

HYGIENIC-SANITARY AND MICROBIOLOGICAL QUALITY OF MEAT FROM THE MARANHÃO TOCANTINE REGION SLAUGHTERHOUSE

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SUMMARY: Beef is a product of high protein value and is one of the main sources of nutrients for humans, but despite its qualities, it and its derivatives are susceptible to changes due to chemical, physical or microbiological reactions. During handling and processing, these products are easily contaminated by microorganisms. If contamination occurs and if the environment is favorable for multiplication, they may alter the organoleptic characteristics of the food, thus causing its deterioration and possibly could endanger the health of the consumer. So, this research has as its objective to evaluate the hygienic-sanitary and microbiological quality of the deriving foods from slaughterhouse at Imperatriz, Maranhão. Bovine meat samples were analyzed to detection of total coliforms and thermotolerants, *Escherichia coli* and *Salmonella*, beyond sample swabs in surfaces to colony counting, pH analysis and verification list application of the physical-structural and hygienic-sanitary conditions. Beyond the slaughterhouse is group three in relation to the installations conditions and in the counting of colony forming unit, there was contamination in all the collected samples, results obtained vary from 0.02×10^2 to 3.0×10^2 UFC/mg. However, in the establishment, fails happened in the Good Manufacturing Practices, in which induced the product exposure to microbiological contamination and food quality commitment.

Keywords: Animal protein. Inspection. Slaughter.

QUALIDADE HIGIÊNICO-SANITÁRIA E MICROBIOLÓGICA DE CARNES ORIUNDAS DO MATADOURO-FRÍGORÍFICO DA REGIÃO TOCANTINA DO MARANHÃO

RESUMO: A carne bovina é um produto de elevado valor proteico, sendo uma das principais fontes de nutrientes para os seres humanos, porém, apesar de suas qualidades, ela e seus derivados estão passíveis de alterações decorrentes de reações químicas, físicas ou microbiológicas. Durante a manipulação e processamento, esses produtos são facilmente contaminados por micro-organismos. Se ocorrer contaminação e se o ambiente for favorável para a multiplicação dos mesmos, eles podem alterar as características organolépticas do alimento, causando assim, sua deterioração e possivelmente poderá colocar em risco a saúde do consumidor. Deste modo, objetivou-se com este trabalho avaliar a qualidade higiênico-sanitária e microbiológica das carnes oriundas do matadouro do Município de Imperatriz, Maranhão. Foram analisadas amostras de carnes bovinas para detecção de coliformes totais e termotolerantes, *Escherichia coli* e *Salmonella*, além de amostras de swabs em superfícies, como mãos, facas e bebedouro para contagem de colônias, análises de pH e aplicação da lista de verificação das condições físico-estruturais e higiênico-sanitárias. Houve contaminação em todas as amostras coletadas, além do matadouro se enquadrar no grupo três em relação as condições das instalações e na contagem de unidade formadora de colônias

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obteve-se resultados que variaram de $0,02 \times 10^2$ a $3,0 \times 10^2$ UFC/mg. Portanto, no estabelecimento, houve falhas nas Boas Práticas de Fabricação, que induziram a exposição do produto a contaminação microbiológica e comprometimento da qualidade do alimento.

Palavras-Chave: Proteína animal. Inspeção. Abate.

INTRODUCTION

The meat production chain is a growing sector due to the growing consumer population (SANTOS *et al.*, 2012). According to Palma (2010), in the food chain, meat is an important food as it is a relevant source of protein, and it contains all the essential amino acids, associated with a low caloric content. Additionally, it is an excellent source of essential fatty acids, B vitamins, namely cobalamin, and the minerals iron and zinc.

Despite the meat nutritional importance, it is an excellent culture medium for the growth of desirable, spoilage and pathogenic microorganisms due to its intrinsic characteristics. (pH close to neutrality, high water activity) (ALCANTARA, 2012). The involvement of meat and meat products in the occurrence of Foodborne Diseases (DVAs) is due to the fact that many pathogens belong to the natural microbiota of animals (digestive tract, pharynx, tonsils, nostrils, lymph tissue) thus contaminating the carcasses during slaughter. These agents are also transported from the contaminated environment to the carcasses by the handler, utensils, food and water (MATSUBARA, 2005).

Most municipal slaughterhouses, especially small ones, do not meet the minimum hygiene requirements along the slaughter flowchart, do not offer security for the handlers in production and, mainly, they do not guarantee a meat free and protected of physical, chemical and biological contamination from man, animals and the environment. According to Santos *et al.* (2009), there is a need for microbiological assessment of foods to verify and ensure that microbiological standards and specifications are met. Therefore, this type of analysis is configured as a tool in determining food quality.

Brazil currently has the largest commercial herd of beef cattle in the world and dominates the second place among beef producing countries and assumed the world leadership in volume of exported meat (IBGE, 2018). According to the Department of Economic Research and Studies (DEPEC), in 2018, Brazil had a share of 35.9% of production in the world scenario and consumption of 38.6%, which proves the Brazilian potential as a manufacturer of meat products. The state of Maranhão has the second largest contingent of cattle in the Northeast region, with 6.885.265 animals, and its share in the slaughter of cattle in 2015 was 2.8%, second only to the state of Bahia. This scenario in Maranhão is the result of significant changes in the production process, mainly related to animal specialization and health (DEPEC, 2019; PAIVA *et al.*, 2018;

SOUZA *et al.*, 2018). Thus, the aim of the study was to evaluate the hygienic, sanitary and microbiological quality of fresh beef from the municipal slaughterhouse of Imperatriz.

MATERIAL AND METHOD

Verification of the Refrigerator-Slaughterhouse Physical-Structural and Hygienic-Sanitary Conditions

In the establishment visited, a checklist prepared according to the norms established in the Resolution of the Collegiate Board of Directors was used to assess the hygienic and sanitary conditions (RDC) n° 216, of 2004 (BRASIL, 2004). The checklist was elaborated with eight verification items, distributed in handlers' evaluations, meat storage and environmental hygienic conditions, as follows: Block 1 - building and facilities; Block 2 - equipment, furniture and utensils; Block 3 – integrated control of vector and urban pest; Block 4 - water supply; Block 5 - waste management; Block 6 - manipulators; Block 7 - storage and transport of prepared food; Block 8 - documentation (good manufacturing practices manual (MBPF) and standard operating procedures (POPs) and records.

After applying the checklist, the sum of conformity to the items evaluated and the classification of the municipal slaughterhouse in one of the groups was performed, according to Matos *et al.* (2012): Group 1 - 76% a 100; Group 2: 75% a 51%; and, Group 3 - of 50% to 0% of the itens.

Meat Sample Collection

Fifty fragments of different beef carcasses from the Municipal slaughterhouse of Imperatriz were collected, with an average weight of 100 grams for each sample, in which, units were removed to compose the analytical sample (25g) from five different locations on the carcass, being in the chest and loin region. The collection was divided into two stages, with subsequent analyses, observing all precautions with regard to hygiene and food safety, so that there was no contamination caused by the researchers' manipulation. (BRASIL, 2003; SILVA, 2008).

The samples were collected aseptically and placed in coolers containing an ice pack, both previously sanitized and taken immediately to the Phytopathology, Microbiology and Food Laboratory – LFMA, of the State University of the Maranhão Tocantins Region – UEMASUL, for subsequent microbiological analysis of total coliforms, thermotolerant coliforms, *Escherichia coli* and *Salmonella sp.* The samples were stored in individual and original packaging, in a refrigerator at the temperature of -20 °C until the time of analysis, as RDC N° 12 of 2001 from ANVISA (BRASIL, 2001).

Surface Swabs

Swabs were aseptically collected from the hands of 10 facility handlers, of two knives used for the killing process and the drinking fountain tap, using the *swab-test* method, for counting possible contaminants. Microorganisms were removed from hands, considered sanitized, by the handlers themselves, randomly chosen (NUVOLARI *et al.*, 2019). The material from the hands was collected by rubbing swabs with circular movements in the palm region, dorsal and between the fingers. Soon after collection, the *swabs* were immersed in sterile test tubes, containing 9mL of saline solution to 0,85%. The culture used was Standard Count Agar, for research of mesophilic aerobic bacteria and stored in a bacteriological greenhouse at 35 °C per 24 hours (SILVA *et al.*, 2017).

Isolation and identification of total coliforms, thermotolerants and *Escherichia coli*

The samples were homogenized, preceded by an initial dilution of 1:10 (10^{-1}), in which the 25g of the sample was added to 225ml of peptone water 0,1%. Subsequently, the second dilution was performed following the same protocol mentioned above (TORTORA; FUNKE; CASE, 2016). In the presumptive test for total coliforms, Lauryl Sulfate Tryptose Broth (LST) was used, in which, three appropriate sample dilutions were selected and, with a 10ml pipette, a series of three tubes was inoculated into the broth, adding 1.0ml of the dilution per tube with 10ml of broth. Then, the incubation of Lauryl Sulfate Tryptose Broth tubes containing an inverted Durhan tube at 35 °C for 24 hours was carried out and it was observed the presence of growth with gas production inside the tube. The confirmatory test for total coliforms was performed from each positive tube from the previous step. An elevation of the culture from the positive tubes was transferred to tubes with Brilliant Green Bile Broth (VB). The tubes were incubated at 35 °C for 24-48 hours and observed for growth with gas production inside the inverted Durhan tubes. The Most Probable Number of total coliforms NMP/g or ml was determined (TORTORA; FUNKE; CASE, 2016).

The thermotolerant coliform test was performed from each positive tube from the previous step. A loaded elevation of the positive culture was transferred to *E. coli* (EC) Broth tubes. The tubes were incubated in a bacteriological incubator at 44.5 °C (most foods) for 24 hours and were observed if there was growth with gas production inside.

Isolation and identification of Salmonella

The identification of microorganisms was carried out in accordance with the *American Public Health Association* (APHA, 2001). Microbiological analyzes were performed at the UEMASUL Microbiology Laboratory. Initially, the pre-enrichment step was carried out, which aimed to recover injured cells, achieved by incubating the sample under non-selective conditions for at least 18 hours. Peptone water was used. 225ml of pre-enrichment broth was added and the sample was homogenized. The vials were incubated at 35 °C for 18-24 hours, with the lids slightly loosened. Subsequently, the selective enrichment step was performed, at this stage, the bottle with pre-enrichment broth was gently shaken and transferred 1 ml to 10 ml of Tetrathionate Broth (TT) and 1 ml to 10 ml of Selenite Cystine Broth (SC).

Tubes were incubated at 35 °C for 24 hours. In the differential plating step, the selective enrichment tubes were shaken in a *vortex* mixer and an elevation of the Tetrathionate Broth was striated on a Hectoen Enteric Agar plate (HE), Bismuth Sulfite Agar (BS) and Xylose Lysine Deoxycholate Agar (XLD). The plates were incubated inverted at 35 °C for 24 hours and it was verified if there was development of *Salmonella sp.* typical colonies (TORTORA; FUNKE; CASE, 2016).

Data analysis

Data were entered, checked and processed in Excel 2012 (Microsoft Office®) where descriptive analysis was applied to present the simple and relative frequencies of the data. The data obtained through observations, questionnaires, interviews, laboratory results were compared with the RDCs N° 275 and 16 and legislation, in addition to research that work on the same theme.

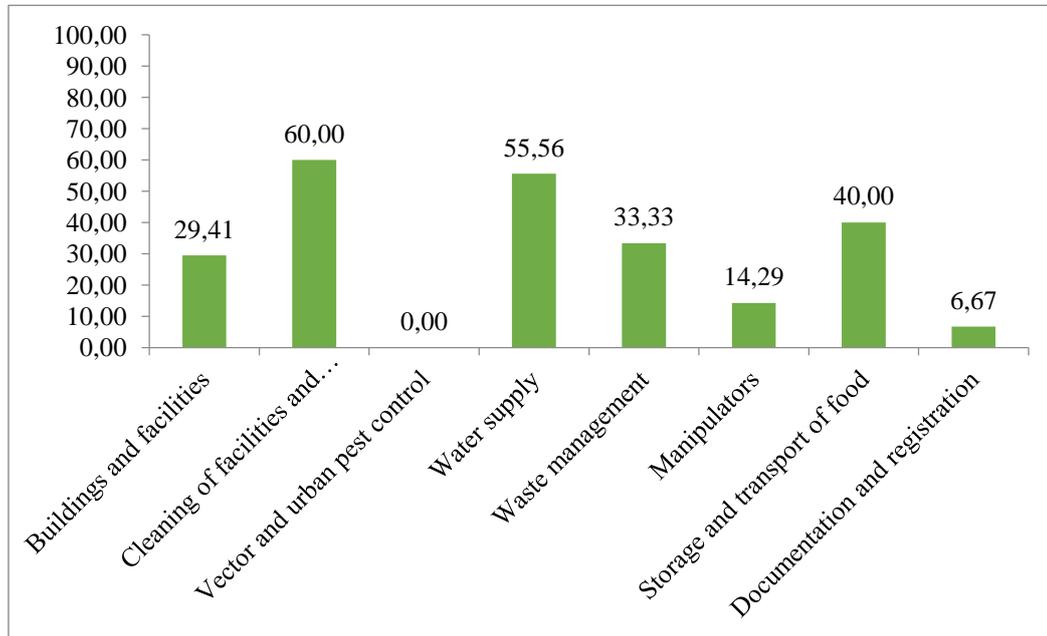
RESULT AND DISCUSSION

Checklist of Physical-Structural and Hygienic-sanitary Conditions of the Slaughterhouse

The values in relation to the fulfillment of the itemized items vary between 100% and 76%, 75% and 51% and 50% and 0%, allowing the classification of the slaughterhouse of *fresh* beef into group 1, group 2 and group 3 respectively, as established by RDC n° 275/2002, from ANVISA, which determines that all items on the checklist must be met by establishments (BRASIL, 2002). The Municipal Slaughterhouse of Imperatriz obtained an average of 30% in relation to the compliance established by the RDC n° 275, thus fitting into group 3. The highest percentages of nonconformities were found in blocks 3 and 8, which refer to vector and urban

pest control and Manual of Good Manufacturing Practices and Standard Operating Procedures, respectively (Figure 1).

Figure 1. Compliance rates (%) in the Municipal Slaughterhouse of Imperatriz.



Block 1 - Building and Facilities; Block 2 - Facilities Sanitation, Equipment, Furniture and Utensils; Block 3 – Integrated Control of Vector and Urban Pest; Block 4 - Water Supply; Block 5 - Waste management; Block 6 - Manipulators; Block 7 - Storage and Transport of Prepared Food; Block 8 - Documentation and Record.

With regard to the percentage of items served per block, according to RDC n° 275, it was observed that block 3, which corresponds to the control of urban vectors and pests, was the one with the most nonconformities, because the facilities, equipment, furniture and utensils were not free from vectors and pests, like flies and ants, as there was not, at the time of application of the research, a set of effective and continuous actions to control them. In group 3, where it was observed that the degree of compliance with BPF was below that established in the legislation, since it is determined that the items evaluated have percentages of compatibility greater than 76% in order to increasingly reduce the risk of food poisoning (BRASIL, 2002).

Block 8, which concerns documentation and records, presented only 6.67% of compliance, once, the establishment does not have a Manual of Good Manufacturing Practices and Standard Operating Procedures, according to RDC n° 216, because it is through these that the handlers will base themselves in order to be able to guarantee a safe processing of the meat. The introduction of Good Manufacturing Practices in food industries limits or exterminates the risks of possible contamination and spread of microorganisms through these foods (CARDOSO, 2004). According to the RDC n° 216/2004 (BRASIL, 2004), physical installations such as floors, walls and ceilings

must have smooth, impenetrable and washable wrappings. In addition, they need to be kept intact, free from cracks, crevices, leaks, leaks, infiltrations, molds, peeling and other defects that can convey contaminants to the food.

In a study carried out by Costa (2013), in Recife (PE), of the 21 establishments visited, 11 (52,38%) had broken floors, other damage and leaks. Already in research conducted by Miranda and Barreto (2012), in 12 establishments selling meat in the state of Bahia, it was observed that 33.3% of the minimarkets had walls, very worn floors and countertops, being able to generate greater moisture and consequently proliferation of microorganisms. The block that obtained the highest percentage of compliance was 2 (60%), which corresponds to the cleaning of facilities and utensils, once, the disposal of waste complies with the provisions of legislation, the slaughter area is sanitized as often as necessary and always after the end of the day and the sanitizers used are standardized by the Ministry of Health.

In a study by Costa (2013), it was verified that of the 21 establishments visited, in 18 (85,71%) there was dirt and blood from meat, demonstrating the lack of hygiene in the workplace. In this context, Soto *et al.* (2006), researching the sanitary conditions of four supermarkets in the municipality of Ibiúna (SP), verified that the cleaning and disinfection procedures of the place were done inappropriately, possibly due to poor storage of products or use of inappropriate products. RDC n° 216/2004 (BRASIL, 2004) recommends that the facilities, the equipment, furniture and utensils must be kept in appropriate hygienic and sanitary conditions and the cleaning and sanitization operations performed frequently and by proven employees, ensuring the maintenance of these conditions and minimizing the risk of food contamination.

Microbiological Analysis

As for the results obtained from *swabs* on surfaces of the Municipal Slaughterhouse of Imperatriz-MA, all samples collected had a high rate of contamination in the first collection, where 90% were uncountable. In the second collection, the values ranged from 0.05×10^2 to 3.0×10^2 UFC/ml. All samples were collected in the same place, at the same time and under the same hygienic conditions (Table 1).

Table 1. Plate counting by the hand *swab* method, knives and drinking fountains in the cattle wing of the municipal slaughterhouse of Imperatriz - MA.

Samples	Microorganism count (UFC/ml)	
	1° Collect	2° Collect
M1	Inc	$3,0 \times 10^2$
M2	Inc	Inc
M3	Inc	$3,0 \times 10^2$

M4	Inc	0,05 x 10 ² Est
M5	3,0 x 10 ²	3,0 x 10 ²
M6	Inc	3,0 x 10 ²
M7	Inc	3,0 x 10 ²
M8	Inc	3,0 x 10 ²
M9	Inc	0,2 x 10 ² Est
M10	Inc	Inc
F1	Inc	-
F2	Inc	-
B	Inc	-

Legend: Est: estimated count; Inc:Uncountable

In reference to the *swab* analysis of the manipulators' hands, Brazilian legislation does not have a microbiological standard for it, however, as the handlers' hands are in direct contact with the food, they can be sources of contamination if there is not proper hand hygiene, especially after using the bathroom.

The results found in the samples collected from the handlers' hands, of knives and drinking fountains, reminds us that the work that the handler performs in the quality of the food that will reach the consumer's table is very important, as well as the correct cleaning of equipment and utensils that are used by them, since they can also contaminate the carcass. In a study carried out in a municipality in the state of Bahia, by Matos (2012), 100% of the *swabs* from the hands of the beef handlers presented values above the microbiological standard determined as regular (10²UFC/mãos) of *Staphylococcus aureus*. The found values of *S. aureus* ranged between 6 × 10² a 2,9 × 10⁴UFC/hand. In the same study, total coliform values were found between < 10 and 2,8 × 10³UFC/hand. For counting total coliforms in the hands of food handlers, there are no pre-established criteria in Brazilian legislation. The results obtained in the microbiological analyzes of total coliforms, thermotolerant, *E. coli* and *Salmonella* of beef samples from the municipal slaughterhouse of Imperatriz – MA (Table 2).

Table 2. Total Coliforms, thermotolerant, *Escherichia coli* (NMP/g) and *Salmonella* (25g) *Salmonella* of beef samples from the municipal slaughterhouse of Imperatriz – MA

SAMPLES	1° COLECT				2° COLECT			
	Coliforms 35°C (NMP/g)	Coliforms 45°C (NMP/g)	<i>E. coli</i> (NMP/g)	<i>Salmonella</i> (25g)	Coliforms 35°C (NMP/g)	Coliforms 45°C (NMP/g)	<i>E.coli</i> (NMP/g)	<i>Salmonella</i> (25g)
1	400	300	-	+	300	300	-	+
2	300	300	-	+	300	300	-	+
3	300	300	-	+	400	400	-	+
4	300	300	-	+	300	300	-	+
5	300	300	-	+	300	300	-	+
6	400	300	-	+	300	300	-	+
7	300	300	-	+	300	300	-	+
8	400	300	-	+	300	300	-	+

9	300	300	-	+	300	300	-	+
10	300	300	-	+	400	400	400	+
11	300	300	-	+	400	400	400	+
12	300	300	-	+	400	400	400	+
13	400	400	-	+	400	400	400	+
14	300	300	-	+	400	400	400	+
15	400	400	-	+	300	300	-	+
16	300	300	-	+	300	300	-	+
17	300	300	-	+	400	400	400	+
18	400	400	-	+	300	300	-	+
19	300	300	-	+	400	400	400	+
20	300	300	-	+	400	400	400	-
21	700	400	-	+	300	300	-	+
22	300	300	-	+	400	400	400	+
23	300	300	-	+	300	300	-	+
24	300	300	-	+	300	300	-	+
25	300	300	-	+	400	400	400	-

In the first collection, 100% of the presence of *Salmonella* and no evidence of *E. coli* in the analyzed samples. In the second collection, there was a variation in the result, in which, of 25 samples, 44% were positive for *E. coli* and 76% positive for *Salmonella*. The importance of hand hygiene care, the group of total coliform bacteria is extremely important so that there is no contamination through it and does not generate public health problems. In this group we find a reference for the hygiene conditions of the manufacturing processes, since they are naturally inactivated when sanitizers are used and are able to colonize several areas of product processing, when sanitation is not done correctly. They can also be a reference for errors in the post-processing of pasteurized foods, like milk, because they are quickly destroyed by heating and do not survive heat treatment. (SILVA *et al.*, 2005).

The appearance of total coliforms in foods that have been processed is seen as a valid indication of post-sanitization or post-thermal treatment contamination, suggesting hygienic failures during product processing and storage or lack of heat treatment since they are not sporulated microorganisms (LIMA SILVA *et al.*, 2015). Thus, it is extremely important to investigate the microbiological quality of the meat consumed by the population of the municipality. In the present work, there was the presence of total and thermotolerant coliforms in all samples collected, with values ranging from 300 to 700 NMP/g. In the current legislation for fresh beef there is no microbiological standard regarding the presence of this bacteria, however, in the Annex II, in the RDC n°12/2001, asks that although there is no microbiological standard, they can cause serious health risks to the consumer, by the bacteria that cause several enteropathies.

Matos *et al.* (2012) in his study of the analysis of the health profile of fresh meat sold in supermarkets, using the most likely number method (NMP) of total coliforms, obtained populations that varied between $1,0 \times 10^2$ to $3,1 \times 10^4$ UFC/g, and all analyzed samples showed total coliforms. Costa (2017), in a similar study, obtained 100% of presence of total coliforms in their samples, with values ranging from $2,3 \times 10^1$ to $4,6 \times 10^2$.UFC/g. The bacteria group of *E. coli* is another significant indicator when we talk about the hygiene and quality of beef. Regarding the determination of its presence in the analyzed meat samples, of 50 samples, adding the two collections, 11 samples (22%) produced gas in Durham tubes, which is indicative of positivity. In the current legislation for raw in natura beef there is no microbiological standard in relation to this bacteria, however, in Annex II, in the RDC n° 12/2001 demands that, despite the lack of a microbiological standard for *E. coli*, they can cause serious health risks to the consumer, since this bacterium is the cause of several enteropathies.

The investigation of *E. coli* bacteria in a sample of thermotolerant coliforms is extremely important, since its indication of fecal contamination is superior to other members of the group, such as some strains of *Enterobacter* and *Klebsiella* (JAY, 2005). Most subgroups of *E. coli* are already in the intestines normal microbiota of humans and warm-blooded animals, but some serotypes can be pathogenic to these beings, not being classified as a part of your intestinal microbiota. Matos (2012) in his work, of 8 samples collected, only 1 (12,5%) presented count of $2,0 \times 10$ UFC/g, considering the detection limit of the established method. Damer *et al.* (2014), present that 92,85% of the samples collected in his research were contaminated by this bacterium, showing that there was fecal contamination in any of the processing steps that these meats were subjected to, since its natural habitat is the intestine of warm-blooded animals.

According to Brasil (2010) the most common bacteria in foodborne disease outbreaks are *Salmonella* and *E. coli*. According to the RDC n° 12 of 2001, raw beef is suitable for human consumption only when it lacks *Salmonella sp.* in 25g of the meat. Regarding the research of *Salmonella* in the meat collected for analysis in this work, there was growth of typical colonies in 88% of the samples, which demonstrates the lack of hygiene on the part of meat handlers and shows levels considered high and who label these meat samples as unfit for consumption, as they pose serious risks to the health of the population.

Damer (2014) in his study, he evidenced the presence of *Salmonella* in 14,28% of 14 analyzed samples. Ferreira and Simm (2012), by analyzing 6 samples of ground beef, they identified *Salmonella sp.* in one of them (16,67%) and Souza *et al.* (2020) analyzed the ground beef from three districts in the southern zone of the city of Macapá-AP and 100% of the samples collected in the butchers of the three neighborhoods analyzed were positive for thermotolerant

coliforms and for *Staphylococcus coagulase*. Lopes (2011) analyzed the presence of *Salmonella* on cowhide and carcasses after skinning and after washing, and the results indicate that, of the 200 animals analyzed, 31 (15,5%) present *Salmonella* sp. in leather and 7 (3,5%) animals were positive in the carcass after skinning and 6 (3%) after washing. Rosina and Monego (2013), in their studies, did not detect contamination by *Salmonella* sp. In none of their samples, demonstrating that the ground beef samples investigated are all within the standard of the Resolution RDC n° 12 of January of 2001 (BRASIL, 2001). On the other hand, in the study carried out by Chagas *et al.* (2017), no presences of *Salmonella* sp. in its samples of beef from slaughterhouses in the state of Pará, which recommends that you have quality of the raw material and efficiency in good handling practices. The appearance of this microorganism can cause various foodborne illnesses, as food poisoning/intoxication (ARÇARI, 2011). In Brazil, the *Salmonella* bacteria predominates in notifications of disease outbreaks caused by contaminated food (BRASIL, 2010).

CONCLUSION

The high levels of contamination in the samples collected demonstrate poor hygienic and sanitary conditions of meat from the slaughterhouse in Imperatriz, Maranhão. In this context, it is considered of great importance the application of public policies in the sense of assistance to activities involving food of animal origin in the Tocantins Region, in the field of training and not just inspection. Good manufacturing practices must be implemented through the routine habits of everyone involved in the production process and thus reduce the risk of food poisoning.

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