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## RESEARCH ARTICLE

# ADULTERATION WITH HORSEMEAT AND BACTERIOLOGICAL QUALITY OF SAUSAGES AND MORTADELLA SAMPLES IN COSTA RICA

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**ABSTRACT**

**Background:** Ready to eat meat represents an important diet component worldwide and Costa Rica does not escape this trend. Generally, these products are made from poultry, beef and pork meat; however, the fraudulent replacement for horsemeat has been reported. **Objective:** The aim of this work was to determine the potential contamination with potential foodborne pathogens and the adulteration with undeclared horse meat of 120 mortadella and 120 sausage samples acquired in the four provinces that conform the Central Valley of Costa Rica. **Methods:** Bacteriological analysis included the Most Probable Number (MPN) of *Escherichia coli* and *Staphylococcus aureus,* presence of *Listeria monocytogenes, Salmonella* spp, and *Clostridium perfringens*. Presence of horse's DNA was determined in 73 sausage and 42 mordatella samples using real time PCR methodology. **Results:** Results obtained show the presence of pathogenic microorganisms and horsemeat in the samples analyzed. A total of 47 (21,4%) isolates of *E. coli,* 59 (26,8%) of *Staphylococcus aureus* and 16 (7,3%) of *Listeria monocytogenes* were obtained from the 240 samples analyzed. No isolates of *Salmonella* spp and *Clostridium perfringens* were obtained. An overall positivity of 48,7% was determined for horsemeat, including a 54,7% positivity for mortadella samples and 45,2% for sausage. **Conclusions:** Data obtained shows that there is an important fraud in ready to eat meat products, and also that the contamination or mishandling of these products may represent a risk for public health.



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## INTRODUCTION

According to the World Health Organization, food borne illnesses are considered one of the top public health issues affecting mainly children, elder people, and immunosuppressed patients. In a study published in 2015 it was estimated that around 600 million people across the world got sick for consuming contaminated food, which causes approximately 420000 deaths (World Health Organization, 2017). The public health impact caused by food borne diseases in Latin America and the Caribbean is due to the ingestion of contaminated food and water that could cause enteritis and diarrheic illnesses in 70% of the cases of vulnerable population (World Health Organization, 2007). Ready to eat meat represents an important diet component worldwide. In countries like Germany, the consumption of these products ascends to 50% of all meat products.

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Costa Rica does not escape to this trend; an important percentage of the national meat production derives to the processing of this kind of products either by big companies or many artisanal and informal ones, the majority of which are not certified by the Health Ministry (Godfray *et al*., 2018). In Costa Rica, an increase in the consumption of ready to eat meat products is clear; according to the Health Ministry, in 2008 the production was of 20 tons and in 2013 it surpassed 25 tons. Generally, these products are made from poultry, beef and pork meat; however, the fraudulent replacement for horsemeat has been reported in other countries where it is associated with a lower quality product and constitutes a potential public health threat due to fewer hygienic controls of the slaughtering conditions (Walker, 2013). Food fraud is defined as the addition of non authentic substances, or the removal or replacement of authentic substances with other ingredients without the consumer's knowledge (Moore, 2012). The main problem associated with the adulteration with other meat species, besides the use of lower commercial quality materials, are the potential health risks for the consumer due to unhygienic slaughtering conditions and the potential

contamination with foodborne microorganisms (Dalvit, 2007). In 2013, a scandal broke down in Europe when it was discovered that food industries were manufacturing products with horsemeat instead of beef as they specified in their labels. Horsemeat has the same protein content as beef but less fat; nevertheless, this type of meat oftentimes doesn't have the sanitary controls required for food safety, so it could cause outbreaks associated with food contamination, as well as allergic reactions in people susceptible to the protein (Sentradeu, 2014). According to CDC data, it was estimated that in 2016 several cases of Salmonellosis were associated with ground beef adulterated with horsemeat that occurred in various states across the United States. Cases of *Trichinella spiralis* associated with consumption of undeclared horsemeat have been documented as well across the world, demonstrating the lack of sanitary controls (Grundy, 2012). Due to the previously stated facts, food controls in terms of innocuity and identification of its components to avoid frauds has been a topic of debate among governments as well as food companies; especially for high-demand products like grains and cereals. However, in meat products there are no enough studies to evidence this problem. Determining the potential contamination and adulteration of the most commonly consumed ready to eat meats such as mortadella and sausage will be useful in order to generate appropriate recommendations for the Health Ministry, producers and consumers regarding the health risks associated with their consumption.

## MATERIALS AND METHODS

**Sampling:** A total of 240 samples of ready to eat meat commercially processed products, including 120 sausage and 120 mortadella samples were acquired from retail stores located in the four provinces that conform the Central Valley of Costa Rica (Heredia, Cartago, San José, Alajuela). Specifically, for each province a total of 60 samples (30 mortadella and 30 sausage) were acquired. These two products were selected since they represent the most consumed ready to eat meat products in Costa Rica. Sample size was determined according to the number of butcheries where they are sold in each province, for a homogeneous distribution over the four provinces. The label of the products analyzed were checked in order to determine if horsemeat was declared as ingredient. Samples were transported at 4 to 6°C to the Food Microbiology Laboratory, University of Costa Rica, and were processed within 24 h.

**Presence of horse's DNA in ready to eat meat samples:** A total of 115 samples, including 73 sausages and 42 mordatella were analyzed for the presence of horse's DNA. Briefly, DNA was extracted by mixing 1 g sample with 750 µl of lysis buffer, 50 µl of SDS 10% and 10 µl of Proteinase K, incubated at 65°C for 6 h. DNA was obtained by adding 500 µl of NaCl solution 6M, centrifuging 15 min at 10000 x g. To 500 µl of the supernanant 300 µl of ethanol 70% was added and after centrifuging for 3 min at 6000G. The pellet was dried and dissolved in Tris EDTA buffer H 7,5 and kept at -20°C until used. The quality and purity of the DNA extracted was determined using the NanoDrop 2000 (Thermo Scientific) equipment. Also, DNA integrity was determined by electrophoresis using 1% agarose gel. PCR real time reactions were done using the Rapid Finder Equine ID Kit (Thermo Scientific). A Step One thermocycler (Applied Biosystems) was used. Reaction mixture had a final volume of 25 µl (7,5 µl primers, 12,5 µl master mix and 5 µl sample and DNA controls with a concentration of 10ng/µl. Amplification protocol was of 95°C for 10 min, 36 cycles at 95°C for 15 s and 60° for 1 min. Data interpretation was done according to the fluorescence detection in the FAM and VIC channels.

**Bacteriological analysis**

**Most probable number (MPN) *Escherichia coli:*** 10 g of each sample were diluted with 90 mL of sterile peptonized water (PW) 0,1% and homogenized in a Stomacher. Decimal dilutions were prepared also using PW. One mL of each dilution was inoculated into 3 tube series of lauryl triptose broth (LTB) and furtherly incubated at 35°C for 48h for a MPN analysis. From each positive tube (turbidity and gas formation) *Escherichia coli* (EC) broth and triptone broth media were inoculated using sterile wooden applicator sticks and incubated for 24 h at 44,5°C. EC tubes that presented gas formation were interpreted as fecal coliforms, and triptone tubes where indol formation was demonstrated using Kovacs reactive were confirmed as *E. coli.*

**MPN *Staphylococcus aureus:*** From the decimal dilutions previously prepared, 3 tubes of tripticase soy broth (TSB)+10% NaCl were inoculated and incubated for 48 h at 35°C. After incubation, a loop was transferred into Baird Parker agar and also incubated at 35°C for 24 h. Suspicious colonies were plated onto nutrient agar plates for biochemical confirmation.

***Clostridium perfringens* isolation:** For the isolation of *C. perfringens*, 25 g of each sample was diluted in 225 ml of Butterfield broth and plated on triptose sulfite cicloserin agar (TSC). Plates were incubated under anaerobic conditions at 35°C for 24 h. Suspicious colonies were plated onto blood agar plates for confirmation.

***Listeria monocytogenes* isolation:** A 25 g sample was homogenized in 225mL of University of Vermont Medium (UVM) broth and incubated at 35°C for 24 h. After this period, 1mL of the incubated broth was transferred to 10mL of Fraser broth and incubated at 35°C for 48 h. This enrichment media was streaked onto Oxford agar plates and incubated at 35°C for 24 h. Presumptive colonies were confirmedusing Vitek 2 system

***Salmonella* spp. Isolation:** The methodology recommended by the International Organization for Standardization (9) was followed. Briefly, 25 g of each sample was weighed and homogenized with 225 mL of simple lactose broth (SLB). This pre enrichment was incubated overnight at 35°C. Following incubation, 1 mL of the preenrichment broth was transferred to 10 mL tubes of tetrathionate broth and Rappaport-Vassiliadis broth. Both media were incubated at 35°C for 24 hours.

For isolation of *Salmonella* species, each enriched medium was streaked onto selective xylose lysine deoxycholate (XLD), Hecktoenand bismuth sulphite agar plates and incubated at 35°C for 24 hours. *Salmonella* suspected colonies than included red colonies with or without black central precipitate for XLD agar, green-blue colonies with or without black central precipitate for Heckton agar and grayish colonies for sulphite agar were plated onto nutrient agar plates for biochemical confirmation.

## RESULTS

Results obtained show the presence of pathogenic microorganisms and horsemeat in the samples analyzed, nevertheless, no label declared the presence of horsemeat as an ingredient, only porcine, beef and pork meat appeared as ingredients. Sample distribution according to the province of origin is shown on Table 1. Labelling is intended to give to the consumer enough information related to product's identity. In this study, 12,1% of the sausage samples acquired were completely unlabeled, as 3,3% of the mortadella samples analyzed. Labelled products fulfilled the Executive Order partially, since they lacked both lot number and caducity date, making traceability impossible.

When the ingredient list was verified, none declared horsemeat in their preparation, nevertheless, results obtained reveal the presence of this ingredient, as shown below. Table 2 describes the percent of positivity for horsemeat present in sausages and mortadella, according to the province of origin. For the molecular analysis, only 115 samples were tested, nevertheless results show that both mortadella and sausage samples are commonly formulated with horsemeat although it is not reported in the labels as ingredient. An overall positivity of 48,7% was determined, 54,7% of mortadella samples and 45,2% of sausage samples presented horsemeat. Alajuela the province that presented the greatest number of ready to eat meat samples adultered with horsemeat (58,1%), followed by Heredia province (51,7%). Also, mortadella samples had a greater percent of positivity than sausage samples for all provinces evaluated except for Alajuela. Table 2 shows the prevalence of potentially pathogenic microorganisms in ready to eat meat samples analyzed. A total of 47 (19,6%) isolates of *E. coli,* 59 (24,7%) of *Staphylococcus aureus* and 16 (6,7%) of *Listeria monocytogenes* were obtained from the 240 samples analyzed.

12,1% of mortadella samples and 7,5% of sausage samples were positive for *E. coli* whereas 15,1% of mortadella samples and 9,6% of sausage samples were positive for *S. aureus*. The percentage of positivity for *L. monocytogenes* found was 6,7% (16/240), coming 11 (4,6%) from mortadella and 5 (2,1%) from sausage. No isolates of *Salmonella* spp and *Clostridium perfringens* were obtained. Heredia was the province that presented the highest number of *E. coli* (36,1%) and *S. aureus*(32,2%) isolates, whereas Cartago presented the greatest percent of positivity for *L. monocytogenes* (50,0%).

## DISCUSSION

Actually, knowledge about the composition of food has become a priority for consumers, trying to avoid any kind of adulteration that might represent a threat for human health. In this research, 48,7% of ready to eat meat samples presented horsemeat, a fact that demonstrates fraud because of the substitution of ingredients declared on the label. The lack of an equine production facility in Costa Rica suggests that the conditions in which these animals are sacrificed are not the optimal ones, especially because horses may be sacrificed when they have finished their productive life, have suffered an important injury and even when they present some kind of heath problem and have to be discarded (Stanciu, 2015). A previous study done in Costa Rica, to detect horsemeat in mortadella and sausage samples with an immunoenzymatic assay showed a 20% of positivity, lower than the one reported in this paper (Chaves 2017,unpublished data). This difference in positivity might be due to the high sensibility (99%) and specificity (99,9) of molecular methodologies, and also to the fact that DNA is very stable, resisting even processing conditions like high temperatures. In addition, data obtained from our study is very trustworthy, since the amplification of an internal standard validates the test. Ready to eat meat products include a wide range of food products obtained with different technologies including different thermal treatments

**Table 1. Sample distribution and label verification as stated by the Executive Order Nº 35079-MEIC-MAG-S, 2009 according to the province of origin**

| Province | Labelled sausage samples n(%) | Unlabelled sausage samples n(%) | Labelled mortadella samples n(%) | Unlabelled mortadella samples n(%) |
|---|---|---|---|---|
| San José | 23 (25,3) | 7 (24,1) | 28 (25,0) | 2 (25,0) |
| Alajuela | 20 (21,9) | 10 (34,5) | 29 (25,9) | 1 (12,5) |
| Heredia | 26 (28,6) | 4 (13,8) | 29 (25,9) | 1 (12,5) |
| Cartago | 22 (24,2) | 8 (25,6) | 26 (23,2) | 4 (50,0) |
| Total (n=240) | 91(37,9) | 29 (12,1) | 112 (46,7) | 8 (3,3) |

**Table 2. Positivity percentage for the adulteration of ready to eat meat products with horsemeat according to the province of origin**

| Province of origin | Number of sausage samples analyzed | Number of sausage samples positive for horsemeat n (%) | Number or mortadella samples analyzed | Number of mortadella samples positive for horse meat n(%) | Total positivity n (%) |
|---|---|---|---|---|---|
| San José | 17 | 7 (41,1) | 10 | 5 (50) | 44,4% |
| Alajuela | 22 | 12 (54,5) | 9 | 6 (66,7) | 58,1 % |
| Heredia | 18 | 8 (44,4) | 11 | 7 (63,6) | 51,7% |
| Cartago | 16 | 6 (37,5) | 12 | 5(41,6) | 39,3% |
| Total | 73 | 33 (45,2) | 42 | 23 (54,7) | 48,7% |

**Table 3. Potentially pathogenic microorganisms isolated from ready to eat meat samples, according to the province of origin**

| Province of origin | *Escherichia coli* n (%) | *Staphylococcus aureus* n (%) | *Listeria monocytogenes* n (%) | *Clostridium perfringens* n (%) | *Salmonella* spp. n (%) |
|---|---|---|---|---|---|
| Cartago | 11 (23,4) | 10 (16,9) | 8 (50) | 0 | 0 |
| Alajuela | 10 (21,3) | 18 (30,5) | 0 | 0 | 0 |
| San José | 9 (19,1) | 12 (20,3) | *6 (37,5)* | 0 | 0 |
| Heredia | 17 (36,1) | 19 (32,2) | 2 (12,5) | 0 | 0 |
| Total | 47 (19,6) | 59 (24,6) | 16 (6,7) | 0 | 0 |

designed for killing microorganisms coming from ingredients, equipment and personnel (11).Additional thermic process is not necessary before its consumption, (Liu, 2016) so the absence of any pathogenic microorganism is mandatory. However, results obtained show the presence of *E. coli* in 19,6% of samples analyzed, demonstrating fecal contamination. The origin of this contamination might be associated to post processes conditions, since the absence of *Salmonella* spp and *Clostridium perfringens* corroborate that the thermic treatment applied is correct. *S. aureus* is an organism that tolerates desiccation and can survive in in-hospital environments, but it is a bad competitor (13).This bacterium was found in 24,6% of samples, a contamination that might be due to the exposure of finished product to contaminated surfaces of even inadequate manipulation. As Fijalkowskik*et al*. manifest (14), a repetitive manipulation by personal and the constant contact with equipment and surfaces increases the risk of cross contamination of food. The amount of *S. aureus* found is not enough for the production of enterotoxin and the generation of a foodborne intoxication during the shelf life of these products. Nevertheless, preformed toxin may be present in raw ingredients, as this molecule is resistant to thermal treatment (Das Dores, 2013). *Listeria monocytogenes* is a foodborne pathogen that causes listeriosis, a fatal disease with more than 15% mortality rate (Jordan, 2018). It represents a risk for elderly and immunocompromised individuals, and ready to eat foods are highly associated to this pathogen, since there is no further anti-microbial step between its production and consumption. The isolation of *Listeria monocytogen*es in 6,7% of the samples analyzed reveals the risk that consumption of sausages and mortadella might represent for vulnerable populations. Similar studies have reported the isolation of this bacteria also from ready to eat meat products (Wang *et al*., 2015). The ability of *Listeria* to form biofilm and its adhesivity to plastic and metal surfaces in production environments is highly associated to its widespread in different environments (Stepanovic *et al*., 2004). The results obtained in this work show that there was no isolation of *Cl. perfringens* in the samples analyzed. The frequency of *Cl. perfringens*in processed meat products has been described as lowworldwide (Mahami *et al*., 2012), because of the thermic process used for their production, which eliminates vegetative forms of this bacteria, as well as the addition of nitrites that may affect *Clostridium* sp. growth.

There was no isolation of *Salmonella* spp in the samples analyzed. Several researches have demonstrated that in normal conditions, *Salmonella* is found in very low quantities in food, because of a competitive inhibition done by other microorganisms (Zhang, 2007). Also, some *Salmonella* species may apply survival strategies when they are in stressful environments, such as a metabolic decrease that leads to a viable non cultivable state (Storage *et al*., 2019; Piennar, 2016). The absence of *C perfringens* and *Salmonella* isolates demonstrate that samples analyzed had an effective thermal treatment, and that the contamination with other microorganisms as described above may be due to post thermal mishandling or cross contamination. The overall results obtained demonstrate that there is an important mishandling of sausage and mortadella products in Costa Rica. The presence of *E.coli* demonstrates contamination with fecal material and the important isolation rate of *L. monocyto*genes demonstrates the potential risk related with these products, especially if they are consumed without any additional heat treatment. Same time, fraudulent practices were revealed, since the presence of horsemeat not declared on the label occurs in an important number of samples

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