Staphylococcus aureus CONTAMINATION EVALUATION IN RABBIT CARCASSES (*Oryctolagus cuniculus)* THROUGH WASHING AND SUPERFICIAL SAMPLING

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ABSTRACT

Staphylococcus aureus (*S. aureus*) is widely distributed in animal population intended for meat production. It is an opportunistic pathogen, which causes multiple skin infections, deep and septicaemia in animals and humans through food and interspecies contact. In this study, two methods were compared to estimate *S. aureus* microbial load in rabbit carcasses. Sampling was undertaken in the sacrifice area of a family production farm in Toluca Valley. Three samples with three repetitions were tested by one of the following methods: in method A (MA) an area of 2.5 X 2.5 cm was delimited in four zones of the carcass, in which a swab was scrubbed vertically and horizontally. Swabs were placed in 10 mL 2% peptone water. In method B (MB), the carcass was washed in 400 mL of peptone water, from which 30 mL were taken. Baird Parker agar plates were inoculated and placed at 37°C for 48 hrs. to determine *S. aureus* microbial load in colony forming units (CFU). Results for MA were $4.8X10^{-4}$ UFC/cm^2 and MB $5.0X10^{-4}$ UFC/mL ($P > 0.05$). There was no statistical difference within methods to estimate carcass microbial load, therefore suggesting the use of superficial sampling due to accesibility.

Key words: *Staphylococcus aureus*, sampling, surface, microbial load, carcass.

Introduction

Staphylococcus aureus is pathogenic for humans and animals (Morales and Ruiz, 2005). It colonizes skin, infects wounds, contaminates medical-surgical material, with the possibility of producing superficial and deep infections, sepsis and septic syndrome (Díaz *et al.*, 2006). In animals it may cause chronic skin infections an mastitis as well (Viana *et al.*, 2011), and may contaminate meat and other foods from animal origin (López *et al.*, 2011). Foodborne diseases are a priority in public health; foodborne intoxications caused by *S. aureus* (Jordá *et al.*, 2012) are usually atributted to enterotoxin producing strains (SEs), which may be isolated from milk, milk products, meat and meat products (Velázquez, 2005). Carcasses contaminated with *S. aureus* may be carriers of foodborne infections, due to deficient hygiene and sanitary quality during sacrifice and meat handling (Hernández *et al*, 2007). *S. aureus* contaminates meat via surfaces, equipment and tools because of its capacity for environmental and food workers' skin colonization. *S. aureus* in meat is frequently related to human and environmental contamination (Yasser *et al.*, 2009). In rabbits, *S. aureus* is responsible for several infectious processes, nevertheless, it is also frequently isolated from apparently healthy people, producing staphylococcosis which affects a limited number of animals by low virulence strains. Another type of infection is caused by high virulent strains which can infect a large number of animals and disseminate to other communities (Rodríguez *et al.,* 2004, Peton and Le Loir, 2014). *S. aureus* in contaminated food tend to be frequent in underdeveloped countries causing food intoxications and community and epidemic acquired infections related to animal and food origin strains (Morales and Ruiz 2005, López *et al*, 2011). Treatment of diseases caused by *S. aureus* represent important expenses to the countries' health services (Hernández *et al.*, 2007). Prevention of foodborne diseases related with *S. aureus* involve microbiological count evaluation of the carcass, process and products, to decrease food contamination and health risk (Directive 64/433/EEC; SENASICA, 2010; Manual de procedimientos, 2004). The objective of this study was to evaluate washing and surface sampling in rabbit carcasses from family slaughterhouses, to determine the microbial load by *S. aureus*.

Material and Methods

Rabbit carcasses were randomly chosen from the sacrifice line in a family type slaughterhouse in a farm from the Toluca Valley. Three samples with three repetitions were taken for the following methods: MA carcass superficial sampling and MB carcass washing. In MA, samples were taken from regions proposed by European Communities (Directive 64/433/EEC 2001), in which the surface sampling template was modified to 2.5 x 2.5 cm. A sterile swab moistured in 2% peptone water for 5 seconds was rubbed ten times vertically and horizontally. Samples were refrigerated at 4°C until studied in the laboratory (NOM-109-SSA1-1994). MB was undertaken using the Guatemaltecan Procedure Manual (2004), in which it is stated that the carcass is introduced in a sterile 30X45 cm plastic bag with 400 mL. 2% peptone water. The closed bag was swayed and inverted at least 30 times per minute to wash the carcass. Thirty mL of this washing solution were placed in a sterile container and the sample was refrigerated under the same conditions as the other one. For CFU *S. aureus* counting in plates, the method described in NOM-115-SSA1-1994 was used. 1 mL was taken from MA transport solution after homogeneization. Serial tenfold dilutions were prepared (NOM-109-SSA1-1994), from which 0.1 mL of dilutions 10^{-1} to 10^{-6} were cultured in Baird Parker agar and incubated at 37°C for 24 h. Characteristic *S. aureus* CFU were counted. Total count was estimated using the following formulae: UFC/cm2 = (N*F/A) * D and UFC/mL = Number of counted colonies x Dilution factor / mL of the cultured simple (Manual de procedimientos, 2004). Results were evaluated using analysis of variance in a random design with two treatments and three repetitions per treatment ($p < 0.05$).

Results and Discussion

Results for the sampling methods used for microbiological carcass counts were: **MA** $4.8X10^{-4}$ UFC/cm^2 y en **MB** $5.0X10^{-4}$/mL y ($p > 0.05$). When comparing both methods, CFU numbers were similar. Statistically, there was no difference between both methods for evaluating contamination in rabbit carcasses. Nevertheless, this study suggests that surface sampling may

represent an accesible way for estimating microbial load in sanitary screening of family slaughterhouses. MB, which is used for sampling hens, might be inconvenient due to bigger volume handling (Manual de procedimientos, 2004). *S. aureus* is an important pathogen that should be evaluated in rabbit carcasses due to potential risk of animal origin strains that could contaminate foods when processing meat, and derivate in foodborne intoxication and infections by MRSA strains in human population (López *et al.,* 2011). This agent is sanitarily important in public health due to strain diversity that may carry risk factors for human health.

Conclusion

It is concluded that MA method may be used for screening microbiological contamination by *S. aureus* in rabbit carcasses from family slaughterhouses.

References

- Díaz J. A, Rios D, Gaona M. A, Matheus L. M. 2006. Apoptosis de células endoteliales inducida por aislamientos colombianos de Staphylococcus aureus. BISTUA. 4(1): 21-28.
- Hernández S.S., Zúñiga E.A., Sánchez O.I., Castro R.J., Román G.A., Santos L.E., 2007. Condiciones microbiológicas en el proceso de sacrificio en un rastro municipal del estado de Hidalgo, México. Vet. Méx., 38 (2): 187-195.
- Jordá B.G., Marucci S.R., Guida M.A., Pires S.P, Manfredi A.E. 2012. Portación y caracterización de *Staphylococcusaureus* en manipuladores de alimentos. Revista Argentina de Microbiología (2012) 44: 101-104
- López V.M., Velázquez O.V., Alonso F.M.U., Díaz Z.S., Pulido G.G. 2011. Identificación de cepas de *Staphylococcus aureus* resistentes a la meticilina (ORSA/MRSA) y con resistencia borderline (BORSA) aisladas de vacas lecheras en el valle de Toluca. XXXV Congreso Nacional de Buiatría.
- Manjarrez L.A.M., Díaz Z.S., Salazar G.F., Valladares C.B., Gutiérrez C.A.C., Barbabosa P.A., Talavera R.M., Alonso F.M.U., Velázquez O.V. 2012. Identificación de biotipos de *Staphyloccocus aureus* en vacas lecheras de producción familiar con mastitis subclínica en la región centro-este del Estado de México. Rev.Mex. 3: 265-274.
- Ministerio de Agricultura, Ganadería y Alimentación de Guatemala. MANUAL DE PROCEDIMIENTOS PARA MUESTREO MICROBIOLOGICO EN .ESTABLECIMIENTOS CERTIFICADOS MAP-SOIC-04. Sistema Oficial de

Inspección de Carnes – SOIC. Dirección de Inocuidad de los Alimentos, Viceministerio de Sanidad Agropecuaria y Regulaciones. 2ª. Edición, Guatemala, Guatemala. 2004

- Norma Oficial Mexicana NOM-092-SSA1-1994, Bines y Servicios. Método para la Cuenta de Bacterias Aerobias en Placa y la norma Nom-109-SSA1-1994.
- Norma Oficial Mexicana Nom-109-SSA1-1994, Bienes y servicios, Procedimientos para la Toma, manejo y transporte de Muestras de Alimentos para su Análisis Microbiológico.
- Norma Oficial Mexicana NOM-115-SSA1-1994, Bienesy Servicios. Método para la Determinacion de *Staphylococcus aureus* en Alimentos.
- Morales M.M., Ruiz de Chávez R.C. 2006. Diferencias en la resistencia a los antimicrobianos de cepas de staphylococcusaureus obtenidas de diversas fuentes de aislamiento Rev. Centro de Investigación.7: 45-64, México
- Official Journal of the European Communities 2001. Directive 64/433/EEC on health conditions for the production and marketing of fresh meat. L 165/48.
- Official Journal of the European Communities. 2001. Directive 71/118/EEC on health problems affecting the production and placing on the market of fresh poultry meat. L 165/48.
- Ortega C., Simón M.C., Alonso J.L., Mateo A., 2009. Caracterización y riesgos para la salud pública de la antibiorresistencia de *Staphylococcusaureus*en la cunicultura intensiva. *Rev. sci. tech. Off. int. Epiz.*, 2009, 28 (3), 1119-1128
- Peton V, Le Loir Y. 2014. Staphylococcus aureus in veterinary medicine. Infect Genet Evol. 21:602-615.
- Rodriguez C.J.M., Santos J.A., Otero A., Garcia L.M.L. 2004. Microbiological Quality of Rabbit Meat. Journal of Food Protection 64(5): 966-971.
- Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria. Dirección General de Agroalimentación, Acuícola y Pesquera. Programa nacional de Reducción de Patógenos. México, SENASICA 2010.
- Viana D., Selva L., Callanan J.J., Guerrero I., Ferrian S., Corp J.M. 2011. Strains of Staphylococcus aureus and pathology associated with chronic suppurative mastitis in rabbits. The Veterinary Journal. 190(3): 403-407.
- Velázquez M.M. 2005. Surgimiento y diseminación de Staphylococcus aureus meticilinorresistente. Salud Pública de México, 47: 381-387.
- Yasser H., Al-Tarazi A., Mohamad A., Albetar b, Akram R. Alaboudi 2009. Biotyping and enterotoxigenicity of Staphylococci isolated from fresh and frozen meat marketed in Jordan. Food Research International. 42(3): 374-379.