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SALMONELLA IDENTIFICATION BY COMPARING BACTERIOLOGICAL AND MOLECULAR METHODS

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Abstract

This present study attempts comparing classical method ISO 6579:2002 with a quick, polymerase chain reaction (PCR) for the identification and isolation of Salmonella carrier pigs without clinical signs by collecting feces and sanitation tests on the surface of carcasses.
After analyzing the samples (n=51) by the two methods (Bacteriological and PCR) has resulted a higher number of positive samples (43.13%) by PCR, compared to the bacteriological method, where only 35.29% were positive samples. After sequencing and introduction into the gene pool (GeneBank), it was found that the most common serovars isolated from the feces were S. Typhimurium, S. Newport, from the surface of carcasses were S. Typhimurium, S. Typhi, S. Newport and S. Enteritidis serovars, and on the surface of working equipment S. Typhimurium and S. Agona.

Keywords*: Identification, isolation, method, pigs carriers, Salmonella spp., serovars.*

INTRODUCTION

The quick identification of infection depends on the correct identification of pathogens and for prevention is required an adequate monitoring. Detailed identification and characterization of this large group of bacteria is a difficult task which requires a combination of different approaches, and for confirmation it appeals to a reference laboratory (N.G. et al., 1996).
To prevent food contamination, foodborne outbreaks and for a quick diagnosis is necessary the use of rapid methods (Hein et. al, 2006; N.G. et al., 1996). So far, the bacteriological method was considered to be the "gold standard" for the isolation and identification of pathogens in food.
However, the cultivation of microorganisms methods requires high volume of work and time, and is not suitable for routine testing of large numbers of samples (Bohachuk et al., 2007; Klerks et al., 2004; Myint et al., 2006).
Therefore, the desire to make a quick diagnose and have a favorable cost-benefit throughout the food chain, finding quick ways continues to be a major concern for the industry and public health authorities. Because of these requirements, polymerase chain reaction (PCR) has become a method increasingly used in the last decade (Malorny et al., 2004; Myint et. Al., 2006; Wolffs et al., 2006), PCR test is a rapid, sensitive and specific detection of pathogens (Bohachuk et al., 2007; Malorny et al., 2004).
PCR molecular method, after 1988, experienced an explosive growth, being the reaction which has revolutionized molecular biology of nucleic acids. The technique is based on a key enzyme in the reaction called Taq polymerase, a polymerase stable at high temperatures (~ 94 ˚ C) in which molecules of DNA, usually double-stranded, dissociate, and thus each strand can be copied.
This technique is important for fast identification of the *Salmonella* spp. carrier from pigs, especially when there are free of *Salmonella* spp. groups, or when it is desired to

control the efficiency of the cleaning and disinfection procedures in a short time.
The question of whether this method can be used in pig farms and feces samples, dust, and so on, which are naturally contaminated with an unknown amount of *Salmonella* spp. containing a large amount of bacterial DNA.
Based on the subject was considered appropriate to conduct investigations of molecular biology and the conditions of our country, in order to make new contributions to the knowledge of Salmonella present in Romania.

MATERIALS AND METHODS

The study was conducted from December 2012-July 2013 on a total of 51 samples, of which 15 were number of fecal samples, 29 sanitation samples collected from the surface of carcasses from several stages of the technological flow and 7 samples from the equipment.
The samples were analyzed in the Hygiene laboratory using two methods in parallel, SR EN ISO 6579:2002 and polymerase chain reaction (PCR), after isolation and identification the samples were sent to sequencing.

RESULTS AND DISCUSSION

Analyzing the samples by the two methods (PCR and bacteriological), has resulted a higher number of positive samples (43.13%) by PCR, than by the bacteriology method, where was achieved only 35.29% positive samples. The results could be attributed to active microorganisms (*Salmonella* spp.) because the presence of DNA was revealed, but they could not grow on culture media (7).

Table 1 illustrates the results of comparing the two methods, PCR molecular method and bacteriological method, of the 51 samples analyzed.

Isolation of *Salmonella* spp. from feces by bacteriological method can be hampered because of the relatively small number of *Salmonella* spp. microorganism in feces (Davies et. al 2000).

Tabel 1

Comparison of PCR and ISO

Samples	Nr.	PCR		ISO	
		n(+)	%	n(+)	%
Feces	15	9	60	10	66.66
Cases	29	11	37.93	7	23.12
Equipment	7	2	28.58	1	14.28
TOTAL	51	22	43.13	18	35.29

By PCR analyze of *Inv*A *Salmonella* Gene using inv A-139 and A-141 primers has shown the presence of clear light bands 284 bp. (Figure 1). The size of the amplification products suggested that microorganisms genetically characterized belong to *Salmonella's* gender. According to the literature by using inv A primers *S. Thiphymurium* serovars, *S. Enteritidis, S. Agona, S. Typhi, S. Cubana* can be highlighted (Eleni et al., 2006; Erik et al., 2007; Ohud et al., 2012; Sandra Maria Ferraz Castagna et al., 2005).

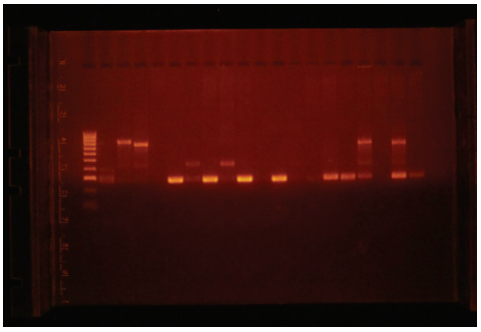

Figure 1 Molecular diagnosis of the *Salmonella* gender by PCR-based on the of amplification *inv*A *Salmonella* gene

After analyzing the samples sent to sequencing and introduction them into the gene pool (GeneBank), it was found that the most common serovars isolated from feces and identical to PCR in 96% are:
-*Salmonella enterica* subsp. *enterica* serovar *Newport* and *Salmonella enterica* subsp. *enterica* serovar *Typhimurium DT104.*
On the surface of carcasses from slaughter pigs the most frequently isolated serovars in 99% and 100% from the gene bank were:- *Salmonella enterica* subsp. *enterica* serovar *Newport*, *Salmonella enterica* subsp. *enterica*

serovar *Typhi*, *Salmonella enterica* subsp. *enterica* serovar *Enteritidis* and *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* var. 5.

The analysis of the samples taken from the working surface of the equipment in terms of the PCR are the same at a rate of 99% isolates of the gene bank:

-*Salmonella enterica* subsp. *enterica* serovar *Typhimurium* and *Salmonella enterica* subsp. *enterica* serovar *Agona.*

Following this study, it appeared that the most common serovars were: *S. Thiphymurium, S. Agona, S. Enteriditis, S. Newport,* which corresponds with literature data (Rostagno et al., 2006).

Similar results were obtained by Chantong et al., published by Sujate Chaunchom (2003), which isolated *S. Rissen* (45.9%), *S. Typhimurium* (10.8%) in the slaughterhouse *S. Stanley* (11.7%).

Following a study made by EFSA (2008), in 2006-2007, on a total of 387 frames, regarding the frequency of isolated serovars in the Member States, *S. Typhimurium* was the most common serovar identified on the surface of pig carcasses, representing 49.4%, followed by the *S. Derby* (24.3%) followed by *S. Infantis*, *S. Brandenburg* and *S. Bredeney* (3.4%, 2.1% and 1.8%).

S. Typhimurium was the most frequently isolated serovar in 10 Member States. *S. Derby* is the second serovar isolated in seven Member States, Belgium, Czech Republic, Denmark, France, Ireland, Latvia and the UK.

CONCLUSIONS

- Until now, the bacteriological method is referred to as "gold standard" for the isolation and identification of *Salmonella* spp. in various products.
- However, this method requires a longer time to obtain results and a high workload.
- This method is not suitable for examining an increased number of samples.

From 51 samples examined by both methods, we obtained a total of 22 (43.13%) positive samples by molecular methods and a number of 18 (5.29%) by bacteriological method.

- From the results obtained in this study, molecular methods (PCR) may be used to identify faster the *Salmonella* spp. micro-organisms taken from different pig samples. Most frequently identified serovars were:
- *S. Typhimurium* and *S. Newport* in feces;
- *S. Typhimurium, S. Typhi, S.Newport* and *S. Enteritidis* on the surface of carcasses;
- *S. Typhimurium* and *S. Agona* on work equipment.

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