

## MICROBIAL CONTAMINATION LEVELS IN RABBIT CARCASSES OBTAINED FROM POPULAR MARKETS IN TOLUCA VALLEY, MEXICO

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### ABSTRACT

Microbial load in 125 rabbit carcasses from popular local markets (supermarkets, butcheries and markets) in Toluca Valley, Mexico was evaluated using plate microdilution to estimate mesophilic and coliform total count (colony forming units/surface, CFU), and *E. coli*, *S. aureus*, *Salmonella spp.* and *Listeria monocytogenes* isolations. The lowest mesophilic and coliform CFUs was observed in samples from supermarkets ( $1.50\pm 0.92$  and  $0.8\pm 0.61$ ) compared to higher microbial load in markets ( $2750\pm 1310$  and  $2463\pm 918$ ) ( $P<0.001$ ). Isolation frequency of pathogens considered as public health risk were: *E. coli* (62.4%), *S. aureus* (36.0%) and *Salmonella spp.* (1.6%). *E. coli* hemolytic (HLYI) and CR+ strains were found and *S. typhimurium* and *S. anatum* serotypes ( $P<0.001$ ). No *Listeria monocytogenes* isolations were obtained with the bacteriological procedures used. It is concluded that the microbial contamination levels of rabbit carcasses evaluated in the different popular markets in Toluca Valley is related with its marketing, in which markets are considered as critical risk for food safety and public health.

**Key words:** Microbial contamination, Rabbit carcasses, Popular markets, Commercialization.

### INTRODUCTION

Rabbit production in an important activity in the State of Mexico's regional socioeconomic development, and is considered nationally important according to SAGARPA (2005). Rabbit production spreading in the state has favoured family microenterprise creation and productive chain integration in the rabbit-product system, which have permitted the rapid emerging rabbit meat market in our entity (Gobierno del Estado de México, 2000). Actually, commercialization and production distribution is made in a free marketing system to supply commercialization centers like supermarkets (big commercial chain markets in urban areas). Butchers directly offer the product in settled shops and mobile markets have traditionally compered different basic food products in urban and suburban areas. When consumer sectors in urban areas evolved, food supply through big commercial markets increased, with the necessity of higher quality products, directed to satisfy consumers (García, 2006).

Due to culinary and nutritional rabbit meat characteristics, these easily adapt to food diets of different human being populations. The demand for safe and innocuous meat products for human consumption forces the maintainance of good production practices in rabbit farms (Liste *et al.*, 2006), which contributes to diminishing microbial contamination risk as well as chemical products presence in rabbit meat. Carcass handling and commercialization should maintain optimal conditions to improve quality and food safety (FAO, 2003). Actually food industry has technology, transformation and manufacturing processes required to preserve industrialized meat and products, necessary for accomplishing quality product expectations in web sector values (Liste *et al.*, 2006; Pozo Lora *et al.*, 1980).

Rabbit meat has its own physical and organoleptic characteristics, which permits offering fresh carcass conservation as well as processed through packaging, which should be maintained at adequate refrigeration temperatures. Cold chain maintains organic and nutritional characteristics, by avoiding possible physical and microbiological meat alterations (Anónimo, 2007).

Microbiological contamination is one of the main risk conditions that affect meat quality and consumers health, because their importance to human health such as: *Salmonella spp.*, *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes*, which are object of public health certification because of the potential risk they represent in diseases transmitted by food (Plym and Wierup, 2006). In Mexico the government through the Ministry of Agriculture has promoted regulations regarding production, transformation and commercialization in the product-rabbit system for good production and manufacturing practices. On the other hand, health regulations applicable to rabbit meat production systems are not specific, due to the absence of regulations regarding quality and product safety, in which microbiological conditions should be specified. This situation could compromise the quality and food safety of rabbit meat.

This study was made to evaluate the microbial contamination levels in rabbit carcasses obtained from popular markets in Toluca Valley, México.

## MATERIALS AND METHODS

A transversal study was taken out during spring-autumn 2007, in Toluca Valley, Mexico in the following municipalities: Toluca, Lerma, Metepec and Mexicaltzingo, sampling 43 fresh rabbit carcass selling places.

### Market categories

1. Supermarkets: autoservice commercial center chains with direct selling to consumers, in which refrigerated whole carcasses or pieces were rigidly packed, fluid absorbent towel used, covered with plastic, with presentation label and expiry date.
2. Butcheries: settled commercial location with refrigerated mammal meat variety including rabbit with direct selling to consumers. Carcass cuts were made when sold and packaging at purchasing using plastic covering.
3. Markets: traditional free mobile selling points in urban and suburban areas. Fresh carcass exhibition was made in non refrigerated glass cabinets for direct selling in polyethylene bags.

### Samples and bacteriological isolation

Once we had authorization from commercial locations, 125 swab samples from exhibited rabbit carcasses were taken from 1 cm<sup>2</sup> of the right dorsal region in between T3 and T6 vertebrae, which were placed in 1 ml transport media used for preenrichment (bile red, bile brilliant green, brain and heart infusion and soy trypticase added with sodium chloride 7.5%, BBL-DIFCO, USA). Samples were refrigerated at 4°C during transportation. Sample processing was made by simplified microplate dilution method (Cottral, 1986), in which 100 µl of the 10<sup>-3</sup> dilution was placed on violet red bile agar plates for standard counting of mesophilic bacteria, incubated for 18 to 24 hours to estimate the probable number of colony forming units (CFU), expressed as 10<sup>3</sup>/carcass surface unit (cm<sup>2</sup>), using a Quebec type counter (REPRESA, México).

Isolation and identification of contamination bacteria was made by inoculation of 0.01 ml from each transport media on Vogel-Johnson agar plates added with potassium telurite, brilliant green and MacConkey, incubated at 37°C for 24 h. CFU were identified by growing characteristics and Gram stain. Final identification was made using *Api 20-E* and *Api Staph* standardized methods (VITEK-

MERIAL, France). For *Listeria spp.* isolation, sample incubation was made in listeria broth at 4°C (BBL-DIFCO, USA), and inoculation on listeria agar plates (BBL-DIFCO, USA) and blood agar plates (BIOXON, México), incubated at 37°C for 48 h. *E. coli* isolations were transferred to blood agar base plates prepared with rabbit 7.5% v/v washed red blood cells and trypticase soy agar plates added with 0.001 g/ml 69% congo red (SIGMA, México), for determination of *E. coli* hemolytic strains (HLY I) and positive congo red strains (CR+).

### Data evaluation

Data were evaluated using descriptive statistics (Daniel, 2002) using average of mesophilic and total coliform CFU, compared in between market categories evaluated by t Student test with alfa (0.05) and observed frequencies for different pathogen types: *Salmonella spp.*, *S. aureus* and *E. coli*.

## RESULTS

Table 1 shows microbial load in rabbit carcasses evaluated in popular Toluca Valley markets. The lowest number of mesophilic and total coliform CFU were identified in commercialized carcasses from supermarkets (1.50±0.92 and 0.8±0.61 respectively) compared to higher loads in markets (2750±1310 and 2463±918) (P<0.001).

**Table 1:** Bacterial load in rabbit carcasses from popular Toluca Valley markets

Market category	Number of sampled places	Sample number from each place (%)	CFU Total mesophilic count	CFU Total coliform count
Supermarket	7	23 (18.4)	1.50 ± 0.92	0.8 ± 0.61
Butchery	19	37 (29.6)	53.5 ± 47.89	37.9 ± 11.54
Market	17	65 (52.0)	2750 ± 1310	1920 ± 850
Total	43	125 (100.0)	3370 ± 1761	2463 ± 918

(P<0.001), CFU probable number expressed as 10<sup>3</sup>/cm<sup>2</sup> surface unit. Average counts ± SD.

In the processed samples (n=125), the highest frequency for public health potential risk was obtained for *E. coli* (62.4%), followed by *S. aureus* (36.0%) and *Salmonella spp.* (1.6%), in which hemolytic *E. coli* and CR+ strains were found. *Salmonella* serotypes were *S. typhimurium* and *S. anatum* (P<0.001). No *Listeria monocytogenes* isolations were identified (Table 2). The isolation frequency for *E. coli* and *S. aureus* was higher in markets and butcheries (P<0.001). *Salmonella spp.* was found in markets only.

**Table 2:** Public health risk pathogen identification in rabbit carcasses from popular Toluca Valley markets

Pathogen	Number of positive isolations	Isolation percentage (%)
<i>E. coli</i> <sup>a</sup>	78	62.4
<i>S. aureus</i>	45	36.0
<i>Salmonella spp.</i> <sup>b</sup>	2	1.6
Total	125	100.0

n =125 carcass samples; (P<0.001), <sup>(a)</sup> hemolytic *E. coli* 23/78 and 11/ 78 CR+, <sup>(b)</sup> *S. typhimurium* and *S. anatum*

## DISCUSSION

Microbial contamination levels were determined in rabbit carcasses obtained from popular markets: supermarkets, butcheries and markets in Toluca Valley, Mexico, in which different important microbial load and public health pathogen presence was identified depending on carcass commercialization origin. These differences mark the risk level of microbial contamination from exogen and endogen sources, produced by product manipulation during sacrifice and carcass processing (Baldelli *et al.*, 1995). It is also possible that a minimum contamination of fresh meat

originated from *S. aureus* carriers could have occurred. The degree of microbial contamination has influence on direct organoleptic and health carcass characteristics (García-López *et al.*, 2006), causing penalization during meat health inspection, because they are considered as non appropriate products for human consumption, depending on the degree of contamination, because of the risk implied in food diseases and intoxications (Rodríguez-Calleja *et al.*, 2006). The high microbial *E. coli* contamination found in carcasses from markets suggests a major public health risk, probably due to *E. coli* HLY I and CR+ strains, which can be considered as enteropathogens and enteroinvasive phenotypes, and should be evaluated using other *in vitro* and molecular procedures for confirmation of ETEC and EIEC human health risk phenotypes. The same happened with *Salmonella spp.*, in which *Salmonella typhimurium* has been widely recognized in foodborne diseases (Khosrof *et al.*, 2002). On the other hand, market commerce is traditionally linked to mexican society popular culture, since prehispanic dates, which has slowly evolved to mobile popular markets. Nevertheless, the high contamination loads could be associated to poor hygiene conditions as well as bad product handling due to inadequate infrastructure in these markets with non observance of food control (Mendoza *et al.*, 2000).

Mesophilic bacteria contamination levels is considered as environmental and product processing contamination, related with total coliform levels. When microbial load exists in carcasses, a fire effect can be triggered by carcass conservation temperature during sale (Khalafalla, 1993).

It is important to consider carcass handling during slaughter and hygiene during processing, which have direct influence on potential basal pathogen load in human health which could be related to *E. coli* and *S. aureus* contamination. This risk can be diminished if adequate sacrifice and hygiene practices are observed in rabbit slaughterhouses, strengthened by maintaining the cold chain during transport, distribution and carcass commercialization (Rodríguez-Calleja *et al.*, 2004).

It is possible that in traditional meat markets health quality and potential risks exist, which could produce food diseases and intoxications (NOM-064-ZOO; García-López *et al.*, 2006). *Listeria monocytogenes* contamination demands a permanent screening program for possible evaluation of human health risk from rabbit carcasses, using other analytical processes (Augustin and Carlier, 2006). It is necessary to identify *S. aureus* risk associated to enterotoxin production and meticillin resistance strains which have been identified in other meat types (Blanc *et al.*, 2006; Volovich and Ladaniï, 1974).

It is concluded that the microbial contamination levels in rabbit carcasses evaluated in different popular market types in Toluca Valley, Mexico show differences depending on the market type, considered as critical risk in markets.

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