stability in water (Scandurra, 2013). For these reasons, the present study aimed to assess the microbiological, chemical and physical quality of drinking water in commercial turkey farms supplied with either tap or well water. A number of factors putatively associated with water quality (e.g., environmental conditions, husbandry practices, season, water recirculation system, etc.) were also investigated, and water quality at different sampling sites along the farm water pipeline was assessed.

MATERIALS AND METHODS

Sample Collection

Samples from 14 turkey farms supplied with well water (WW) and 14 supplied with tap water (TW) were analyzed. Both groups were randomly selected within the densely-populated poultry area (DPPA) of North-Eastern Italy. This area is characterized by the highest density of poultry in Italy and one of the greatest in Europe (Mulatti et al., 2011). Farms under study were operational for an average of 17 ± 12 years (SD) and consisted of 4 ± 2 sheds housing 10.000-70.000 turkeys in total; birds therein were 55 ± 35 days of age. Water wells were 65 ± 45 m deep underground. Seventeen farms had a system for water recirculation *vs.* 11 without such system. In order to guarantee pipeline hygiene, all farmers declared to apply a pipeline sanitization protocol as part of their normal operating procedures with stabilized hydrogen peroxide at each new production cycle (concentration of 2–3%) and continuously when the drinking system was operating (concentration of 25–50 ppm).

Water samples were collected in 2012–2013 in late winter (February-March) and in mid-summer (July-August) at 3 sampling sites: the water source (A), the tank at the beginning of the nipple line where medicines are mixed for administration via water (B) and the end of the nipple line (C). Water temperature at the source and pH at A, B, C were also measured. Samples were collected in sterile containers, delivered to the laboratory at 4°C and processed within 24 h for microbiological analyses and 48 h for chemical and physical analyses.

Chemical and Physical Analyses

Ammonia concentration was determined by spectrometric indophenol assay (APAT, 2003), while the concentration of nitrate, sulphate and phosphate was determined by ionic chromatography (APAT, 2003). Chromium, copper and iron concentrations were quantified by atomic absorption spectrophotometry (APAT, 2003). The EDTA (ethylenediaminetetraacetic acid) titration method was used to determine the level of hardness (APAT, 2003).

Microbiological Analyses

Water samples were tested for total bacterial count at 22°C, enumeration of *Enterococcus* spp., enumeration of *E. coli*, presence of *Salmonella* spp. While specific legislation for animal drinking water is not available, analyses were performed according to European Council Directive 98/83/EC on the quality of water intended for human consumption (European Commission, 1998b). A national reference guideline (ISS, 2007), which set analytical methods for water intended for human consumption, was also considered. Analyses for enumeration of *Enterococcus* spp. and *E. coli* were performed according to UNI EN ISO 7899-2 (UNI, 2003) and UNI EN ISO 9308-1 (UNI, 2002), respectively. Enumeration of total bacterial count at 22°C and 37°C were performed according to UNI EN ISO 6222 for colony count (UNI, 2001), and according to ISO 6887-1 (ISO, 1999) and ISO 8199 (ISO, 2005a) for sample dilutions and for membrane filtration method. For the detection of *Salmonella* spp., 1 L samples were tested by membrane filtration method and then processed according to ISO 6579 (ISO, 2002).

To detect the presence of *Campylobacter* spp., both cultivation and real-time PCR were performed. Isolation was performed according to the standard method for *Campylobacter* detection in water (ISO, 2005b), which was slightly modified (SCA, 2002; Williams et al., 2012). Briefly, water samples were filtrated through a sterile membrane with a pore size of 0.2 μm (Sartorius, Goettingen, Germania) in a vacuum pump system. Then, each membrane was inoculated into 50 mL of Exeter broth (Mast Diagnostics, Merseyside, UK)

and incubated at 41.5°C for 48 h under microaerobic conditions. Two hundred μL of each broth culture were streaked onto Karmali agar (OXOID, Basingstoke, UK), after passive filtration (Giacomelli et al., 2012), and incubated at 41.5°C for 48 h under microaerobic conditions. Suspected *Campylobacter* colonies were examined by multiplex end-point PCR for genus and species identification (Yamazaki-Matsune et al., 2007). For real-time PCR, water samples were filtrated as described above and filters inoculated and vortexed in 5 mL of deionized water. Three mL of the suspension were centrifuged at 5,000 rpm for 10 min and the pellet resuspended in 200 μL of PBS. The DNA extraction was carried out by using the High Pure Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions, and the multiplex real-time PCR assay by using Taqman® probes and specific primers for *C. jejuni* and *C. coli* detection, as previously described (Toplak et al., 2012).

Statistical Analyses

An explorative data analysis was performed to examine the distributions of chemical and physical parameters. Given the high skewness of all these parameters, median and interquartile range were used as descriptive statistics, and, for each parameter, linear quantile mixed model targeting the median was adopted to assess the association with the type of water source (TW or WW), the sampling site (A, B or C) and the season (winter or summer), that represented the fixed effects in the model. To take into account the hierarchical structure of the sampling design, the farm was included in the model as grouping factor, whereas sampling site and season were additionally declared in the random part of the model. A General Linear Model (GLM) was used to evaluate the association between mean temperature and type of water source, season and their interaction. The outcomes of microbiological analyses, expressed as presence/absence, were modelled using Generalized Linear Mixed Models (GLMMs), choosing the binomial distribution as the response distribution. Major water properties (pH, salinity, hardness and iron) and farm characteristics, such as water source, presence of a supply system with water recirculation and birds age, were included in the models as fixed effects as putative risk factors. According to sampling design, random effects of farms, sampling site and season were also included. To facilitate the evaluation of the results and the discussion, water parameters were classified into categories according to their observed distribution. The software R 3.3.0 was used to perform linear quantile mixed models, whereas software SAS v.9.4 was adopted for the other statistical analyses.

RESULTS

The results of microbiological, chemical and physical analyses by source of water supply, sampling site and season are given in Tables 1 and 2. Regarding water temperature, a significant interaction between water source and season was found ($P < 0.01$): WW was significantly colder than TW (17.4 ± 3.6 *vs.* 20.6 ± 2.8) in summer, but not in winter (14.6 ± 2.1 *vs.* 13.3 ± 2.7). Chemical, physical and microbiological quality profiles of WW and TW were mostly within the limits of TW for humans (Counc. Dir. 98/83/EC). Median values of pH, sulphate, nitrate, iron and salinity levels were significantly different between TW and WW ($P < 0.05$). Except for pH, the same was found between winter and summer. Concerning the sampling site, copper median concentration was significantly higher at site B ($P < 0.001$) and C ($P = 0.041$) compared to A. Both sources had a lower microbiological quality at site B and C (e.g., up to 10^3 CFU/100 mL for *E. coli*) than A (Tables 1 and 2).

Salmonella enterica subsp. *enterica* serotype Kentucky was isolated only from WW at site A, both in summer and winter from the same farm. *Campylobacter* spp. was isolated only twice in a farm supplied with WW and from another one supplied with TW at site B and C in winter. Positivity at real-time PCR was found for *C. coli* in 30.4% of samples and for *C. jejuni* in 14.9% of samples. Table 2 shows the associations between *Campylobacter* spp. positivity at real-time PCR and source of water supply, season and sampling site; only season was found to be significant, with winter posing a higher risk for *C. jejuni* positivity in water. Table 3 shows the associations between putative risk factors for real-time PCR positivity to *C. jejuni*, *C. coli* and for total microbial count: low salinity and high hardness were identified as risk factors for presence of *C. coli* and *C. jejuni*, respectively.

DISCUSSION

While surface water is a recognized vehicle of disease transmission for poultry (Amaral, 2004), the present study showed that WW given to commercial turkeys in North-Easter Italy is of satisfactory quality for the analysed parameters and therefore represents a suitable alternative to TW for livestock. Most turkey farms presented levels of nitrates lower than the limits for potability (50 mg/L). High levels of nitrates can be related to contamination with residential, industrial or agricultural waste. In humans, consumption of drinking water with high nitrate levels (i.e. higher than 50 mg/L) may cause hypertrophy of the thyroid (Van Maanen et al., 1994). The toxicity for poultry has been reported at levels higher than 50 mg/L for chickens and 75 mg/L for turkeys, while in broilers nitrate levels greater than 20 mg/L may have a negative effect on growth and feed conversion (as reviewed by Carter and Sneed, 1996). Also naturally occurring chemicals (i.e. copper and iron) were within the recommended limits for poultry (Carter and Sneed, 1996). Copper levels higher than 600 μg/L produce bitter flavor that may reduce water consumption (Carter and Sneed, 1996). Iron

Table 1. Chemical and physical analyses of drinking water in 28 turkey farms supplied with either well or tap water during summer and winter of 2012–2013 in North-Eastern Italy. Water samples were collected twice in 2012–2013 (in winter and in summer) at 3 sampling sites: the water source (A), the beginning (B) and the end (C) of the nipple line. Data are expressed as median values with interquartile range (within parentheses). Significance (Sig.): *P < 0.05, **P < 0.01, ***P < 0.001.

Parameter	*Limits of potability* Dir.98/83/EC	*Maximum acceptable level*	Water source			Sampling site				Sampling season		
			Tap water	Well water	Sig.	A	B	C	Sig.	Summer	Winter	Sig.
pH	*6.5–9.5*	*>6[b]; 5–8[c]*	7.7 (0.4)	7.5 (0.4)	*	7.6 (0.5)	7.6 (0.4)	7.6 (0.4)		7.7 (0.4)	7.6 (0.4)	
Sulphate (mg/L)	*250*	*250[b]; 200[c]*	31.1 (27.0)	36.8 (59.9)	*	30.8 (45.1)	32.0 (42.4)	31.8 (47.9)		27.5 (31.5)	33.4 (59.6)	**
Phosphate (mg/L)			0.0 (0.1)	0.1 (0.5)		0.1 (0.3)	0.0 (0.2)	0.0 (0.2)		0.0 (0.4)	0.0 (0.2)	
Nitrate (mg/L)	*50*	*25[b,c]*	16.2 (11.8)	5.5 (17.8)	***	13.3 (15.8)	12.3 (17.11)	12.3 (18.0)		11.9 (17.3)	15.0 (18.4)	**
Copper (μg/L)		*600[b,c]*	4.5 (12.6)	3.1 (22.2)		1.9 (3.1)	10.4 (22.3)	6.7 (26.5)	**	3.2 (11.4)	6.9 (18.5)	
Chromium (μg/L)[a]	*50*		1.3 (1.8)	0.0 (0.1)		0.2 (1.7)	0.0 (1.4)	0.0 (1.3)		0.0 (1.2)	0.9 (1.8)	
Ammonia (mg/L)[a]	*0.5*		0.0 (0.0)	0.0 (0.0)		0.0 (0.0)	0.0 (0.0)	0.0 (0.0)		0.0 (0.0)	0.0 (0.2)	
Salinity (PSU)			0.3 (0.1)	0.4 (0.3)	***	0.3 (0.2)	0.3 (0.2)	0.3 (0.2)		0.2 (0.1)	0.3 (0.2)	***
Hardness (mg/L)		*110[c]*	240 (78)	245 (145)		240 (104)	252 (108)	240 (101)		260 (127)	240 (74)	
Iron (μg/L)	*200*	*300[b,c]*	1.1 (4.8)	3.5 (19.4)	*	1.1 (4.8)	1.8 (7.1)	2.3 (7.7)		1.1 (4.8)	2.8 (9.4)	*

[a]Statistical testing not performed because of insufficient non-zero observations.
[b]Carter and Sneed, 1996
[c]Aviagen Turkey Inc., 2015

and copper are contained in valves, pipes and fittings and are present in coatings and alloys. This can explain the higher concentrations at site B and C compared to A, as the main source of contamination is often the corrosion of interior plumbing (WHO, 2011). Iron levels higher than 300 μg/L affect flavor, turbidity and color of water, as well as staining of plumbing fixtures (WHO, 2011). Although a high level of iron (600 ppm) in water was not found to affect broiler performances (Fairchild et al., 2006), it may form chelates with apramycin and tetracyclines, thus limiting the therapeutic effect of water medication (Scandurra, 2013).

Well water had higher levels of hardness in comparison to TW. However, samples from both sources often presented levels higher than 200 mg/L, which is known to be critical for causing calcium deposits in the pipeline (Enne et al., 2006). Hard water has not been demonstrated to have either a positive or a negative direct impact on poultry health and performance (Carter and Sneed, 1996). However, hardness can decrease drugs solubility, preventing animals from receiving an effective dose (Scandurra, 2013). Although pH was within the range of acceptability for potable water (European Commission, 1998), it was significantly lower in WW. A level of pH lower than 5.5 can create problems to the urinary and digestive systems, bone demineralization and fragility, as well as corroding the pipeline and being incompatible with some medicines and vaccines (Enne et al., 2006). Water pH ranging between 6.0 and 6.3 is suspected of having negative effects on poultry performances (Carter and Sneed, 1996). On the other hand, Grizzle et al. (1996) found that a water pH of 6.25 did not negatively affect broiler growth in comparison with a water pH of 6.75. Rather, a water pH of 5.75 negatively affected it in comparison with a water pH of 6.25 and 6.75, respectively. Only a few samples had a high level of ammonia, whose contamination can derive from industrial and agricultural waste (WHO, 2011). To the best of our knowledge, we are not aware of any studies describing the effect of high levels of water ammonia on poultry health. However, ammonia can react with chlorine to reduce free chlorine and to form chloramines (WHO, 2011).

Most samples presented a total microbial count (a general indicator of pipeline hygiene) under the limits for TW, and a very low fecal contamination (i.e. presence of *E. coli* and *Enterococcus*). Instead, a significant decrease in microbiological water quality was found at the nipple line. Water samples varied in hardness and iron levels. High levels of iron and hardness are known to be risk factors for biofilm deposition and bacterial proliferation in the pipeline (Wingender and Flemming, 2011). Specifically, iron promotes the growth of bacteria that derive their energy from the oxidation of ferrous iron to ferric iron (WHO, 2011). The formation of biofilms increases with the flow velocity of water (Lehtola et al., 2006); therefore, bird age is indirectly a further risk factor, as when birds are young the limited water consumption may be associated with a low

Table 2. Microbiological analyses of drinking water in 28 turkey farms supplied with either well or tap water during summer and winter of 2012–2013 in North-Eastern Italy. Water samples were collected twice in 2012–2013 (in winter and in summer) at 3 sampling sites: the water source (A), the beginning (B) and the end (C) of the nipple line. Significance for P < 0.05.

		Water source			Sampling site				Sampling season		
Parameter	Category	Tap	Well	*P*	A	B	C	*P*	Summer	Winter	*P*
Total microbial count 22°C	$>10^3$ UFC/mL (%)	60.7	46.4	0.29	5.4	71.4	83.9	<0.001	58.3	48.8	0.09
E. coli and Enterococcus	$>10^2$ UFC/100 mL (%)	15.5	35.7	0.44	7.1	35.7	33.9	<0.001	31.0	20.2	0.06
Campylobacter jejuni	PCR+ (%)	15.5	15.5	0.55	10.7	19.6	16.1	0.66	8.3	22.6	0.09
Campylobacter coli	PCR+ (%)	32.1	28.6	0.21	26.8	35.7	28.6	0.79	17.9	42.9	<0.001

Table 3. Risk factors for RT-PCR positive samples to *Campylobacter jejuni*, *Campylobacter coli* and for total microbial count (TMC 22°C) $>10^2$ in 28 turkey farms sampled in North-Eastern Italy in summer and winter 2012–2013. Continuous variables were classified on the basis of the data distribution. Significance for P < 0.05.

		Campylobacter coli			*Campylobacter jejuni*			TMC 22°C $>10^2$		
Parameter	Category	n.	%	*P*	n.	%	*P*	n.	%	*P*
Supply system with water recirculation	Yes	42	35.0	0.08	19	15.8	0.87	66	55.0	0.83
	No	9	18.8		7	14.6		24	50.0	
Birds age (days)	≤30	13	31.0	0.35	4	9.5	0.45	20	47.6	0.58
	30–50	18	37.5		10	20.8		27	56.3	
	51–75	12	28.6		6	14.3		26	61.9	
	>75	8	22.2		6	16.7		17	47.2	
pH	≤7.36	39	28.2	0.94	39	12.8	0.86	39	12.8	0.49
	7.37-7.63	45	37.8		45	13.3		45	13.3	
	7.64-7.83	41	29.3		41	19.5		41	19.5	
	>7.83	43	25.6		43	16.3		43	16.3	
Salinity (PSU)	≤0.21	42	35.7	*0.04*	42	16.7	0.11	42	4.8	0.80
	0.22-0,28	39	38.5		39	10.3		44	15.9	
	0.29-0,38	43	20.9		43	14.0		48	20.8	
	>0.38	44	27.3		44	20.5		34	20.6	
Hardness (mg/L)	≤191	42	21.4	0.40	42	4.8	*0.05*	42	16.7	0.37
	192–240	44	34.1		44	15.9		39	10.3	
	241–300	48	29.2		48	20.8		43	14.0	
	>300	34	38.2		34	20.6		44	20.5	
Iron (μg/L)	≤ LOD	47	23.4	0.55	47	8,5	0.76	47	8.5	0.35
	LOD – 2.28	37	40.5		37	21.6		37	21.6	
	2.29-12.22	42	26.2		42	11.9		42	11.9	
	>12.22	42	33.3		42	21.4		42	21.4	

water flow. Yet, poor microbiological water quality was not significantly associated with its physical and chemical properties, nor with the presence of a water recirculation systems and birds' age. Taken together, these results call for improvements in microbiological water quality as directly related to sanitization procedures applied by farmers, which need to be reviewed. Farmers had also declared to routinely use different commercial products to guarantee pipeline hygiene. However, as previously suggested (Sparks, 2009), the efficacy of these products may largely differ depending on water properties.

Isolation of potentially pathogenic microorganisms was generally uncommon. However, *S.* Kentucky was isolated from the same WW samples in different seasons at the water source, raising some concerns on the potential role of WW as vehicle of *Salmonella* transmission. Also *Campylobacter* was rarely isolated, although the positivity detected by real-time PCR ranged between 8% to 43%, irrespective of the sampling site. Pipeline hygiene was not influenced by season, while *Campylobacter* positivity was higher in winter. This is in accordance with previous findings which identified an optimal *Campylobacter* survival at low temperatures (around 4°C) (Thomas et al., 1998).

The low frequency of *Campylobacter* isolation confirms the well know limitation of culture-based procedures in isolating the microorganism from water samples, which is likely to underestimate the true prevalence due to the high susceptibility of *Campylobacter* to suboptimal environmental conditions (Thomas et al., 1998; Chaisowwong et al., 2012). Moreover, the bacterium can be present in water also as viable but nonculturable forms, which are able to survive under adverse conditions (Rollins and Colwell, 1986). For these reasons, real-time PCR is a valid support to detect the microorganism in water and to understand possible routes of transmission. While no significant association between *Campylobacter* spp. presence and source of water supply was found, low salinity and high hardness were identified as risk factors for presence of *C. coli* and *C. jejuni*, respectively. Moreover, there was a tendency towards significance (P = 0.08) in the association between presence of a water recirculation system and *C. coli*. As previously suggested (Sahin et al., 2015), *Campylobacter* transmission to birds is more likely to

occur from the farm environment through water, rather than from the water source itself (Bull et al., 2006; Mughini-Gras et al., 2016).

In conclusion, the results of the present study call for improvements in sanitization procedures for farm drinking water pipelines, highlighting also issues related to drinking water characterized by high levels of hardness and iron. While water recirculation systems, bird age, and most chemical and physical water properties did not seem to be associated with microbiological water quality, low salinity and high hardness were specific risk factors for *C. coli* and *C. jejuni* presence, respectively. Although *Campylobacter* spp. isolation from water samples was problematic, detection of *Campylobacter* spp. genetic material showed that this zoonotic pathogen is highly prevalent in the farm pipeline.

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