

VOLUME02ISSUE01

"DIAGNOSIS OF SALMONELLA ENTERITIDIS IN TISSUES AND INTESTINAL
CONTENT OF EXPERIMENTALLY INFECTED CHICKENS IN CHILE BY POLYMERASE CHAIN REACTION"

SÁNCHEZ, P; BORIE C; NAVARRO C.

Abstract

Salmonella Enteritis's is the most involved Salmonella serotype in Food-borne Illness in our country, being poultry products the main source in human infection. For this reason, its diagnostic and control becomes more and more important to avoid outbreaks that jeopardize public health. Bacteriological culture is the standard method used for the detection of S.E in animal tissues samples and takes at least 4 to 7 days to deliver results. To reduce diagnostic times, without reduce sensitivity and specificity, new and alternative methodologies have been developed and Polymerase Chain Reaction (PCR) has taken a privileged place obtaining an important reduction of detection times and processing a great number of samples simultaneously.

The objective of this work was to implement standard PCR test for the detection of S.E by using specific primers to detect invA gene. For this purpose, a first stage was performed in which the PCR technique was implemented using enrichment broth inoculated whit bacteria, followed by the establishment of the minimal detectable bacterial concentration that the technique was able to detect, obtaining a minimum of 10¹ CFU/ml. After, the implemented test was used to detect S.E in 53 samples of tissues and intestinal content of experimentally infected chickens that were positive to bacteriological culture and 126 negatives samples. In the first case all the samples are positive while in the second experience from the 126 negative samples, only 6 obtained amplification bands (4,8%), being 3 of them a pool of organs, and 3 intestine and intestinal content. Thus, it can be concluded that the implementation of standard PCR test for the detection of S.E in tissues and intestinal content samples from chickens was successful, with a high bacterial detection capacity and an execution time of 3 days, maximum (being 2



of them incubation un enrichment broth). This diagnostic test should be used as a complement for the traditional technique presently used.

KEYWORDS

PCR, Inva, Diagnostic Method, Salmonella.

REFERENCES

Alexandre, M., Pozo, C., Gonzalez, V., Martínez, M. C., Prat, S., Fernández, A., Fica, A., Fernández, J., Heitmann, G. 2000. Detección de Salmonella enteritidis en muestras de productos avícolas de consumo humano en la Región Metropolitana. Revista médica de Chile. 128(10): 1075-1083.

Antunes, P., Mourão, J., Campos, J., Peixe, L. 2016. Salmonellosis: the role of poultry meat. Clin Microbiol and Infect 22: 110-121

Desmidt, M., Ducatelle, R., Haesebrouck, F. 1996. Pathogenesis of Salmonella enteritidis phage type four after experimental infection of young chickens. Veterinary Microbiology. 56: 99-109.

Freschi, C. R., De Oliveira E Silva Carvalho, L. F., Bruno De Oliveira, C. J. 2005. Comparison of DNA-extraction methods and Selective Enrichment broths on the detection of Salmonella Typhimurium in swine feces by polymerase chain reaction (PCR). Brazilian Journal of Microbiology. 36(4): 363-367.

Galán, J. E., Curtis, R. 1991. Distribution of the invA, -B, -C, and -D genes of Salmonella typhimurium among other Salmonella serovars: invA mutants of Salmonella typhi are deficient for entry into mammalian cells. Infection Immunology. 59(9): 2901-2908.

Gwida, M., AL-Ashmawy, M., 2014. Culture versus PCR for Salmonella Species Identification in Some Dairy Products and Dairy Handlers with Special Concern to Its Zoono-



tic Importance. Veterinary Medicine International, vol. 2014, Article ID 502370, 5 pages, 2014. https://doi.org/10.1155/2014/502370.

Heredia, N., García, S. 2018. Animals as sources of food-borne pathogens: A review. Animal nutrition 4: 250-255

Kasturi, K., Drgon, T. 2017. Real-Time PCR Method for Detection of Salmonella spp. in Environmental Samples. Appl Environ Microbiol 83: e00644-17.

Kwan, J., Littledike, E. T., Keen, J. E. 1996. Use of the polymerase chain reaction for Salmonella detection. Letters in Applied Microbiology. 22: 46-51.

Löfström, Ch., Knutsson, R., Engdahl Axelsson, Ch., Radström, P. 2003. Rapid and Specific Detection of Salmonella spp. in Animal Feed Sample by PCR after Culture Enrichment. Applied and Environmental Microbiology. 70: 69 – 75.

Lorenz, T. 2012. Polymerase Chain Reaction: Basic Protocol Plus Troubleshooting and Optimization Strategies. J Vis Exp. (63): 3998.

Malorny, B., Hoorfar, J., Bunge, C., Helmuth, R. 2003. Multicenter validation of the analytical accuracy of Salmonella PCR: towards an International Standard. Applied and Environmental Microbiology. 69(1): 290-296.

Nagaraja, K. V., Pomeroy, B. S., Williams, J. E. 1991. Paratyphoid infections. In: Diseases of Poultry, 9th ed. Calnek, B. W., Barnes, H. J., Beard, C. W., Reid, W. M., Yoder, H. W. Jr, eds. Iowa State University Press, Ames, Iowa. pp: 99–120.

Oliveira, S. D., Rodenbusch, C. R., Cé, M. C., Rocha, S. L. S., Canal, C. W. 2003. Evaluation of selective and non-selective enrichment PCR procedures for Salmonella detection. Letters in Applied Microbiology. 36: 217–221.



VOLUME02ISSUE01

Prado, V., Solari, V., Alvarez, I. M., Arellano, C., Vidal, R., Carreño, M., Mamani, N., Fuentes, D., O'ryan, M., Muñoz, V. 2002 Situación epidemiológica de las enfermedades transmitidas por alimentos en Santiago de Chile. Periodo 1999-2000. Revista médica de Chile. 130(5): 495-501

Revolledo, L., Ferreira, A. J. P., Mead, G. C. 2006. Prospects in Salmonella control: Competitive Exclusion, Probiotics, and Enhancement of Avian Intestinal Immunity. Journals of Applied Poultry Research. 15: 341–351.

Sambrook, J., Fritsch, E. F., Maniatis, T. 1989. Gel Electrophoresis of DNA. In: Molecular Cloning. A laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press. USA. pp: 6.2-6.62.

Stone, G. G., Oberst, R. D., Hays, M. P., Mcvey, S., Chengappa, M. M. 1994. Detection of Salmonella serovars from clinical samples by enrichment broth cultivation-PCR procedure. Journal of Clinical Microbiology. 32(7): 1742-1749.

Velilla, A., Terzolo, H., Feingold, S. 2004. Avances en el diagnóstico molecular de Salmonella PCR aplicada a la avicultura y a la microbiología de los alimentos. [online] [consulta: 16 - 01 – 2017]

Whyte, P., Mcgill, K., Collins, J.D., Gormley, E. 2002. The prevalence and PCR detection of Salmonella contamination in raw poultry. Veterinary Microbiology. 89: 53–60.

Wilson, I. 1997. Minireview: Inhibition and facilitation of Nucleic acid amplification. Applied and Environmental Microbiology. 63(10): 3741-3751.

AUTHOR'S AFFILIATION SÁNCHEZ, P



VOLUME02ISSUE01

Department of Animal Preventive Medicine. Faculty of Medicine Veterinary and Animal Sciences. University of Chile.

BORIE C.

Department of Animal Preventive Medicine. Faculty of Medicine Veterinary and Animal Sciences. University of Chile.

NAVARRO C.

Department of Animal Preventive Medicine. Faculty of Medicine Veterinary and Animal Sciences. University of Chile.