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Potential microbiological contamination of effluents in poultry and swine abattoirs

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SUMMARY

Health risks in the effluents of seven swine abattoirs and of seven poultry abattoirs were evaluated with regard to environment degradation and to dissemination of pathogenic microorganisms during the rainy and dry seasons. Supply-water samples from affluents and effluents of the treatment systems at different sites within the abattoir processing system were analysed. Similarly, water samples from the three recipient sites (emission point, 100 m upstream, 100 m downstream) were also analysed. Temperature, free residual chlorine (FRC), total coliform bacteria, *Escherichia coli*, enterococci, identification and serotyping of salmonellae were assessed. Scalding is the most significant stage in the slaughtering chain ($P<0{\cdot}05$) when temperature is taken into account. Temperatures at effluents and at the sampled sites in the water bodies accorded to state and federal legislation standards. Supply waters did not meet the standards for FRC and microbial count standards according to the Ministry of Health and within limits imposed by the Industrial and Sanitary Inspection Regulations for Animal Products. Feather plucking and evisceration in poultry slaughter and the cleansing of carcasses and facilities in poultry and swine slaughtering had the highest contamination impact. The three loci at the water bodies were above the microbiological standards for classes II and III sites, in conformity with Law 8468 of the state of São Paulo, Brazil and Conama. *Salmonella* was found at several sites during slaughter, at both types of abattoirs, including in the effluent treatment system. This showed that these sites were the dissemination sources of the microorganism.

INTRODUCTION

Water-transmitted diseases have been the cause of high mortality rates in rural areas. Some deaths have been linked to water polluted by non-treated waste originating from domestic and industrial activities. Nigeria is a typical example of such a situation. To make matters worse, contaminated water is also used for abattoir cleaning [1, 2].

Many researchers have reported discharge of waste water into streams and degradation in the ecology of water systems. Benka-Coker & Ojior [1] associated human health with the possibility of accumulation of pathogenic enteric microorganisms by aquatic organisms.

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During the last 10–15 years many countries have detected a serious and dramatic increase in salmonellosis. Swine and poultry are the most common *Salmonella* reservoirs among different animal species [3–12], and it has been shown that only a few colony-forming units (c.f.u.) are necessary for infection in predisposed subjects [7].

Recent research [12–14] has confirmed that the slaughter of these animals produces a high probability of carcass contamination by potential pathogenic microorganisms, such as *Salmonella* spp., and that there is no possibility for the total elimination of health risks in the process.

Past investigations [12, 15–17] have shown that the consequences of *Salmonella* in water may be very serious. Several studies [1, 18–23] have actually indicated a strong link between the presence of *Salmonella* spp., and enterococci with faecal coliform bacteria density over 1×10^3 c.f.u./100 ml.

Few specific references exist with regard to *Salmonella* in poultry and swine abattoir effluents in spite of increasing concern about this bacterium at different processing sites within the abattoirs. This fact may be linked to the microorganism's characteristics, especially to its being a bad competitor when compared to other microorganisms; mainly faecal contamination indicators which are present in high quantities in effluents.

Research was undertaken during the rainy and dry seasons to enhance physical characterization by temperature and concentrations of free residual chlorine (FRC) recording, microbiological characterization by counting total coliform bacteria, *Escherichia coli* and enterococci, and the determination of the prevalence of *Salmonella* in water and waste water from poultry and swine abattoirs and in the recipient water bodies.

MATERIALS AND METHODS

Characterization of abattoirs

Seven poultry and seven swine abattoirs in the state of São Paulo, Brazil, were analysed. Whereas four poultry abattoirs were under the supervision of the State Inspection Service (SISP), three came under the Federal Inspection Service (SIF). In contrast, four swine abattoirs were supervised by SIF and three by SISP. Details about the systems of treatments are given in Tables 1 and 2.

Characterization of sample sites, collection and transport of samples

Water-sample sites in poultry abattoirs were supply source, scald tank, plucking tank, evisceration, carcass cleaning, pre-cooling tanks, cooling tanks, cleaning of facilities, affluent and effluent of treatment system, emission site in the stream, 100 m upstream from the emission site, and 100 m downstream from the emission site.

Water-sample sites in swine abattoirs were supply source, scald tank, carcass cleaning, cleaning of facilities, affluent and effluent of treatment system, emission site in the stream, 100 m upstream from the emission site, and 100 m downstream from the emission site.

Collection procedures took place during normal working days, in the morning, between May and September 2003 (dry period) and between January and March 2004 (rainy period). The collection procedures, per abattoir, were done once a week, in each period.

Supply water from the two different types of abattoirs was collected according to APHA [24]. Waste water from the slaughtering room, affluents and effluents, waste treatment system and water from the three sites of the receiving stream were collected in 1000-ml sterilized polyethylene flasks. Measuring tapes were used to determine sites 100 m upstream and 100 m downstream from the emission site of effluents in the receiving stream.

In the poultry abattoirs, the number of samplings per day was 13. Therefore, in both the dry and rainy period there were 91 samples per week. In the swine abattoirs, there were 9 samples per day, giving 63 samples per week in both the dry and rainy period.

Samples were transported in isothermal boxes with ice to the Biomass Laboratory of the Department of Rural Engineering and to the Water and Food Analyses Laboratory of the Veterinary Department and Animal Reproduction of the Faculty of Agrarian and Veterinary Sciences in Jaboticabal, São Paulo, Brazil.

Laboratory analyses

Temperature rates

Temperature was registered by Corning PS (Corning, NY, USA) 16 digital thermometer.

Concentrations of FRC

Concentrations of FRC were determined according to Hanna Instruments Inc. [25] by *N*,*N*-diethyl paraphenyl (DPD).

Table 1. *Characteristics of poultry abattoirs (ABT) in which samples were collected between May and September 2003 (dry season) and January and March 2004 (rainy season) in the interior of the state of São Paulo, Brazil*

	ABT 1	ABT 2	ABT 3	ABT 4	ABT 5	ABT 6	ABT 7
Inspection	State	State	State	Federal	Federal	State	Federal
Animals killed per day	24 000	13 000	5000	135 000	16 128	8000	105 000
Recovery of blood	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Staff	130	80	25	738	70	80	480
Deposit of human excretion	Stream	Cesspit	Cesspit	Municipal treatment station	Abattoir treatment station	Municipal treatment station	Cesspit
Water source supplying abattoir	Well, depth >20 m	Well, depth >20 m	Well, depth >20 m	Well, depth >20 m	Well, depth >20 m	Well, depth >20 m	Water source and well, depth >20 m
Summary of treatment of affluent	Admission	Primary treatment, secondary treatment (2 aired stabilizing pools and one polishing pool), stream	Primary treatment; secondary treatment (3 stabilizing pools), fertilizing irrigation	Primary treatment, secondary treatment (2 aerobic pools and 1 anaerobic pool), stream	Primary treatment, secondary treatment (1 selected stabilizing pool and 1 anaerobic pool), stream	Primary treatment, secondary treatment (1 aired stabilizing pool), stream	Primary treatment, secondary treatment (3 anaerobic stabilizing pools), stream
Volume of affluent (yearly average)	720 m^3/day	200 m^3/day	75 m^3/day	2400 m^3/day	458 m^3/day	120 m^3/day	1575 m^3/day
Class of receiving body	4	2	No data	3	2	2	2
Cleaning (pre-cleaning, detergency and hygienization)	Daily	Daily	Daily	Daily	Daily	Daily	Daily

Table 2. *Characteristics of swine abattoirs (ABT) in which samples were collected between May and September 2003 (dry season) and between January and March 2004 (rainy season) in the interior of the state of São Paulo, Brazil*

	ABT 1	ABT 2	ABT 3	ABT 4	ABT 5	ABT 6	ABT 7
Inspection	State	Federal	State	Federal	State	Federal	Federal
Animals killed per day	60	250	35	110	150	700	1000
Recovery of blood	Yes	Yes	No	Yes	Yes	Yes	Yes
Staff	12	68	10	80	13	92	286
Deposit of human excretion	Municipal treatment station	Cesspit	Cesspit	Municipal treatment station	Cesspit	Cesspit	Cesspit
Water source supplying abattoir	Well, depth >20 m	Well, depth >20 m	Well, depth >20 m	Well, depth >20 m	Well, depth >20 m	Well, depth >20 m	Well, depth 20 m
Summary of treatment of affluent	Municipal treatment station	Primary treatment, secondary treatment (5 selected stabilizing pools), fertilizing irrigation	Primary treatment, secondary treatment (1 selected stabilizing pool), fertilizing irrigation	Primary treatment, secondary treatment (1 selected stabilizing pool), fertilizing irrigation), stream	Primary treatment, secondary treatment (1 selected stabilizing pool), fertilizing irrigation), fertilizing irrigation	Primary treatment, secondary treatment (1 anaerobic stabilizing pool and three selected pools), stream	Primary treatment, secondary treatment (2 anaerobic stabilizing pools, 1 aerobic pool, one drying pool and 1 level pools), stream
Volume of effluent (annual average)	12 m^3/day	100 m^3/day	17 m^3/day	55 m^3/day	90 m^3/day	350 m^3/day	500 m^3/day
Class of receiving body	2	No data	No data	2	2	2	2
Cleaning (pre-cleaning, detergency and hygienization)	Weekly	Daily	Daily	Daily	Weekly	Daily	Daily

Determination of most probably numbers (MPN) of total coliform bacteria and Escherichia coli [24]

Samples were first diluted in peptone water 0·1% (Oxoid, Basingstoke, Hants, UK); 10 ml of the sample were added to 90 ml of the diluent, producing a dilution of 10^{-1}. Successive decimal dilutions were obtained from the above original dilution.

Coliform bacteria were counted by the chromogenic-fluorogenic-hydrolysable substrate technique. A Colilert flask (Idexx Laboratories Inc., Westbrook, ME, USA) was added to 100 ml of sample or its dilutions, followed by homogenization, transference to a tray (Idexx) and the deployment of a model sealer 1295.00 1E-E (Idexx) in which the sample was distributed in cells and then sealed.

Yellow cells were counted after incubation at 35 °C/24 h. The Most Probable Number (MPN) of total coliform bacteria per 100 ml of sample was obtained from a specific MPN table. UV light was focused on the tray and the MPN of *E. coli*/100 ml of sample was obtained by the number of fluorescent cells and with the MPN table mentioned above.

Determination of MPNs of enterococcus [24]

Initially samples underwent the same dilution processes described previously.

In samples where microbial counting was expected to be low, detection of enterococci was performed by the fluorogenic substrate technique. A sample of 100 ml water or its dilution was mixed with Enterolert fluorogenic substrate (Idexx) and transferred to a tray (Idexx) after homogenization. The tray was transported to model sealer 1295.00 1E-E (Idexx) where the sample was distributed in cells and the tray sealed. After incubation at 41 °C for 24 h, fluorescent cells were counted under UV radiation. MPN of enterococci/100 ml of sample was obtained using the specific MPN table.

In samples where a higher microbial accumulation (>10 per ml) was expected the multiple tube method was used for enterococcus detection. Dilutions of 10^{-1} and subsequent dilutions were distributed in a set of five tubes per dilution. Tubes contained culture medium Chromocult Enterococci Broth (Merck KGaA – 64271; Darmstadt, Germany). Reading was done after incubation for 24 ± 4 h at 44 °C: tubes with a greenish-blue colour were positive and indicated the presence of enterococci. The number of positive tubes were checked by the MPN table and results were expressed in MPN/100 ml.

Isolation and serotyping of Salmonella *spp. bacteria*

Sample pre-enrichment was done according to Barros *et al.* [26]. Enrichment phases, selective plating and presumed identification were done according to Barros *et al.* [26]; Holt *et al.* [5] and Bonardi *et al.* [14].

Isolates, suggesting *Salmonella* spp., were tested with antigen by agglutination tests with polyvalent serums O Poli A-I (Bacto Laboratories Pty Ltd, Liverpool, NSW, Australia) and H (Difco; Becton Dickinson & Co., Sparks, MD, USA). The Adolfo Lutz Institute of São Paulo, Brazil performed the serotyping.

Analysis of results

Tukey's test at the 5% significance level was employed for analysis of the results [27]. See Steel & Torrie [28] for the program and descriptions used.

RESULTS AND DISCUSSION

Average temperatures

Table 3 shows that average temperatures ranged between 9 °C and 54 °C during the dry season and between 7 °C and 57 °C during the rainy season in sampling sites of the poultry abattoirs. Water from scalding and plucking had significantly higher average temperatures compared with those at other sampling sites, whereas water from cooling had significantly lower average temperatures. Scald-tank water average temperature reached 54 °C and 57 °C respectively during the dry and rainy seasons; water average temperature from the plucking tank reached 31 °C and 29 °C respectively during the dry and rainy seasons; water average temperature from the cooling tank reached 9 °C and 7 °C respectively during the dry and rainy seasons.

Table 4 shows mean temperatures in water samples in swine abattoirs. Temperatures ranged between 20 °C and 61 °C and between 21 °C and 61 °C respectively during the dry and rainy seasons.

It has been verified that only the water of the scald tank had significant temperatures ($P < 0{\cdot}05$) when compared with those of other sampling sites. No significant differences ($P > 0{\cdot}05$) were detected between the poultry and swine abattoirs with regard to mean temperature rates in the water of supply source, affluents, effluents and water bodies (emission site, 100 m upstream, 100 m downstream) either during the dry or rainy seasons.

Table 3. *Average temperatures of supply water in the different phases of the slaughtering process, in affluents and effluents of the waste treatment systems, and in three sites of the recipient water bodies, collected in poultry abattoirs between May and September 2003 (dry season) and between January and March 2004 (rainy season) in the state of São Paulo, Brazil*

	Temperature (°C)	
Sampling site	Dry season	Rainy season
Supply	23^{Aa}	24^{Aa}
Scald tank	54^{Ab}	57^{Ab}
Feather-plucking facility	31^{Ac}	29^{Ac}
Evisceration facility	24^{Aa}	24^{Aa}
Carcass cleaning	23^{Aa}	25^{Aa}
Pre-cooling	24^{Aa}	26^{Aa}
Cooling	9^{Ad}	7^{Ad}
Cleaning of facilities	21^{Aa}	24^{Aa}
Affluent of treatment systems	22^{Aa}	23^{Aa}
Effluent of treatment system	22^{Aa}	26^{Aa}
Emission site in the stream	21^{Aa}	22^{Aa}
100 m downstream from emission site	21^{Aa}	23^{Aa}
100 m upstream from emission site	21^{Aa}	22^{Aa}

In each line values with different capital letters are significantly different by Tukey's test at the 5% significance level. In each column values with different lower-case letters are significantly different by Tukey's test at the 5% significance level.

Table 4. *Arithmetic temperature rate means, of supply water in the different phases of the slaughtering process, in affluents and effluents of the waste treatment systems, and in three sites of the recipient water bodies, collected in swine abattoirs between May and September 2003 (dry season) and between January and March 2004 (rainy season) in the state of São Paulo, Brazil*

	Temperature (°C)	
Sampling site	Dry season	Rainy season
Supply	22^{Aa}	25^{Aa}
Scald tank	61^{Ab}	61^{Ab}
Carcass cleaning	23^{Aa}	24^{Aa}
Cleaning of facilities	27^{Aa}	25^{Aa}
Affluent of treatment systems	23^{Aa}	26^{Aa}
Effluent of treatment systems	20^{Aa}	25^{Aa}
Emission site in the stream	21^{Aa}	23^{Aa}
100 m downstream from emission site	21^{Aa}	21^{Aa}
100 m upstream from emission site	20^{Aa}	21^{Aa}

In each line values with different capital letters are significantly different by Tukey's test at the 5% significance level. In each column values with different lower-case letters are significantly different by Tukey's test at the 5% significance level.

Table 5. *Average of concentrations of free residual chlorine in supply water collected in poultry and swine abattoirs between May and September 2003 (dry season) and between January and March 2004 (rainy season) in the state of São Paulo, Brazil*

	Free residual chlorine	
Supply water (mg/l)	Dry season	Rainy season
Poultry abattoirs	$0{\cdot}36^{Aa}$	$0{\cdot}46^{Aa}$
Swine abattoirs	$0{\cdot}33^{Aa}$	$0{\cdot}39^{Aa}$

In each line values with different capital letters are significantly different by Tukey's test at the 5% significance level. In each column values with different lower-case letters are significantly different by Tukey's test at the 5% significance level.

Although anaerobic degradation rate of organic wastes increases with temperature, the temperature effect is always lower than that found by Van't Hoff's formula which shows that levels of chemical reactions double every 10 °C [29, 30].

Working in Iran on the efficiency of treatment systems for waste abattoirs water, Torkian *et al.* [31] reported similar temperatures at affluent and effluent sites of treatment systems.

Johns [32] found that temperatures at affluents and effluents of abattoir treatment systems vary significantly worldwide. They are frequently low in Europe whereas rates in Australia vary between 30 °C and 35 °C. The same researcher states that in subtropical areas temperature rise is beneficial since the biological systems necessary for waste treatment are more efficient around 37 °C. However, fat emulsification at high temperatures causes a series of problems especially in intensive treatment systems such as plants with activated slime.

It must be emphasized that mean temperatures of effluents in the two types of abattoir and in the two climatic periods (Tables 3 and 5) remain below the 40 °C maximum rate for the emission of effluents in

Table 6. *Average of log units of total coliform bacteria* (*TC*), Escherichia coli (*EC*) *and enterococci* (*ET*), *in log, in supply water of the different slaughtering stages and of the effluents of abattoirs, collected in poultry abattoirs between May and September 2003* (*dry season*) *and between January and March 2004* (*rainy season*) *in the state of São Paulo, Brazil*

	TC		EC		ET	
Sampling site	Dry	Rainy	Dry	Rainy	Dry	Rainy
Supply	$0{\cdot}3^{Aa}$	$0{\cdot}9^{Ba}$	$0{\cdot}1^{Aa}$	$0{\cdot}4^{Aa}$	$0{\cdot}1^{Aa}$	$0{\cdot}2^{Aa}$
Scald tank	$3{\cdot}3^{Ab}$	$3{\cdot}2^{Ab}$	$3{\cdot}0^{Ab}$	$3{\cdot}1^{Ab}$	$4{\cdot}5^{Ab}$	$4{\cdot}4^{Ab}$
Plucking facilities	$6{\cdot}8^{Ac}$	$9{\cdot}0^{Ac}$	$6{\cdot}4^{Ac}$	$8{\cdot}8^{Ac}$	$7{\cdot}1^{Ac}$	$7{\cdot}4^{Ac}$
Evisceration facilities	$7{\cdot}9^{Ac}$	$7{\cdot}9^{Ac}$	$7{\cdot}4^{Ac}$	$7{\cdot}5^{Ac}$	$5{\cdot}1^{Ac}$	$5{\cdot}9^{Ac}$
Carcass cleaning	$5{\cdot}4^{Ac}$	$6{\cdot}7^{Ac}$	$5{\cdot}0^{Ac}$	$6{\cdot}5^{Ac}$	$5{\cdot}8^{Ac}$	$5{\cdot}6^{Ac}$
Pre-cooling	$4{\cdot}0^{Ab}$	$3{\cdot}6^{Ab}$	$3{\cdot}8^{Ab}$	$4{\cdot}5^{Ab}$	$2{\cdot}8^{Ab}$	$3{\cdot}3^{Ab}$
Cooling	$3{\cdot}4^{Ab}$	$3{\cdot}5^{Ab}$	$3{\cdot}0^{Ab}$	$3{\cdot}1^{Ab}$	$2{\cdot}1^{Ab}$	$2{\cdot}9^{Ab}$
Cleaning of facilities	$4{\cdot}4^{Ab}$	$7{\cdot}8^{Bc}$	$3{\cdot}9^{Ab}$	$6{\cdot}8^{Bc}$	$3{\cdot}5^{Ab}$	$6{\cdot}7^{Bc}$
Effluent of abattoir	$7{\cdot}6^{Ac}$	$7{\cdot}9^{Ac}$	$7{\cdot}3^{Ac}$	$7{\cdot}2^{Ac}$	$7{\cdot}4^{Ac}$	$5{\cdot}7^{Ac}$

In each line values with different capital letters are significantly different by Tukey's test at the 5% significance level. In each column values with different lower-case letters are significantly different by Tukey's test at the 5% significance level.

the environment, as ruled by Resolution 357 Conama [33] and Decree 8468 [34].

Under the circumstances mentioned above mean temperature rates at the three recipient sites (emission site, 100 m upstream and 100 m downstream) remain below the 30 °C maximum rate, as ruled by Resolution 357 Conama [33].

Free residual chlorine

Table 5 shows mean concentrations of FRC in the supply water of poultry and swine abattoirs during the dry and rainy seasons.

Mean FRC concentrations in poultry abattoirs were 0·36 mg/l and 0·46 mg/l respectively during the dry and rainy periods, with no significant difference ($P>0{\cdot}05$) between the two collection periods. FRC rates of 0·33 mg/l and 0·39 mg/l were found in the supply water of swine abattoirs, with no significant difference ($P>0{\cdot}05$). There was no difference ($P>0{\cdot}05$) between concentrations of FRC in the supply water of poultry and swine abattoirs (Table 5).

According to Decree 518 of the Brazilian Ministry of Health [35], FRC concentrations in supply water must range between 0·5 mg/l and 2 mg/l. On the other hand, the Industrial and Sanitary Inspection Regulations for Animal Products – Riispoa [36] defines 1 mg/l FRC for water used in industries producing animal products for human consumption. Consequently, Table 5 shows that supply water had lower FRC concentrations during the two periods at the two types of abattoir than those prescribed by Decree 518 [35] and by Riispoa [36]. This is very dangerous because there is not sufficient chlorine to promote disinfection in these water and the risk to the public's health becomes greater.

Microorganisms indicating faecal contamination

Tables 6 and 7 show mean log rates of MPN for total coliform bacteria, *E. coli* and enterococci in supply water during the different slaughtering stages and at the effluent sites of poultry and swine abattoirs respectively.

Whereas evisceration and plucking sites and carcass and facilities cleaning were the most accountable sites for microorganisms in the waste water of poultry abattoirs, carcass and facilities cleaning contributed the most for the presence of microorganisms in swine abattoirs.

Plucking, evisceration and carcass cleaning stages in poultry abattoirs failed to present different rates ($P>0{\cdot}05$) among themselves and with the abattoir's effluent. This fact shows the accountability of these sites for faecal microorganisms, including pathogens, in waste water contamination in poultry abattoirs. Another salient point is the contamination of supply water by microorganisms caused by low FRC.

According to Decree 518 of the Brazilian Ministry of Health [35] absence of total coliform bacteria and *E. coli* in water for human consumption is mandatory. Nevertheless, this has not been verified in supply water in poultry and swine abattoirs during the rainy and the dry seasons.

Table 7. *Average of log units of total coliform bacteria* (*TC*), Escherichia coli (*EC*) *and enterococci* (*ET*), *in log, in supply water at different stages of the slaughtering process and of the effluents of abattoirs collected in swine abattoirs between May and September 2003* (*dry season*) *and between January and March 2004* (*rainy season*) *in the state of São Paulo, Brazil*

	TC		EC		ET	
Sampling site	Dry	Rainy	Dry	Rainy	Dry	Rainy
Supply	$0{\cdot}1^{Aa}$	$0{\cdot}3^{Aa}$	$0{\cdot}1^{Aa}$	$0{\cdot}1^{Aa}$	$0{\cdot}1^{Aa}$	$0{\cdot}1^{Aa}$
Scald tank	0^{Aa}	$0{\cdot}4^{Aa}$	0^{Aa}	$0{\cdot}4^{Aa}$	$0{\cdot}6^{Aa}$	$0{\cdot}8^{Aa}$
Cleaning of carcass	$4{\cdot}6^{Ab}$	$5{\cdot}5^{Ab}$	$4{\cdot}1^{Ab}$	$4{\cdot}8^{Ab}$	$3{\cdot}9^{Ab}$	$3{\cdot}5^{Ab}$
Cleaning of facilities	$5{\cdot}3^{Ab}$	$5{\cdot}8^{Ab}$	$4{\cdot}6^{Ab}$	$5{\cdot}1^{Ab}$	$3{\cdot}9^{Ab}$	$3{\cdot}7^{Ab}$
Effluent of abattoir	$6{\cdot}7^{Ab}$	$7{\cdot}3^{Ab}$	$6{\cdot}1^{Ab}$	$6{\cdot}9^{Ab}$	$4{\cdot}5^{Ab}$	$4{\cdot}0^{Ab}$

In each line values with different capital letters are significantly different by Tukey's test at the 5% significance level. In each column values with different lower-case letters are significantly different by Tukey's test at the 5% significance level.

Riispoa [36] rules that supply water in industries producing animal products for human consumption must contain a maximum concentration of 23 MPN/100 ml. Tables 6 and 7 show compliance to the Riispoa ruling with regard to supply water during the dry and rainy seasons in poultry and swine abattoirs [36]. However, describing verocytotoxin-producing *E. coli* (VPEC) strains in samples of supply water (9·09%) collected in swine abattoirs in France, Bouvet *et al.* [37] insist that supply water may be a potential source of contamination in abattoirs.

As with *E. coli*, enterococci are related to the faecal contamination of water. Although its absence in supply water is mandatory, this fact has not been verified in any sample of supply water from poultry and swine abattoirs (Tables 6 and 7).

High temperatures in the water of scald tanks of both industries, in this study, may have produced low rates of coliform bacteria and enterococci in the water. However, it has been verified that only the temperature of the scald tank water within the swine slaughtering process remained over 60 °C, as registered by Berends *et al.* [38] and by Swanenburg *et al.* [9]. This has contributed to lower microbial counts than those found in scald-tank water in poultry abattoirs (Tables 6 and 7).

Similar results were registered by Camps [3], Baudisova [39] and Bouvet *et al.* [40]. Finding *stx* genes from VPEC strains in water samples of scald tanks (9·67%), the above authors state that regular cleaning and disinfection during the slaughtering process may avoid or, at least, lessen cross-contamination of carcasses within the slaughtering facilities. This statement has been tested and refuted by Cherrington *et al.* [41].

Low temperatures in pre-cooling and cooling tanks of poultry abattoirs (Table 3) may explain the low microbial accumulation in the water of these sampling sites.

Tables 6 and 7 show that, within the poultry slaughtering process, water collected at the plucking, evisceration and carcass cleaning sites had the highest contamination rate at effluents during the dry and rainy periods. The same may be said with regard to facilities cleaning during the rainy period. On the other hand, within the swine slaughtering process, water from carcass and facilities cleaning during the dry and rainy seasons had the highest rate of microorganisms at the abattoirs' effluents.

An increase in faecal indicators has also been noted during the slaughtering process, with a subsequent contamination of the environment by microorganisms, which may be a source of contamination for industrialized food. Adequate hygiene and sanitization should be introduced during and after slaughtering to minimize contamination of the environment and produce safe food.

Table 8 shows mean rates of total coliform bacteria, *E. coli* and enterococci in affluents and effluents of the waste-treatment systems and at the three recipient sites (emission, 100 m upstream, 100 m downstream), during the dry and rainy periods, in the two types of abattoirs.

Contamination rates of coliform bacteria and enterococci, ranging from 10^3 to 10^7, in the affluents and effluents of the treatment systems and at the collecting sites of streams, from the poultry and swine abattoirs analysed in the current research, coincide with those reported by Benka-Coker & Ojior [1] and Fransen *et al.* [42]. These authors investigated the impact of

Table 8. *Average of total coliform bacteria* (*TC*), Escherichia coli (*EC*) *and enterococci* (*ET*), *in log, in water of affluents and effluents of waste treatment systems, and at three sites of receiving stream, collected in poultry* (*P*) *and swine* (*S*) *abattoirs between May and September 2003* (*dry season*) *and between January and March 2004* (*rainy season*) *in the state of São Paulo, Brazil*

	TC				EC				ET			
	Dry		Rainy		Dry		Rainy		Dry		Rainy	
Sampling site	P	S	P	S	P	S	P	S	P	S	P	S
Affluent of treatment system	$7{\cdot}6^{A}$	$6{\cdot}7^{A}$	$7{\cdot}9^{A}$	$7{\cdot}3^{A}$	$7{\cdot}3^{A}$	$6{\cdot}1^{A}$	$7{\cdot}2^{A}$	$6{\cdot}9^{A}$	$7{\cdot}4^{A}$	$4{\cdot}5^{A}$	$5{\cdot}7^{A}$	$4{\cdot}0^{A}$
Effluent of treatment system	$6{\cdot}0^{A}$	$6{\cdot}5^{A}$	$5{\cdot}8^{A}$	$6{\cdot}6^{A}$	$4{\cdot}7^{A}$	$5{\cdot}6^{A}$	$4{\cdot}6^{A}$	$4{\cdot}8^{A}$	$4{\cdot}6^{A}$	$4{\cdot}2^{A}$	$4{\cdot}1^{A}$	$3{\cdot}9^{A}$
Emission site in stream	$5{\cdot}3^{A}$	$6{\cdot}0^{A}$	$5{\cdot}5^{A}$	$5{\cdot}8^{A}$	$4{\cdot}6^{A}$	$5{\cdot}3^{A}$	$4{\cdot}8^{A}$	$5{\cdot}4^{A}$	$4{\cdot}0^{A}$	$6{\cdot}0^{A}$	$4{\cdot}5^{A}$	$5{\cdot}4^{A}$
100 m downstream from emission site	$5{\cdot}9^{A}$	$4{\cdot}9^{A}$	$6{\cdot}7^{A}$	$4{\cdot}9^{A}$	$4{\cdot}8^{A}$	$4{\cdot}0^{A}$	$6{\cdot}2^{A}$	$4{\cdot}3^{A}$	$4{\cdot}5^{A}$	$4{\cdot}2^{A}$	$4{\cdot}9^{A}$	$4{\cdot}0^{A}$
100 m upstream from emission site	$5{\cdot}5^{A}$	$4{\cdot}6^{A}$	$6{\cdot}1^{A}$	$4{\cdot}7^{A}$	$4{\cdot}3^{A}$	$3{\cdot}1^{A}$	$5{\cdot}5^{A}$	$3{\cdot}4^{B}$	$3{\cdot}3^{A}$	$4{\cdot}4^{A}$	$5{\cdot}8^{A}$	$2{\cdot}2^{A}$

In each line values with different capital letters are significantly different by Tukey's test at the 5% significance level.

abattoir waste water and confirmed the faecal features and the organic content of waste.

Effluents of treatment systems, when compared with the affluents, in poultry abattoirs decreased by 1·6 log (in the dry period) and 2·1 log (in the rainy period) for total coliform bacteria, by 2·6 log (in the dry and rainy period) for *E. coli*, and by 2·6 log (in the dry period) and 1·6 log (in the rainy period) for enterococci.

Total coliform bacteria had a reduction rate of 0·2 log (during the dry period) and 0·7 log (in the rainy period), the reduction rate of *E. coli* reached 0·5 log (in the dry period) and 2·1 log (in the rainy period); the reduction rate of enterococci was 0·3 log (in the dry period) and 0·1 log (in the rainy period) in effluents of the treatment systems of swine abattoirs.

In spite of the reduction rates in microbial accumulation of poultry and swine abattoirs analysed in the current analysis, some authors, such as Bastos [22], use log units (LU) to express the removal of pathogens and their indicators so that super-evaluation of coliform bacteria removal expressed by such high numbers as 90% and 99·0% could be avoided.

Low microbial reduction rates reported in the effluents of the treatment systems of poultry and swine abattoirs ($P > 0{\cdot}05$) may be attributed to a lower liquidation of suspended solid matter and colloids and to low operation temperatures (Tables 3 and 4). Based on maximum rates of *E. coli* recommended by the World Health Organization [43] for the reutilization of water in irrigation (10^3 *E. coli*/100 ml), effluents from the two types of abattoirs should not be used in agricultural irrigation.

Further, samples of water at the three recipient sites (emission site of effluent, 100 m upstream, 100 m downstream) of the two types of abattoir were above the microbiological standards of 5×10^3 total coliform (TC)/100 ml and 4×10^3 faecal coliform (FC)/100 ml, stipulated by Decree 8468 [34], and of 5×10^3 TC/100 ml and 1×10^3 FC/100 ml, mandatory by Decree 357 of Conama [33], for class II water. The microbiological framing of water in class III water bodies (20×10^3 TC/100 ml and 4×10^3 FC/100 ml), ruled by Decree 8468 [34] and Conama [33], was not complied with. Therefore, water usage becomes unsuitable for the irrigation of vegetables, fruit trees and shrubs.

The existence of treatment systems without any monitoring and improvement programmes or of pseudo-treatment, such as that of ponds without any protection for aquifers, may explain the deficiencies in quality decrease of the contaminating power of waste water of the abattoirs under analysis.

Faulty microbiological standards of the three samples sites in the water bodies for classes II and III water may have worsened the situation. Moreover, besides making the water dangerous to human health, its potential impact on the environment has been heightened.

Salmonella spp.

Table 9 shows identified serotypes of *Salmonella* according to the origin of samples at the sites of the poultry slaughtering process. Whereas two (14·29%) out of 14 samples from scald tanks of poultry

Table 9. *Numbers of samples analysed, number and percentage of positive* Salmonella *spp. samples, period of the year in which collection was undertaken; serotypes identified according to origin of water samples collected from supply, and from different phases of slaughtering process, from affluents and effluents, from waste treatment systems and at three sites of the receiving stream, collected in poultry abattoirs between May and September 2003 (dry period) and between January and March 2004 (rainy period) in the state of São Paulo, Brazil*

Origin of samples	Total samples		%*	Period	Identified serotypes
	Tested	Positive			
Supply	14	0	0	—	
Scald tank	14	2	14·3	Dry	*Salmonella enterica* subsp. *enterica* 4,5,12:–:–
Plucking facilities	14	1	7·4	Rainy	*Salmonella* Senftenberg
Evisceration	14	0	0	—	
Cleaning of carcasses	14	0	0	—	
Pre-cooling	14	1	7·4	Dry	*Salmonella* Give
Cooling	14	0	0	—	
Cleaning of facilities	12	0	0	—	
Affluent of treatment system	14	0	0	—	
Effluent of treatment system	14	1	7·4	Rainy	*Salmonella enterica* subsp. *enterica* 4,5,12:r:–
Emission site in the stream	8	0	0	—	
100 m downstream from emission site	7	0	0	—	
100 m upstream from emission site	7	0	0	—	
Total	160	5	3·1		4

* Prevalence (in %), of samples positive to genus *Salmonella* spp., in proportion to total from each analysed origin.

abattoirs were contaminated by salmonella, serotype *Salmonella enterica* subsp. *enterica* 4,5,12:–:–, *S*. Senftenberg was found in only one (7·41 %) out of 14 samples in plucking machine tank water.

The above findings corroborate research by Cherrington *et al*. [41], according to whom carcass contamination mainly occurs at the initial stages of the slaughtering process, or rather, the scalding and feather-plucking process where microorganisms such as *Salmonella* spp., *Staphylococcus* spp. and *Clostridium* spp. are the main bacteria in the scald-tank water.

Similar results were reported by several researchers over 25 years ago. Cherrington *et al*. [41] reported that cleaning the scald tank with water jets after several hours of slaughtering was not sufficient to remove *Salmonella*. This was due to the 81–86% prevalence of bacterium isolation with a rise in detection rate in proportion to the processing stage.

In Spain, Carramiñana *et al*. [7] reported two (12·5%) serotypes of *S*. Typhimurium and 11 (68·9%) serotypes of *S*. Enteritidis in water samples from scald tanks with 75% isolation rates. Nevertheless, the fact that water temperature in the scald tank was not above 61 °C (Table 3) may explain the two serotypes of *Salmonella* found in the scald-tank water of poultry abattoirs in the current study.

Isolation of salmonella was possible in only one (7·41%), serotype *S*. Give, out of 14 water samples in pre-cooling tanks in the poultry slaughtering process. Isolation may be related to inefficient water chlorination in the tanks coupled with high microbial accumulation at this sampling site.

Isolation of salmonella, identified as *S. enterica* subsp. *enterica* 4,5,12:r:–, has been verified in one sample (7·41%) out of 14 from effluents of the treatment systems of poultry abattoirs.

On investigating the impact of abattoir effluents on rivers in Nigeria, Benka-Coker & Ojoir [1] isolated 22 bacteria from effluents and from river water samples. Seven were *Salmonella* spp., six were *E. coli*, three were *Staphylococcus* spp., three were *Streptococcus* spp., two were *Shigella* spp. and one was *Klebsiella* spp. Frequent isolations of *Salmonella* spp. and *E. coli* from affluents and from downstream river water samples were also registered. However, no *Salmonella* spp. were found in upstream river water samples.

Table 10. *Numbers of samples analysed, number and percentage of positive* Salmonella *spp. samples, period of the year in which collection was undertaken; serotypes identified according to origin of water samples collected from supply, and from different phases of slaughtering process, from affluents and effluents, from waste treatment systems and at three sites of the receiving stream, collected in swine abattoirs between May and September 2003 (dry period) and between January and March 2004 (rainy period) in the state of São Paulo, Brazil*

Origin of samples	Total samples		%*	Period	Identified serotypes
	Analysed	Positive			
Supply	14	0	0	—	
Scald tank	14	0	0	—	
Cleaning of carcasses	14	0	0	—	
Cleaning of facilities	14	1	7·4	Dry	*Salmonella* Panama
Affluent of treatment systems	14	1	7·4	Rainy	*Salmonella* Typhimurium
Effluent of treatment systems	12	0	0	—	
Emission site in stream	1	0	0	—	
100 m downstream from emission site	1	0	0	—	
100 m upstream from emission site	1	0	0	—	
Total	85	02	2·4		2

* Prevalence (in %), of samples positive to genus *Salmonella* spp., in proportion to total from each analysed origin.

Whereas in 1996 Fransen *et al*. [42] identified *Salmonella* in 84% of effluents of poultry abattoirs, in 2003 Bonardi *et al*. [14] registered the isolation of salmonella in 55 intestinal contents of 150 slaughtered animals (37%). There were 57 isolated *Salmonella* strains which belonged to serotypes *S*. Derby (20 strains), *S*. Typhimurium (11 strains), *S*. Seftenberg (2 strains) and *S*. Give (1 strain).

Table 10 presents *Salmonella* isolation and identified serotypes according to origin of samples from the different sites of the swine slaughtering process.

No sample collected from the scald-tank water in swine abattoirs was positive for *Salmonella* (Table 10). This fact has also been reported by Mafu *et al*. [4] and Pearce *et al*. [44]. The latter researchers have also reported that *Salmonella* was found in 31% of carcasses, after bleeding. Isolates were *S*. Typhimurium, *S*. Derby, *S*. Hadar and *S*. Infantis. However, scalding decreased the incidence of *Salmonella* from 31% to 1%, with *S*. Derby as the isolate.

Corroborating other research work [12, 20] current analysis shows that when water is maintained at a constant temperature of 61 °C (which failed to occur in the scald tanks of poultry abattoirs), scalding decreases the number of microorganisms and pathogens such as *Salmonella* and may thus be considered a critical control point (CCP).

S. Panama was isolated from water during swine abattoir facility cleaning in a single sample (7·41%) out of 14 (Table 10).

Swanenburg *et al*. [11] reported the isolation of *Salmonella* in 101 samples out of a total of 925 from slaughtered swine and in 140 out of 447 samples from abattoir facilities (29·4%). The rate of *Salmonella* from slaughtering facility water was very high, with a 50–70% detection rate in samples per day. The same researchers insist that *S*. Typhimurium and *S*. Derby were the most frequently isolated serotypes in samples from abattoir facilities. During the same year, albeit in another study, the same authors reported *S*. Panama and *S*. Typhimurium in samples from water from abattoir facilities cleaning (57%).

In Holland, Fransen *et al*. [45] isolated *Salmonella* in 92% of effluents from swine abattoirs. Two years later the same authors found *Salmonella* spp. in the crude slime from the activated slime system used in the treatment of waste water from swine abattoir facilities. An equal isolation percentage was obtained from affluents in the treatment systems (Table 10). In this case, however, the serotype was *S*. Typhimurium.

S. Typhimurium is acknowledged to be one of the most dangerous serotypes for public health within the context of 2000 serotypes [11, 46]. While in the United States *S*. Typhimurium has been consistently reported since 1999 in human and animal salmonellosis, in

England, Wales and Scotland it is the next most common salmonella isolated in humans and animals during the last decade. Since the pathogen has substantially decreased in both humans and animals during the last three years, the epidemic may be replaced by another serotype [14].

Several epidemiological studies have shown that there is a worldwide incidence of *S*. Enteritidis and that this serovar has replaced *S*. Typhimurium as the most common serotype currently detected [7].

The emergence of *S*. Enteritidis in table-egg layers and humans has been explained by the combination of two main factors: the extraordinary epidemiology of *S*. Enteritidis infections in laying hens and the centralized rearing of breeding stock [47]. In contrast to most other zoonotic *Salmonella* serotypes, *S*. Enteritidis has been shown to cause colonization of the peri-reproductive tissue of the laying hens [48]. This may lead to colonization of the egg contents during the formation of the egg in the reproductive tract. Due to this ability for vertical transmission parent birds can transmit the infection to their progeny and laying hens can infect eggs produced for consumption.

Abundant data point towards *Salmonella* in chickens and swine prior to slaughtering, at different critical sites within the slaughtering process, in final products and in meals (the sub-products of fatty parts) [38, 49]. It may thus be presumed that bacteria are more frequently found in water output from the abattoir facilities.

According to Nascimento *et al*. [13], there are several reasons why *Salmonella* is not so frequently registered in water and wastewater. The most important are: (1) the treatment of abattoir effluents; (2) the great amount of competing bacteria that cut short the multiplication of salmonella; the latter may not reach detectable levels that would make it of great concern; (3) the failure of the bacterium to survive during long periods under certain circumstances; (4) the low sampling (a single sample) with low periodicity (once a month; sometimes once a year). Simple samplings may not always be used for specific problems. In fact, the results of *Salmonella* isolation may be affected by serotypes in the samples and by the sampling method [9].

Microbiota in abattoir facilities partially reflects the microbiota of slaughtered swine and poultry. Serotypes of *Salmonella* that manage to survive in certain environmental niches may become part of the abattoirs' residential microbiota (home strains) causing slaughtered animals to be contaminated by *Salmonella* by means of this residential microbiota [8].

Prevalence of salmonella in different water samples is greatly related to the contamination hazards from animal carcasses at the end of the slaughtering process and the activity of faecal contents as a potentially contaminating source in water and extensively to both industry facilities and humans [14].

Since a single colony-forming unit may under certain circumstances multiply itself up to several millions, even low rates are significant. When the process is contaminated, *Salmonella* spp. may be isolated from the machinery, waste water and the workers' hands until the next stop and/or until the end of the day when the series machinery is cleaned and disinfected. In this case contamination and cross-contamination are unavoidable during all working hours [12, 20].

Abattoir hygiene has to be kept at high levels since it is mandatory that residential microbiota of *Salmonella* should be absent in slaughtering facilities. Regular cleaning and disinfection of all equipment, even during slaughtering, are necessary, and their respective efficiency checked. Adequate cleaning and disinfection of the series process may prevent the propagation of higher salmonella contamination and, consequently, of cross-contamination with the microorganism for longer periods. However, there is always the risk of contaminations and cross-contaminations during the production series.

It should also be emphasized that technicians should be aware of the risks and hazards of meat products contaminated by waste water with pathogenic microorganisms and faecal bacteria. Since food of animal origin is rich in proteins, it constitutes a substrate for the development of these microorganisms.

CONCLUSIONS

High concentrations of total coliform bacteria and *E. coli* and the identification of *Salmonella* strains in the effluents of treatment systems of both types of abattoirs makes the waste water unsuitable for recycling and it should be considered a potential source of disease agents.

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DECLARATION OF INTEREST

None.

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