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Prevalence of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 in beef from a peripheral country

Prevalência de Escherichia coli produtora de toxina Shiga (STEC) O157:H7 em carne bovina de um país periférico

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Abstract: The objective of this study was to diagnose the hygienic-sanitary quality of raw beef sold in supermarkets and street markets in Recôncavo of Bahia. Twenty-four samples of beef from six municipalities located in the region were evaluated between May and July 2023. The samples were evaluated for the presence of mesophilic and psychrotrophic aerobic microorganisms, total coliforms, *Escherichia coli* and *Salmonella* spp. The *E. coli* isolates were subjected to genotypic characterization using Polymerase Chain Reaction (PCR) to verify the *E. coli* virulence genes, including the gene for Shiga toxin (stx) to identify enterohemorrhagic *E. coli* (EHEC) and the gene for ST-I toxin (stI) to identify enterotoxigenic *E. coli* (ETEC). The results indicate a high microbiological count with a total of 75% of the samples not complying with the parameters established in legislation for one or more microorganisms described. Only 25% of the total establishments checked, corresponding to supermarkets, complied with the legislation. The stI gene was not identified in any of the samples studied and the stx gene was identified in two samples sold in street markets in the municipalities of Cachoeira and Santo Amaro. It is concluded that part of the beef sold in supermarkets and street markets in the six municipalities has unsatisfactory microbiological quality, which may pose risks to the health of consumers.

Keywords: public health; genetic characterization; microbiology.

Resumo: O objetivo deste estudo foi diagnosticar a qualidade higiênico-sanitária da carne bovina in natura comercializada em supermercados e feiras livres no Recôncavo

da Bahia. Vinte e quatro amostras de carne bovina provenientes de seis municípios da região foram avaliadas entre maio e julho de 2023. As amostras foram avaliadas quanto à presença de microrganismos aeróbios mesófilos e psicrotróficos, coliformes totais, Escherichia coli e Salmonella spp. Os isolados de E. coli foram submetidos à caracterização genotípica por meio da Reação em Cadeia da Polimerase (PCR) para verificar os genes de virulência de E. coli, incluindo o gene para toxina Shiga (stx) para identificar E. coli enterohemorrágica (EHEC) e o gene para toxina ST-I (stI) para identificar E. coli enterotoxigênica (ETEC). Os resultados indicam uma contagem microbiológica elevada, com um total de 75% das amostras em desacordo com os parâmetros estabelecidos na legislação para um ou mais microrganismos descritos. Apenas 25% do total de estabelecimentos fiscalizados, correspondentes a supermercados, estavam em conformidade com a legislação. O gene stI não foi identificado em nenhuma das amostras estudadas e o gene stx foi identificado em duas amostras comercializadas em feiras livres nos municípios de Cachoeira e Santo Amaro. Conclui-se que parte da carne bovina comercializada em supermercados e feiras livres nos seis municípios apresenta qualidade microbiológica insatisfatória, o que pode representar riscos à saúde dos consumidores.

Palavras-chave: saúde pública; caracterização genética; microbiologia.

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Introduction

Currently, Brazil occupies a prominent position on the world stage as the second largest producer and exporter of beef (IBGE, 2023, IMMET, 2023). This leading role is a reflection of the significant modernization of the sector, which has contributed to increase productivity and improve product quality. Beef trade, in particular, represents an activity of great relevance to the national economy, in addition to

play a fundamental role in food security (RODRIGUES & COSTA, 2021).

Recognized as one of the proteins of animal origin with the highest biological value and wide versatility, beef is available in several cuts and forms of presentation, being present in a notable way in the population's diet (MONTEIRO et al., 2018).

Under ideal conditions, the muscle tissue of animals is considered sterile (CUNHA et al., 2022). However, its intrinsic characteristics — such as pH close to neutrality, high water activity and rich nutritional composition — offer an environment conducive to the growth and multiplication of microorganisms (AMORIM et al., 2019).

Furthermore, meat can be exposed to different contaminants throughout the production chain — from slaughter, skinning and evisceration, until it reaches the points of sale. Along this path, irregularities related to the storage, handling and display of the product are common, often without due control and inspection by the competent bodies. These factors increase the risk of contamination, compromising the safety and quality of the meat (JÚNIOR et al., 2020; ARAÚJO et al., 2021).

In this context, controlling the presence and multiplication of microorganisms becomes an essential criterion to ensure the sanitary and commercial quality of the product, since high levels of contamination not only favor its deterioration, but also represent serious risks of the consumer health (MAIA & OLIVEIRA, 2023).

With the growth of national and international trade in animal products, the global spread of foodborne pathogens has become a growing concern. It has been demonstrated that food of animal origin can act as vectors for pathogenic microorganisms such as *Salmonella* spp., Shiga toxin-producing *Escherichia coli* (STEC) and *Listeria monocytogenes*. Beef, in particular, is occasionally consumed raw or undercooked, which increases the risk of ingesting viable pathogens, either directly or through cross-contamination. In this scenario, *Salmonella* spp., *L. monocytogenes*, STEC and *Staphylococcus* spp., especially *Staphylococcus aureus*, stand out among the main agents causing foodborne diseases, being recognized as serious public health problems at a global level (VARGAS et al., 2021; EFSA, 2022; RINN et al., 2024).

According to the World Trade Organization (WTO), more than 3 million children under the age of five die

annually from foodborne diseases, often associated with severe diarrhea (ZHAO, 2019; BAI et al., 2024).

In Brazil, microbial contamination is identified as the main cause of food poisoning outbreaks. Among the most frequently identified microorganisms are strains of *Salmonella*, *Escherichia coli* O157, *Listeria monocytogenes*, and *Shigella*, which may be present in meat products and pose serious risks to human health (PAUDYAL et al., 2018; BAI et al., 2024).

It is important to note that meat can harbor multiple pathogens simultaneously, further aggravating the risks to public health. In some countries, such as China, the presence of these bacteria in meat products is strictly prohibited by health regulations.

In view of this scenario, the present study aimed to evaluate the microbiological quality and perform the genotypic characterization of *Escherichia coli* isolates from raw beef sold in supermarkets and street markets in the Recôncavo Baiano region.

The intention is to contribute with relevant data for the promotion of food safety and protection of consumers health in the municipalities analyzed.

Material and Methods

Collection, sample preparation and microbiological analysis

The study was developed in twelve beef marketing units, located in six municipalities (Santo Antônio de Jesus, Cruz das Almas, Muritiba, São Félix, Cachoeira and Santo Amaro), located in the Recôncavo region, state of Bahia. Two collections were carried out in each municipality, with two samples of 200 g of raw beef (beef chuck) being obtained in each collection, thus making a total of 24 samples, in the period between May and July 2023.

In order to preserve their original characteristics, the samples were sent to the Animal Microbiology and Parasitology Laboratory of the Federal University of Recôncavo of Bahia (UFRB), Cruz das Almas campus.

For microbiological analyses, regarding the presence of mesophilic and psychrotrophic aerobic microorganisms, total coliforms and Escherichia coli, the samples were initially prepared aseptically and subjected to the serial dilution process until the third dilution (10^3) was reached, with the aim of reducing the contamination load per unit volume, making it possible to count the colonies in Petri dishes.

Then, 1mL (1000 µL) aliquots of each dilution were added to sterile Petri dishes, and then the specific culture media for each microorganism was poured, using the "Pour Plate" deep plating technique.

For the analysis of total coliforms and E. coli, the Chromocult® Coliform Agar culture medium was used. As for the Mesophilic and Psychrotrophic Aerobic Microorganisms, the Standard Counting Agar (PCA) medium was used.

After inoculation of the samples, the plates were incubated inverted in an incubator at 37ºC for 24 hours to determine total coliforms and E. coli, and for 48 hours to determine mesophilic aerobes. The psychrotrophic bacteria were incubated in a refrigerator at 7ºC for ten days. After the incubation period, colony counts were performed using the standard plate count technique, to express the results in Colony Forming Units per gram of sample (CFU/g). Finally, the data were subjected to statistical analysis using the Statistical Analysis System – SAS, and the results expressed in log CFU/g.

Colonies suggestive of E. coli were preserved in Eppendorf microtubes containing Brain Hear Infusion (BHI) broth with 20% glycerol and kept at –18ºC for subsequent DNA extraction and Polymerase Chain Reaction (PCR) analysis.

The analysis for the research of Salmonella spp was performed using the Compact Dry SL® chromogenic enzymatic method (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan), performed according to the manufacturer's recommendations. After the incubation period, the results were expressed qualitatively, regarding the presence or absence of the agent.

DNA extraction and PCR

For DNA extraction, the samples were initially recovered in BHI broth at 37ºC for 24 hours in a microbiological incubator, and then sent to the Genetics Laboratory – LIPAGE, of the Federal University of Recôncavo of Bahia (UFRB), Santo Antônio de Jesus campus.

The Eppendorf microtubes containing 1 mL of the bacterial culture suspension were centrifuged at 13,200 rpm for 5 min. The supernatant was discarded, and then 800 µL of sterile milliQ water were added. After homogenization, the samples were again subjected to the centrifugation process, under the same conditions described previously.

Finally, the supernatant was discarded and 80 µL of sterile milliQ water were added. Then, the samples were subjected to a temperature of 96ºC for 10 min in a water bath.

The supernatant was again discarded, and the samples were kept at -20ºC until the time of analysis.

The extracted DNA was subjected to PCR to identify the virulence genes stI and stx, using the following reagents and volumes: 2.5 µL of PCR buffer; 0.5 µL of dNTP mix; 0.75 µL of MgCl2; 0.2 µL of Taq DNA polymerase; 1.0 µL of primers 1 and 2; 3.0 µL of template DNA, with the final volume adjusted to 25 µL with sterile milliQ water.

The amplification reactions were performed in an Amplitherm® TX96 Plus thermocycler, according to the conditions, primer sequence and fragment size (bp) described in Table 1.

The amplification products, together with SYBR Green Life Technologies®, were subjected to electrophoresis in a 2% agarose gel in a horizontal system, with the negative control of both genes studied. The run was performed with a GSR® 200STD digital source, in 1 hour, at 100 Volts and 40 mA.

The products were then observed in a Loccus L-PIX ultraviolet photodocumenter.

Table 1. Primer sequence, amplified fragment size and conditions used in PCR for detection of the virulence-associated gene.

gene/serotype	Primer sequence 5'-3'	(bp)	PCR conditions
stx / EHEC	TTT ACG ATA GAC TTC TCG AC CAC ATA TAA ATT ATT TCG CTC	227	5 min 94ºC/ 35 cycles of 1 min 94ºC, 3 min 48ºC and 4 min 72ºC/10 min 72ºC
stI / ETEC	TTA ATA GCA CCC GGT ACA AGC AGG CTT GAC TCT TCA AAA GAG AAA ATT AC	147	5 min 94ºC/30 cycles of 1 min 94ºC, 1 min 55ºC and 1 min 72ºC/10 min 72ºC

Results and discussion

E. coli O157, Shigella and Salmonella are common foodborne pathogens that can cause a variety of illnesses, ranging from fever and diarrhea to abdominal pain and even

death. Consequently, rapid, sensitive and reliable detection of these pathogens is essential to mitigate the associated risks. In the field of foodborne pathogen detection, rapid and accurate detection methods have a significant impact on food safety and public health.

The mean values of the standard plate count for aerobic mesophilic microorganisms, psychrotrophic microorganisms, total coliforms and Escherichia coli, as well as the presence or absence of Salmonella, are shown in Table 2.

According to Normative Instruction No. 161 of July 1, 2022, of the National Health Surveillance Agency (ANVISA), which establishes the microbiological parameters for raw beef, it determines the maximum standard of 106 CFU/g (6 log CFU/g) for the presence of mesophilic aerobic microorganisms, 10^2 CFU/g (2 log CFU/g) for Escherichia coli and the absence of Salmonella in 25 g of the sample. The legislation does not establish parameters for psychrotrophic microorganisms and total coliforms, however, the counting of these microorganisms is considered important, as they are indicators of the hygienic and sanitary conditions of food.

The analyses indicate the presence of mesophilic aerobic microorganisms in 100% of the samples studied, according to the average values shown in Table 2. Of these, 66.66% (8/12) present contamination above the maximum limit of 106 CFU/g (6 log CFU/g), established by law, with average counts ranging from 4.345 to 8.845 log CFU/g. The count of these microorganisms is considered important for determining the microbial load in food, since most pathogenic microorganisms that cause foodborne diseases are mesophilic. In other words, they develop well at temperatures between 20ºC and 45ºC.

Similar results were found by Rinn et al. (2024) and Junior et al. (2023) who also described contamination by mesophiles in 100% of meat samples, with 71% of samples showing a contamination level greater than 1.5 x 10^6 CFU/g in butcher shops in the city of Nampula, Mozambique.

The Heterotrophic Mesophilic Microorganism (HMM) count is used as an indicator of adequate processing conditions, to maintain the cold chain and to predict the shelf life of raw meat (GONZÁLEZ-GUTIÉRREZ et al., 2020; RINN et al., 2024).

In general, for raw meat, it is reported that high detection frequencies of HMM, between 7 log CFU/g and 8 log CFU/g, are associated with lower

quality, earlier onset of texture and odor changes, and generally reduced shelf life (GONZÁLEZ-GUTIÉRREZ et al., 2020; RINN et al., 2024).

Additionally, high MMH values in raw meat, as found in our study, combined with high detection frequencies of other hygiene-indicating bacteria, such as E. coli, notably elevated in this study, indicate unhygienic conditions in the production chain or inadequate temperature during transportation and storage (RINN et al., 2024).

There was growth of psychrotrophic microorganisms with average values ranging from 4.035 logs UFC/g to 6.922 log UFC/g. Although Brazilian legislation does not refer to the total count of psychrotrophic microorganisms, the high count of this group of microorganisms directly influences the shelf life of the product, and may cause undesirable changes in the texture, color, odor and flavor of the meat, making it inappropriate for consumption.

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Table 2. Average results of microbiological analyses of raw beef sold in supermarkets and street markets in Recôncavo of Bahia, expressed in log UFC/g.

Samples	Mesophiles	Psychrotrophics	Total coliforms	*Escherichia coli*	*Salmonella* spp.
ESTAB1	5,173	6,032	5,235	3,835	absence
ESTAB2	4,471	4,425	4,315	1,585	absence
ESTAB1	6,855	5,595	5,415	3,555	absence
ESTAB2	7,689	6,434	3,274	3,033	presence
ESTAB1	6,665	6,922	5,405	3,054	absence
ESTAB2	4,655	4,618	4,233	1,860	absence
ESTAB1	7,395	4,035	4,265	3,235	presence
ESTAB2	8,845	5,845	5,455	3,870	presence
ESTAB1	7,911	6,185	5,605	3,250	presence
ESTAB2	7,595	6,275	5,325	4,933	presence
ESTAB1	6,760	4,447	4,465	3,955	absence
ESTAB2	4,345	4,605	3,215	1,885	absence

SAJ: Santo Antônio de Jesus, CDA: Cruz das Almas, MUR: Muritiba, SAF: São Félix, CAH: Cachoeira, SAA: Santo Amaro, ESTAB1: open market, ESTAB2: supermarket.

Some authors cite psychrotrophic counts below 2 log CFU/g as indicators of low contamination, counts between 3 and 4 log CFU/g as intermediate contamination, and high contamination when counts between 5 and 6 log CFU/g are observed (FUNG et al., 1980; RINN et al., 2024, FUNG et al., 1980)

Based on this classification for counting psychrotrophic organisms, five (41.66%) establishments presented average values considered intermediate and seven (58.33%) presented high contamination. Slightly similar results were described by Velho et al. (2015), where 37.5% of the samples of fresh beef sold in supermarkets in Mossoró-RN presented intermediate contamination and 68.5% presented high contamination, with average values ranging from 3.24 logs UFC/g to 6.64 logs UFC/g.

As observed in Table 2, total coliforms were isolated in 100% of the samples analyzed, with averages ranging from 3.215 log CFU/g to 5.605 log CFU/g. The results found for this group of microorganisms are similar to those presented by Marcanzoni et al. (2018), who, when analyzing beef sold in the city of Chapecó - SC, also observed a high degree of contamination, with the presence of total coliforms in 100% of the samples.

Regarding the occurrence of E. coli, the lowest average values observed and which meet the parameters established by law correspond to supermarkets in the municipalities of Santo Antônio de Jesus, Muritiba and Santo Amaro, representing 25% (3/12) of the total establishments verified in this study.

In another study, Oliveira et al. (2008), when analyzing 10 samples of beef sold in supermarkets in João Pessoa-PB, detected the presence of this agent in 70% of the samples and the presence of coliforms in 100% of the samples studied, data that corroborate the present study.

According to Alves et al. (2018), when present in food, *E. coli* indicates the occurrence of contamination by material of fecal origin, directly or indirectly, thus suggesting the exposure of the product to unsatisfactory hygienic-sanitary conditions during its handling. In addition, contamination by *E. coli* indicates the possible presence of other enteric pathogens, as well as the occurrence of pathogenic strains responsible for the occurrence of diarrhea, hemorrhagic colitis, uremic and septicemia syndrome.

For *Salmonella*, the legislation establishes the absence of the microorganism in 25 g of raw meat.

Therefore, the beef samples sold in the municipalities of Cruz das Almas, São Félix and Cachoeira are considered unfit for consumption.

Of these, three come from street markets and two come from supermarkets. Lower values were observed in a study developed by Oliveira et al. (2019), aimed to evaluate beef cuts sold in supermarkets in the city of Barra do Garças - MT, where none of the samples analyzed indicated the occurrence of the pathogen.

On the other hand, when analyzing samples of beef, pork, and goat meat sold in two municipalities in Angola, for Salmonella contamination, Maia & Oliveira (2023) observed results superior to those found in this study, with the presence of the agent in all samples of beef analyzed. Considering the same study, similar results were found for total coliforms, where the authors also report the occurrence of contamination by these microorganisms in 100% of the samples.

The authors correlate the high degree of contamination with the poor conditions of display and marketing of the meat, without refrigeration and exposed to the constant presence of flies, insects, and dogs. As well as the hygiene conditions of the handlers. Velho et al. (2015) identified the pathogen in five (63%) of the eight supermarkets and seven (88%) of the stalls in public markets selling beef in the city of Mossoró-RN.

The stx gene was identified in two of the *E. coli* isolates (Figure. 1), which came from samples sold at street markets in the cities of Cachoeira and Santo Amaro. Both samples exceeded the maximum limit established by law for contamination by mesophilic aerobes and *E. coli*, in addition to the occurrence of contamination by Salmonella in the sample from the street market in Cachoeira.

This result was superior to that found by Marquezine et al. (2022) that, when evaluating 215 samples of beef cuts produced in meat processing plants in the state of São Paulo, did not identify the stx gene in any of the samples. In another study developed by Stella et al. (2020), evaluating swab samples from the carcass of 154 cattle slaughtered in a slaughterhouse in the city of Mineiros - GO, none of the samples presented the stx gene.

Higher numbers were observed in a study developed by Tarazi et al. (2021), where a total of 410 diarrhea fecal samples of human and animal were analyzed, in addition to samples of ground meat collected in retail markets

in northern Jordan. Of this total, 194 E. coli isolates were identified, of which 57 were classified as EHEC (stx).

The stI gene was not identified among the strains analyzed, and there was no genotypic characterization of enterotoxigenic *E. coli* (ETEC).

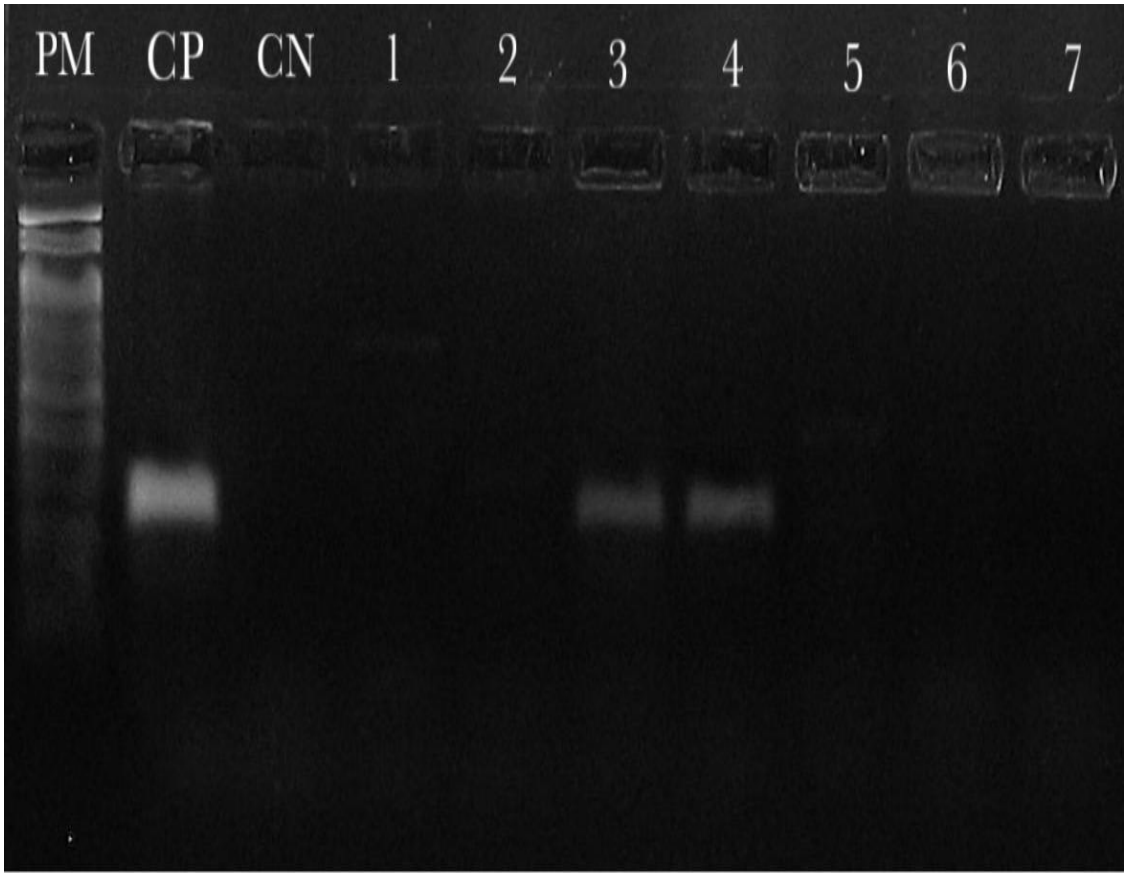

Figure 1. Photograph of the 2% agarose gel of the PCR for the stx gene found in *E. coli* samples isolated from beef. Fragment size 227bp, MW: Molecular Weight 100bp, CP: Positive Control, NC: Negative Control, positive samples 3 and 4.

Beef with poor sanitary quality sold in Recôncavo of Bahia represents a serious problem with impacts that extend from local trade to state, national and international ones. Several studies and analyses indicate high levels of contamination by microorganisms such as *Escherichia coli*, *Salmonella*, total coliforms, mesophilic and psychrotrophic bacteria in samples of beef sold in street markets and supermarkets in the region. This situation highlights significant deficiencies in hygiene practices, maintenance of the cold chain and proper handling of the product, directly

compromising the food safety of consumers. At the local level, the main consequence is the loss of consumer confidence. When the population perceives the health risk, they tend to avoid consuming meat from certain points of sale, which directly affects the livelihood of small traders and butchers, especially those who operate in street markets. In addition, the beef produced and sold in Recôncavo is now seen as a product of lower value, economically devaluing what could be an important source of income for the region. Many of these establishments also do not have the necessary structure to meet health requirements, further aggravating the problem.

At the state level, the losses are even greater. The image of the agri-food sector in Bahia is damaged, mainly because this state is one of the main meat producers in the Northeast.

When a region systematically presents meat with a high degree of contamination, there is a negative effect on the reputation of the entire state, affecting the acceptance of the product in more demanding consumer centers, such as Salvador and tourist cities.

The state health surveillance department is pressured to intensify its actions, which increases operating costs and can generate tensions with local traders. In addition, low-quality meat makes it difficult to sell to other regions of the state, reducing the internal competitiveness of the product.

In the national context, the problem can result in informal trade barriers, especially in distribution networks that value strict quality and traceability criteria. Although meat from the Recôncavo region is not directly included in the export market, its poor health reputation harms meatpacking plants, cooperatives and local entrepreneurs who wish to expand their markets to other regions of Brazil. There is also a considerable risk to public health, since contaminated meat can contribute to outbreaks of foodborne diseases, generating costs for the health system, lawsuits and distrust regarding the effectiveness of health inspections.

On the international scene, even though the region's meat is not destined for large-scale export, the growing demand for traceability and food safety in global markets creates an indirect requirement on the entire national production chain. Recurrent cases of contamination in certain regions of the country may compromise Brazil's image as a reliable supplier, even influencing import policies in partner countries. Furthermore, studies conducted in African and Asian countries reveal

similar patterns of contamination in contexts of precarious infrastructure, lack of effective inspection and inadequate handling of meat — showing that the meat health problem is not exclusive to Recôncavo or Brazil, but is part of a global food crisis that requires international cooperation, harmonization of health standards and strengthening of epidemiological surveillance networks.

Given this scenario, it is urgent to implement public policies and intervention strategies that prioritize the training of handlers, improvements in the infrastructure of points of sale, increased inspection and incentives for the formalization of the production chain.

The problem of low-quality meat in the Recôncavo of Bahia is not only a local challenge, but a reflection of structural weaknesses with inter-territorial and international implications, which, if not corrected, will continue to compromise the health of consumers and the competitiveness of the Brazilian production chain on the global stage.

Conclusions

Beef sold in the municipalities of Santo Antônio de Jesus, Cruz das Almas, Muritiba, São Félix, Cachoeira and Santo Amaro has a high bacterial load, compromising the quality of the product and posing a risk to the health of consumers, given the presence of microorganisms that indicate contamination of fecal origin and are pathogenic to humans.

It is essential to encourage health education programs, aiming to spread knowledge among meat handlers and traders about the importance of food safety, personal hygiene and good handling practices. As well as, to consumers, regarding the risks involved in the practice of purchasing and consuming products of animal origin without a guarantee of origin.

Then, there must be greater control over health inspections carried out by the competent bodies on the practice of marketing products of animal origin, as well as the qualification and training of these services, especially in cities in the interior and street markets, where contamination rates tend to be even higher.

Therefore, the public authorities play an important role in promoting One Health through programs, policies and legislation aimed at public health.

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