NON DESTRUCTIVE STANDARIZED METHOD TO DETERMINE BACTERIAL CONTAMINATION BY *Staphylococcus aureus* IN RABBIT CARCASSES

GARDUÑO-GUADARRAMA V.[1], VELÁZQUEZ-ORDOÑEZ V.[2], ALONSO-FRESÁN M.U.[2*], ZAMORA-ESPINOSA J.L.[2], TALAVERA-ROJAS M.[3], DOMÍNGUEZ-VARA I.A.[4], MENDOZA-BECERRIL J[4].

1.-Programa de Ciencias Agropecuarias y Recursos Naturales – UAEM. Área salud animal. Centro de Investigación y Estudios Avanzados en Salud Animal. Km 15.5 carretera Toluca-Atlacomulco, Estado de México.
2.-CIESA. Facultad de Medicina Veterinaria y Zootecnia. Universidad Autónoma del Estado de México. Cuerpo Académico en Salud Animal (UAEM-CA-3 Salud Animal).
3.-CIESA. FMVZ. UAEM. [4] FMVZ. UAEM. El Cerrillo Piedras Blancas, México.
**Corresponding author*:muaf@uaemex.mx

ABSTRACT

Rabbit's meat is increasingly consumed worldwide, regardless of its microbial counts. There is not an specific method to determine the microbial count in rabbit carcasses. This is the main reason for developing a non destructive surface sampling, taking into consideration the sampling method for big species to determine microbial counts for *Staphylococcus aureus*. Fifteen rabbit carcasses were sampled, taking a different surface sample per group (five rabbits per group). Wet swabs with peptone water were scrubbed in different regions: R1 in the thigh, R2 in the back, R3 in the ribs and R4 in the shoulder (one rabbit per region). The four regions were sampled in one rabbit from each group. The surface sampled for Group 1 was 2.5 X 2,5 cm; Group 2, 5 X 5cm and Group 3, 10 X 10cm. Serial decimal dilutions were made and cultured in Baird Parker Agar for *Staphylococcus aureus* growth. The best surface for sampling was the smallest. *S. aureus* CFU/cm^2 in the samples exceed the national and international maximum limits, representing a hygiene alert due to possible *S. aureus* strain dissemination.

Key words: surface sampling, non destructive method, *Staphylococcus aureus,* rabbit carcass, CFU/cm^2.

Introduction

Over the years, it has been undoubtable the people's interest on achieving and maintaining a healthy life according to today's needs and demands, through the implementation of optimal diet strategies directed towards disease prevention. Therefore, it has been necessary to look for new food sources, as well as screening their microbiological quality. Meat is one of the main foods providing nutrients such as proteins, lipids and vitamins amongst others. Nevertheless, there are controversies nowadays regarding its nutritional role, due to the fact that consumers consider that high ingestion is correlated with health problems, including obesity and cardiovascular diseases, therefore reducing its consumption (Schönfeldt and Gibson, 2008). This is the reason why people tend to modify their lifestyle by exploring new healthy diet habits, in which rabbit meat (*Oryctolagus cuniculus*), stands out as a nutritious and healthy election (Hu and Willett, 2002; Hernández, 2008; Hernández and Dalle, 2010; Simonová *et al.,* 2010). Nevertheless, there has not been developed specific sampling methods to determine its bacterial load without affecting the carcass presentation quality. Several pathogens which could be present in raw meat might as well contaminate the workers' hands during processing and handling of the meat, with posterior transmission to other food, equipment and other workers as well (Rodríguez, 2002). To guarantee the microbiological quality of rabbit's meat, a screening program should be followed, in compliance with microbiological criteria which could demonstrate that the implemented measures for quality assurance in meat maintains an adequate control of microorganisms.

Material and Methods

Fifteen rabbit carcasses were randomly selected in a slaughterhouse in Toluca Valley to carry out surface sampling using different area templates. Baird Parker medium was prepared as established in NOM-115-SSA1-1994, (DOF, 1995a). In the same way, peptone water was prepared, which was used as moisturizing solution for the swabs used when sampling (10 mL per sample), from which different dilutions were prepared according to NOM-110-SSA1-1994, (DOF, 1995b).

Samples were taken from different carcass regions: thigh, back, ribs and shoulder (one rabbit per region) according to the European Union approved method (European Directive 2001/471/EC) (U.E., 2001). This sampling technique was performed in three groups with 5 rabbits each, limiting the sampling surface area per group. In the first one, a surface area of 2.5 X 2.5 cm was sampled, rubbing this area in a different region per rabbit: R1 in the thigh, R2 in the back, R3 in the ribs and R4 in the shoulder. In the fifth rabbit the sample was taken by rubbing the same area in the four regions. This same procedure was followed for the other two groups with the difference of sampling surface in group 2 (5 X 5 cm) and group three (10 X 10 cm).

Serial decimal dilutions were performed in accordance to NOM-115-SSA1-1994, (DOF, 1995a), from which 0.1mL of each dilution was cultured in Baird Parker agar, which was distributed using a 90º sterile glass rod. Plates were incubated at 35°C for 48 hours to determine CFU counts in the carcasses. Results were analyzed using variance analysis ($P<0.05$) in a random block experimental design using Megastat for Microsoft office Excel 2007.

Results and Discussion

In Table 1 the microbial contamination is shown at the last dilution where CFU could be counted. Calculation for CFU/cm^2, was obtained according to the formula: Ns = (N*F/A) * D established by (MAP-SOIC, 2004).

Sampled Surface	Region	Rabbit	CFU (1 X 10^{-4})	CFU/cm^2
2.5 X 2.5 cm	1	1	20000	320000
	2	2	40000	640000
	3	3	30000	480000
	4	4	60000	780000
	1+2+3+4	5	130000	520000
5 X 5 cm	1	6	20000	80000
	2	7	10000	40000
	3	8	40000	160000
	4	9	30000	120000
	1+2+3+4	10	90000	90000
10 X 10 cm	1	11	20000	20000
	2	12	30000	3000
	3	13	70000	70000
	4	14	70000	70000
	1+2+3+4	15	160000	40000

The microbial contamination found in all regions and sampled surfaces were different (P<0.05), with the highest numbers probably representing the zones with more exposure to the environment or handling. Nevertheless, regarding the sampling template, the most suitable one was 2.5 X 2.5 cm, because of the rabbit´s size. There were some handling problems when sampling with the 5 X 5 cm template, and the 10 X10 cm template was simply too big for the rabbit carcass. There may have been more sampling errors when using the mid-size or biggest one, and less when using the smallest. A previous study in which microbial load in popular markets was performed, only 1 cm^2 was sampled from the right dorsal region of the carcass, finding 36% of the carcasses contaminated with *S. aureus* (Velázquez *et al.,* 2008). Based on our results, there is a high prevalence of *Staphylococcus aureus* on the rabbit's carcass surface. Mexican legislation

establishes a minimal detection count of 100 CFU/g for solid foods or 10 CFU/mL for liquid ones. Nevertheless, the maximum limits per cm^2 have not been established, but when comparing our results with the limits stated in different international legislations they are above 10^6 CFU/g in food according to Mexican legislation (DOF, 1995a), 10^3 UFC/g according to Venezuelan legislation (MSAS, 1996) and 10^5 CFU/g according to Northamerican legislation (Jablonkin y Bohach, 2001). Heinz and Hautzinger (2007) recommendations for microbiological criteria in fresh meat (total plate counts/cm^2) are: good with less than 10000, critical between 10000 and 100000 and not acceptable with more than 100000. The microbiological counts found in this study might have resulted from rabbit carcass mishandling, representing a potential public health risk if the strains are capable of producing toxins. *S. aureus* can be present in the slaughterhouse's environment or may reach the carcass via cross contamination (USDA, 2005).

Conclusions

Based on these results, it can be inferred that the surface method used for the analysis of big species carcasses can be used for small ones, such as the rabbit carcass, with slight modifications according to surface sampling, concluding that the most suitable one for rabbit carcasses is 2.5 X 2.5 cm. The other two are discarded due to the small surface area of the whole rabbit.

These results do not imply health risk regarding rabbit meat consumption, due to the microorganisms' elimination when cooked, but it rises a sanitary alert because of the possible *S. aureus* strain dissemination regarding the carcasses handling in different distribution points.

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