PHENOTYPIC AND GENOTYPIC DETECTION OF *Salmonella* spp IN NATURALLY AND EXPERIMENTALLY INFECTED BIRDS

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**ABSTRACT**

Poultry is the largest reservoirs *Salmonella*, they are often reported in the products of avian origin and are largely related to foodborne illness in humans. The standard diagnostic method takes about seven days to complete, hindering efforts to control levels and implementation of zero tolerance for this pathogen. Therefore quantification of microbiological techniques and molecular quantification to help assess the risk and accelerate the process have been studied. This study aimed to evaluate the behavior of samples of birds naturally and artificially infected with *Salmonella* spp. Compared to the standard microbiological diagnostic methods qualitative and quantitative Real-time PCR (qPCR) from selective broth. This study was conducted in two experiments. The experiment “A” was performed on commercial farm with infected broilers naturally. Serovars found were serotyped and classified as *Salmonella* enterica subsp Enterica serovar Enteritidis (SE) and *Salmonella* enterica subsp Enterica serovar Senftenberg (SS). The SE was inoculated into hundred and twenty broilers for the experiment “B” in which were kept in laboratory environment. For both tests the samples were subjected to qualitative and quantitative diagnostic standard, and the qPCR from the Rappaport- Vassiliadis broth (RV) and Tetrathionate (TT), settings a standard quantitative test. The qPCR results showed no difference in the use of broth for molecular diagnostic. The technique oh qPCR for these experimental conditions remained satisfactory detection rates, but showed lower sensitivity when compared to the pattern recommended for the isolation of *Salmonella* spp.

**KEYWORDS**: microbiology, molecular biology, quantification of *Salmonella* spp.

**INTRODUCTION**

The enteric diseases in recent years has become one of the biggest challenges for the poultry industry worldwide, resulting in productivity losses, increased mortality among birds and contamination of food for human consumption. The main focus of the *Salmonella* control is returned to public health. It has been shown that this pathogen has several input sources and is continually present in the production, even with all the control programs running, which is not possible to establish zero tolerance, making the establishment of reasonable goals for reducing levels of contamination throughout the production chain and, therefore, be possible to reduce the cases of contamination in humans.

Since 2009 the method of quantification for control of *Salmonella* and *Campylobacter* spp in chicken meat has been working in various sectors and bodies to establish the diagnosis in order to reduce risks to consumer health and assign the best programs control of this pathogen (Borsoi et al. 2010).

The detection process involves the conventional steps of the *Salmonella* for the complete process, it takes at least seven days to confirm a positive result and from three to four
days to confirm a negative result (Maciorowski et al. 2005).

The need for immediate measures to control diseases became the polymerase chain reaction (PCR) a powerful tool in microbiological diagnosis.

The technique called RT-PCR (qPCR), which combines the processes of amplification and detection in the same equipment, and the ability to quantify the micro-organisms in the sample. All the analysis takes near 24-48h, in contrast to the seven days of culture by the traditional method (Chen et al. 2010).

This study aimed to compare the standard microbiological diagnostic methods qualitative and quantitative qPCR in samples of birds naturally infected with Salmonella spp. and artificially infected with Salmonella Enteritidis.

MATERIALS AND METHODS

The work was divided into two phases (A and B). In experiment “A” was selected from a broiler farm, with drag swabs positive for Salmonella spp. According to the methodology employed by the Ministério da Agricultura Pecuária e Abastecimento (MAPA) (Brasil 1995), in which samples were collected using cloacal swabs at the 22 and 32 days old birds for the qualitative and quantitative analyzes. The identification of positives birds was performed with sealed in the leg of the bird and these were divided into three groups.

The experiment “B” was conducted in the experimental block of the do Laboratório Central de Diagnóstico de Patologias Aviárias (LCDPA) located in the Central Animal Facility of the Universidade Federal de Santa Maria (UFSM), Brasil, in which 120 beef heifers, with one-day-old Cobb were lodged in batteries.

The experimental desing consisted of six tramatments with two replications. The chickens were inoculated with 1mL at a concentration of \(1.0 \times 10^8\) CFU/mL in 1 mouth and 2 days of age with Salmonella Enteritidis (SE) isolated from the experiment “A”. The birds 35 days were euthanized and necropsied and cecal tonsils were collected aseptically for microbiological analysis.

The analysis of Salmonella spp. for both experiments was the second qualitative methodology for Salmonella spp. (Brasil 1995, Brasil 2003) and quantitatively, using the techniques found in the literature (Borsoi et al. 2010) with modifications aimed to improve it, always in triplicate. The sowing methods were used for depth (pour plate), bat (spread plate) tested at concentrations of 20, 50, and 1mL and groove.

To perform Real-time PCR– qPCR were aliquoted in all steps of selective enrichment samples were aliquoted in 2.0mL cryotubes and frozen at -20 °C until DNA extraction. Bacterial DNA samples were extracted by NewGen PREPTM Kit(1) and subsequently purified with the kit NewGen preampTM(1). The amplification reaction was performed by qPCR kit SALAmp™ Newgen(1), all produced by Simbios Biotecnologia(1).

The reaction Real-time PCR was performed on specific equipment (Applied Biosoys Step One Plus theme 7300). All procedures were performed according to the indications fabricante1.

The Statistical Analysis using chi-square \((X^2)\) with a significant difference of 5% \((P <0.05)\) using SAS 9.2 for 2010. Standard procedures were used to calculate the sensitivity and specificity of qPCR. The accuracy was calculated by summing the positive - positive and negative - negative, expressed as a percentage (Myint et al., 2006).

RESULTS AND DISCUSSION

There are several methods for quantification of Salmonella spp. and many of them with conflicting results which reinforce the need for standardization. It is known that Salmonella spp. is not a good competitor and is often accompanied by various micro-organisms becoming essential to use means of enrichment for isolation and quantification (Borowsky et al. 2007).

The protocol “C” (using dilutions in the RV and TT broths with subsequent plating on

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XLD and (Xylose lysine desoxycholate agar) VB (Brilliant green agar) where plating it was observed colonies of *Salmonella* spp om 100% of positive samples, or 45% (27/60), marking it possible to count easily, reaching a reliable result. The colonies grew preferentially on XLD agar, but agreeing with the samples seeded in VB agar.

Experiment “A” there was agreement in 32 samples (57.14%) between the microbiological and qPCR, 17 cases (30.36%) positive and 15 negative (26.78%), indicating a satisfactory relationship, but low between the methodologies. Through traditional microbiological test was detected *Salmonella* spp. in 33 samples (58.92%), 17 (30.36%) confirmed by qPCR. The qPCR showed DNA of *Salmonella* spp. in 8 samples (14.28%) characterized as negative in the traditional procedure, suggesting greater sensitivity of this methodology.

In experiment “B” there was agreement in 96 samples (80%) between the qPCR and the microbiological, 20 cases (16.66%) positive and 76 negative (63.33%), indicating a good relationship between methodologies. Through traditional microbiological test was detected *Salmonella* in 31 samples (25.83%), 20 (16.66%) confirmed by qPCR. The qPCR showed DNA of *Salmonella* spp. in 13 samples (10.83%) characterized as negative in the traditional procedure, suggesting greater sensitivity of this methodology.

The accuracy of the test (Table 1) of the qPCR evaluated at this assay was relatively smaller than that obtained in other studies, using other biological specimens (Wilkins et al., 2010). But must consider the limitations obtained for the implementation of the benchmarking.

Serovars found were serotyped and classified as *Salmonella enterica* subsp Enterica serovar Enteritidis (SE) and *Salmonella enterica* subsp Enterica serovar Senftenberg (SS).

There is a general consensus that a standard method for typing of *Salmonella* is difficult to be adapted and the combination of diagnostic methods is useful for epidemiological analysis of isolates (Betancor et al., 2004). Phenotypic and genotypic analyses used together are tools that allow developments in studies of virulence factors of strains.

**CONCLUSION**

For the present study, the quantification protocol using dilutions in RV and TT broths with subsequent plating on XLD and VB was more efficient for counting of *Salmonella* spp. with greater reliability of results, however it is quite onerous.

The results showed no difference in the use of selective broth and Rappaport-Vassiliadis Tetrathionate for molecular diagnosis, but it showed a different behavior depending on the sample.

The technique of qPCR for these experimental conditions remained satisfactory detection rates even when the samples were subjected to freezing for a year and a half, but showed lower sensitivity when compared to the pattern.

Techniques for diagnosis of *Salmonella* spp. should be linked to satisfactory results, taking into account each particular sample.

**REFERENCES**

BETANCOR, L. et al. (2004) Random amplified polymorphic DNA and phenotyping analysis of *Salmonella* enterica serovar Enteritidis

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(VP+) –Positive predictive value; (VP-) – Negative predictive value


