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COMPARISION OF THE BAX® SYSTEM WITH AN IN-HOUSE MSRV METHOD FOR THE DETECTION OF SALMONELLA IN CHICKEN CARCASSES AND PORK MEAT

Paulo R. Franchin[1,3]; Paulo J. Ogliari[1,2]; Dalton F. Andrade[2]; Maura Chiapinoto[3]; Giovana Lemos[3]; Marina Rebelatto[3]; Ivair G. da Silva[3]; Cleide R.V. Batista[1]*

[1]Department of Food Science and Technology, Center of Agricultural Sciences, Federal University of Santa Catarina, Brasil; [2]Department of Informatics and Statistics, Federal University of Santa Catarina, Brasil; [3]Center of Technology Perdigão S/A, Laboratory of Microbiology

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ABSTRACT

A study was performed to compare the analytical procedure of the BAX® System for *Salmonella* PCR assay with the Modified Semi-Solid Rappaport-Vassiliadis (MSRV) method, for the detection of *Salmonella* in naturally contaminated chicken carcass samples (n = 762) and raw pork meat (n = 566). The chicken carcasses samples were collected during slaughtering after defeathering or immediately after evisceration and the raw pork meat collected from the deboned head of recently slaughtered pigs and others deboned raw fresh pork meat. The BAX® System detected 134 *Salmonella*-positive samples in chicken carcasses and 145 samples in pork meat, while the MSRV method isolated 142 and 144 *Salmonella*-positive samples, respectively. No significant difference was observed between the two methods for chicken carcasses and pork meat, according to McNemar test at the 5% level.

Key words: BAX® System, MSRV method, *Salmonella*, chicken, pork meat

INTRODUCTION

Various members of the genus *Salmonella* can cause food intoxication. Foods are commonly tested for the presence of *Salmonella* due to the low infective dose potential of the microorganism (3).

The isolation and identification of *Salmonella* became a problem to meat industry laboratories because of the long time necessary to obtain results with conventional culture methods, such as the ISO 6579 reference method (2) which involves nonselective pre-enrichment, followed by selective enrichment in broth and plating onto differential agar. Suspicious colonies are then confirmed biochemical and serologically. This method can be applied to any type of food and requires 4 to 5 days for the confirmation of the presence of *Salmonella* in a sample (15).

To guarantee microbiological safety during food processing, methods that rapidly detect *Salmonella* are necessary for the opportune identification of the source of contamination (13). For the food industry that retains its products until the results are obtained, the time-consuming nature of conventional methods cause economic losses, with a consequent continuous interest in alternative, faster methods (42).

Various methods for the detection or isolation of *Salmonella* have been proposed, including immunological methods (7,8,18,19,36,43,45), DNA-DNA hybridization (5,17,24), DNA amplification (21,26,27,37), conductance (34) and impedance measurements (20). In addition, motility-based immunodiffusion tests (modified 1-2 test (35,44) and detection methods based on motility on liquid or semisolid media (6,12,15,23,39) have been used.

The method using modified semisolid Rappaport-Vassiliadis (MSRV) agar as selective and differential enrichment medium for *Salmonella* can be stood out because of its simplicity, rapid response and serological confirmation of migrated cultures is

*Corresponding Author. Mailing address: Universidade Federal de Santa Catarina - Depto. de Ciência e Tecnologia de Alimentos. Rod. Admar Gonzaga, 1346, Itacorubi Florianopolis, SC. CEP 88034-001. Tel.: (48) 3331-5380. E-mail: cbatista@mbox1.ufsc.br

obtained 48 h after the beginning of pre-enrichment (10,11) and low cost (32,33). The method has been validated by the Association of Official Analytical Chemists (AOAC) for cocoa products (AOAC 993-07) (9), for dried milk products (AOAC 995-07) (4) and recently in the Netherlands by the Animal Health Service in monitoring programs for the eradication of *Salmonella* at all levels of poultry meat production (14).

More modern molecular techniques for the detection and characterization of microorganisms involve the use of the polymerase chain reaction (PCR). The BAX® system (DuPont Qualicon) for screening *Salmonella* in foods is one of the first commercial PCR-based systems for the detection of pathogens, which simplifies the PCR procedure by combining all necessary reagents (primers, enzyme, deoxyribonucleosides) in a single tablet. The BAX® *Salmonella* test is an automated method that uses PCR technology for the detection of *Salmonella* in foods and has AOAC Research Institute (RI) approval status for *Salmonella* detection in meat, milk, and poultry classes of foods (31).

With the technological advances in the detection methods, food industry laboratories are increasingly confronted with the task to validate the analytical procedures for pathogen detection, sometimes already tested and validated for international organisms, such as International Organization for Standardization (ISO), AOAC, British Standards Institute (BSi).

Based on the above considerations, the objective of the present study was to compare two analytical methods for the detection of *Salmonella* sp in chicken carcass and pork meat samples naturally contaminated collected in the same commercial processing plant. The procedures compared were the BAX® System, which is based on PCR, and the MSRV culture method, which is based on phenotypic characteristics.

MATERIALS AND METHODS

Samples

The following samples were studied: a) chicken carcasses collected during slaughtering after defeathering or immediately after evisceration (n = 762), and b) raw pork meat obtained from the deboned head of recently slaughtered pigs (n = 566). Immediately after collection, the samples were sent to the laboratory for analysis within a maximum interval of 2 h between collection and sample preparation for microbiological analysis.

Assay procedure

Twenty-five grams (± 0.5 g) of the sample was weighed in a sterile Whirl-Pak (Nasco) bag on a Dilumate 3 scale (AES Laboratories, France), 225 ± 0.5 g buffered peptone water (BPW; Oxoid CM: 509) was added, and the mixture was homogenized in a peristaltic homogenizer (MA-440 Marconi, Brazil) for 1 min. The samples were incubated at 36 ± 1ºC for 20-24 h for pre-enrichment. Next, 0.1 ml of the pre-enriched broth was transferred to a plate at three equidistant locations containing selective MSRV medium (Acumedia 7511) supplemented with 1 ml of 2% novobiocin (Inlab 5701, Brazil) solution per liter of previously prepared medium according to manufacturer recommendations.

The plates were incubated at 42 ± 0.5ºC for 24 h and growth was interpreted as follows: plates showing positive motility (migration halo visible based on the difference in the color tone of the medium) were considered to be presumptive positive and submitted to serological test with somatic polyvalent serum (Probac do Brazil). The positive plates that showing positive motility, were transferred from migration edge of growth with a platinum loop onto Brilliant Green Agar, Hektoen Enteric Agar and/or Xylose Lysine Desoxycholate medium (Oxoid CM: 329, 419 and 469, respectively), and incubated for 24 h at 37ºC. Typical colonies were also confirmed based on biochemical tests, initially Triplice Sugar Iron Agar (TSI) (Merck 1.03915) and Urea broth (Merck 1.08483), and serological tests (somatic polyvalent serum and specific serogroups, mainly B, C_1, C_2, D, E (Probac do Brasil). The API 20E system (Biomerieux Brasil S/A) was used as a complementary test during initial identification. Strains considered to be positive were sent to the Oswaldo Cruz Institute, Rio de Janeiro, Brazil, in nutrient agar tubes (Oxoid CM3). Plates showing no motility were considered to be negative for *Salmonella* spp.

Aliquots derived from the same pre-enriched broth were submitted to PCR analysis using the BAX® system protocol (DuPont Qualicon).

Statistical analysis

The methods were compared statistically using the McNemar test: $[|(a - b)| -1]^2/a+b = \chi^2$, where *a* corresponds to samples testing positive by the MSRV method and negative by the BAX® system and *b* to samples testing negative by the MSRV method and positive by the BAX® system ($\chi^2 > 3.84$ is significant at a level of $p = 0.05$) (41). The Kappa index, which indicates the strength of the relationship between the row and column variables of a cross tabulation, in tables 1, 2 and 3, was calculated as described by Sachs, L., 1984 (38). Kappa values of < 0.01 indicate no concordance, those between 0.1 and 0.4 indicate weak concordance, those between 0.41 and 0,60 indicate clear concordance, those between 0.61 and 0.80 indicate strong concordance, and those between 0.81 and 1.00 indicate nearly complete concordance.

RESULTS

Salmonella sp was detected in 20.7% of the 762 chicken carcass samples and in 28.3% of the 566 pork meat samples, based on the BAX® and the MSRV positive data.

In chicken carcass samples, the MSRV method isolated 142 *Salmonella*-positive samples, while the BAX® system detected

134, (Table 1) with no significant difference between the two detection procedures (McNemar test, $\chi^2 = 1.23$, p = 0.2684)

In pork meat the MSRV method isolated 144 *Salmonella*-positive samples versus 145 detected by the BAX® system (Table 1), with the difference being no significant (McNemar test, $\chi^2 = 0.0000$, p = 1.0000).

Considering all samples analyzed, the MSRV method isolated 286 *Salmonella*-positive samples versus 279 detected by the BAX system (Table 1), with no significant difference between the two detection procedures (McNemar, $\chi^2 = 0.507$, p = 0.4764).

The Kappa values 0.823, 0.8559 and 0.840 (Table 1), indicate nearly complete concordance between the MSRV and BAX® system methods in both samples analyzed.

Chicken carcasses and pork meat samples show similar specificity by both methods (MSRV and BAX® system). Chicken carcasses samples showed greater sensitivity in MSRV than to the BAX® system and the pork meat samples showed similar sensitivity by both methods (Table 2).

The concordance for chicken carcasses and pork meat samples was 95.0% (Table 2).

All isolates as positive *Salmonella* culture were sent to the Oswaldo Cruz Institute and were confirmed to belong to the genus *Salmonella*.

Cultures positive for *Salmonella* sp isolated by the MSRV method (39 samples) that tested negative in the BAX® system (Table 1) were submitted to analysis by the BAX® system itself and were confirmed to be positive by the principle of detection of the BAX® system, i.e., PCR.

DISCUSSION

According to Bennett *et al.* (3), the BAX® system was always positive when the cell concentration of *Salmonella* in BPW was 5 x 10^3 CFU/ml or higher and in the MSRV method, according to Smedt and Bolderdijk (11) motility enrichment was always successful when the *Salmonella* cell concentration was at least 60 per ml, irrespective of the high numbers of competitive cells., i.e., 83 times lower quantity than in the BAX system.

This mathematical calculation might theoretically explain the *Salmonella*-negative samples obtained with the BAX® system, but not explain the negative results by MSRV. The MRSV method does not detect non-motile *Salmonella* and this might explain the difference between the two methods.

Another explain to the positive samples by BAX® system and negative by MSRV method, but with a very low probability, is that samples that tested positive with the BAX® system and negative by the MSRV method might be attributed to the physiological state of the cells, because DNA can be quite persistent in dead cells and may be amplified by PCR (16,22,25,28,40), and therefore this technique could not be used to differentiate viable from nonviable food-borne pathogens (30).

Table 1. Results for *Salmonella* sp in naturally contaminated chicken carcasses and pork meat determined with the BAX system and MSRV method.

Sample	Bax			MSRV						Kappaindex
	P	N	Total	P	PA	PD	N	ND	NA	
Chicken	134	628	762	142	118	24	620	16	604	0,823
Pork Meat	145	421	566	144	129	15	422	16	406	0,856
Both	279	1.049	1.328	286	247	39	1.042	32	1.010	0,840

P: samples positive for *Salmonella*; N: samples negative for *Salmonella*; PA: samples positive for *Salmonella* in MSRV and BAX ($MSRV^+$ BAX^+); PD: samples positive for *Salmonella* in MSRV and negative in BAX ($MSRV^+$ BAX^-); ND: samples negative for *Salmonella* in MSRV and positive in BAX ($MSRV^-$ BAX^+); NA: samples negative for *Salmonella* in MSRV and BAX ($MSRV^-$ BAX^-).

Table 2. Concordance, sensitivity, specificity, false positive rate and false negative rate for *Salmonella* sp in naturally contaminated chicken carcasses and pork meat determined with the BAX system and MSRV method.

Sample	*Total Samples*	*BAX Samples(+)*	*MSRV Samples (+)*	*Sensitivity (%)*		*FN (%)*		*Specificity (%)*		*FP (%)*		*Concordance (%)*
				MSRV	*BAX*	*MSRV*	*BAX*	*MSRV*	*BAX*	*MSRV*	*BAX*	
Chicken	762	134	142	88	83	12	17	96	97	4	3	95
Pork Meat	566	145	144	89	90	11	10	96	96	4	4	95
Both	1.328	279	286	89	86	11	14	96	97	4	3	95

FN: False negative rate is 100 – sensitivity rate; FP: False positive rate is 100 – specificity rate.

According to the manual of the manufacturer, the advantages of the BAX® system combine speed with easy and simple handling. In addition, the method yields results in 30 ± 1 h, reduces the potential of errors such as cross-contamination, does not require interpretation by a specialist, and permits effective processing of a large number of samples with up to 96 tests in a single step, thus being highly valuable for routine laboratory use (Manual of the BAX® system).

In contrast, when we used the BAX® system, it is not possible to identify strains in epidemiological trials, except when cultures are obtained from pre-enrichment or selective enrichment broth for the isolation of colonies from these positive samples, a procedure that is performed using traditional culture media.

After the pre enrichment the MSRV method is also simple to use but requires 24h for the incubation time and the confirmation of presumably positive or definitely negative samples. Presumably positive samples can be confirmed by serological tests directly from the MSRV plate, and also isolated by streaking from the external migration zone from MSRV onto differential and selective *Salmonella* media such as XLD or BPLS (Brilliant-green Phenol-red Lactose Sucrose Agar). Colonies isolated after 24 h of incubation can be used for any other biochemical test for definitive confirmation whether or not the isolate belongs to the genus *Salmonella.* Strains can then be submitted to typing.

The MSRV method permits the differentiation of *Salmonella* after 24 h of incubation at 42ºC based on the active motility of these microorganisms on this medium, in contrast to the inability of competitors to migrate at the same velocity; migration zones of *Salmonella* strains can reach a radius of 10 to 40 mm. In the case of development of migration zones equal to or larger than 10 mm, the presence of *Salmonella* can be expected (12,42).

The main interest of the food industry is to find a method that detects the highest possible percentage of positive samples in order to obtain the best response in terms of the efficacy of prevention methods and pathogen monitoring adopted by the company (in the present case *Salmonella* sp), i.e., actions aimed at reducing the frequency of this pathogen. Clearly, the objective is to be sure that the new method is able to detect more pathogens than the method already used in daily laboratory routine. A possible higher frequency of isolation of *Salmonella* sp from naturally contaminated samples would be due to the greater efficacy of the new detection method and could not be attributed to possible failures in the quality programs adopted by the company, for example a pathogen reduction program.

The combination of the two analytical techniques reported here has the advantage that, when the two methods are run together in parallel, a positive or negative result can be obtained within 30 h by the BAX® system. In addition, the microorganism can be isolated from the MSRV plate for strain typing for epidemiological purposes permitting here, at the discretion of the laboratory, the elimination of a possible false-positive result generated by the BAX® system due to the very low possibility of the amplification of DNA from dead cells present in samples as a consequence of industrial processing.

In view of the equivalence of the two methods demonstrated by the statistical test, one or the other can be used for the analysis of *Salmonella* sp in these types of samples, taking into account the cost of the method and the urgency of the need to obtain the results for lot release.

The results of this study agree with another that likewise confirms the good productivity of MSRV agar in chicken carcasses and raw meat samples (1,28,36,39).

MSRV confirmations is not so dependent on operator experience because plates obtained presented much less accompanying flora, if any at all. This fact is used by several authors (De Smedt *et al.*, 1986) and in the AOAC Official Method 993.07 (AOAC, 1995) to attempt direct serological testing of migrated MSRV cultures, considering them nearly pure cultures (28).

In this study the productivity for naturally contaminated chicken carcasses and pork meat was 89.93% for MSRV and 87.73% for BAX® system.

These results demonstrate that the MSRV culture method and the BAX® system do not differ significantly from one another for the samples analyzed and the MSRV methods is as efficient as the BAX® system method in detecting *Salmonella* in raw pork meat and chicken carcasses and could be considered as a routine screening method for motile *Salmonella* in these samples.

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RESUMO

Comparação do Sitema BAX® com o Método MSRV para detecção de *Salmonella* em carcaças de frango e carnes suínas

Um estudo foi realizado com o objetivo de comparar o procedimento analítico de detecção de *Salmonella* com o Sistema BAX® automatizado, baseado na Reação em Cadeia da Polimerase (PCR) com o método de Rappaport-Vassiliadis em Agar Semi-Sólido modificado (MSRV) para detecção de *Salmonella* em amostras de carcaças de frango naturalmente contaminadas (n=762) e retalhos de carne suía (n=566). O Sistema BAX® detectou 134 amostras positivas para *Salmonella*

em carcaças de frango e 145 amostras positivas para *Salmonella* em retalhos de carne suína, enquanto o MSRV detectou 142 e 144 amostras positivas respectivamente. Não houve diferença estatisticamente significativa entre os dois métodos, segundo McNemar ao nível de significância de 5%.

Palavras chave: Sistema BAX®, Método MSRV, *Salmonella*, frango, carne suína

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