International Journal on Veterinary Medicine

Identification of *Salmonella* Choleraesuis in Pigs with Gastrointestinal Problems

Research Article

Volume 1 Issue 1- 2022

Author Details

Méndez Palacios Néstor, Vázquez Flores Felícitas, Méndez Mendoza Maximino, Galicia Domínguez José Alfredo and Mendez Palacios Netzi Naidi*

Facultad de Medicina Veterinaria y Zootecnia, Benemérita Universidad Autónoma de Puebla, Mexico

*Corresponding author

Méndez Palacios Néstor, Faculty of Veterinary Medicine and Zootechnia, Benemérita Universidad Autónoma de Puebla, 7.5 Carretera Cañada Morelos -El Salado Tecamachalco, Pue, México

Article History

Received: December 17, 2022 Accepted: December 20, 2022 Published: December 20, 2022

Abstract

Salmonellosis is a gastroenteric infectious disease that produces significant harmful effects on human and animal health. In pigs, *Salmonella choleraesuis* mainly causes enteritis and the manifestations begin 36 hours after infection with erosions, and edema of the cecal mucosa, at 64 hours later the intestinal wall is thickened and diffuse classification underlying the erosion is observed. The necrotic membrane is affected at 96 hours and at 128 hours the intestinal wall is inflamed. It commonly affects pigs from weaning to 3 or 4 months of age, the septicemic form is the most common that can occur. Conventional diagnosis (bacterial isolation) of this microorganism is laborious and requires investing a lot of time, and special sterility conditions in the laboratory, since it is based on bacterial culture and the identification of the main biochemical characteristics of the microorganism. In the present study, the usefulness of molecular methods as a safe and rapid tool for the identification of *salmonella* variants of epidemiological importance in human and animal health has led to the development of laboratory techniques that allow a clear differentiation between the common flagellated variants of *salmonella* and non-flagellated variants, in addition to distinguishing subspecies between strains that possess flagella.

The objective that was raised was to develop oligonucleotides different from those reported to identify *Salmonella choleraesuis*. The main methodology consisted in the development of specific oligonucleotides, based on the regions of the genomic sequence of the *Salmonella choleraesuis* flagellum that are more conserved and whose similarity between serotypes estimated to be around 99.9%. Several tests were carried out that verified the effectiveness of the designed oligonucleotides and the serotype *Salmonella choleraesuis* could be diagnosed with great efficiency.

Keywords: *Salmonella; Salmonella* Choleraesuis; Serotypes

Introduction

Daniel Elmer Salmon a Veterinarian who is credited with being the first to investigate bacterial diseases, in 1885 he discovered the first strain of *Salmonella* in the intestine of a pig with swine cholera, which was later called *Salmonella choleraesuis* (Boyen, et al. [1]). At that time there were multiple types of *Salmonella* that were taxonomically accepted, however, as a result of subsequent research in genetics population indicating a high degree of DNA similarity, all *Salmonella* isolates could be classified into a single serotype: *Salmonella choleraesuis*, these serotypes were subsequently sub classified into 7 groups based on DNA similarity and host range (Silva, et al. [2]). In 1999, Jean Paul Euzéby proposed the designation of *Salmonella enterica*, thus replacing the species of the genus *Salmonella*, from *Salmonella choleraesuis* to *Salmonella enterica.* Among *Salmonella enterica* serotypes, more than 2,600 serotypes have been reported worldwide. All these or the majority cause 80% of the confirmed cases of salmonellosis in Europe (EFSA [3]), in France specifically during 2009, 38.9%

©2022 Néstor. This work is published and licensed by Example Press Limited. The full terms of this license are available at https://skeenapublishers.com/terms-conditions and incorporate the Creative Commons Attribution – Non Commercial (unported, v3.0) License (http://creativecommons.org/licenses/by-nc/3.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Emample Press, provided the work is properly attributed.

of isolates were of *Salmonella* in humans and 13% in food and animals that corresponds to *Salmonella typhimurium* (Bugarel [4]).

Salmonella Typhimurium and *Salmonella enteritidis* have a wide range of hosts (around 18) and can infect a considerable number of animal species; some others, such as S. *abortusovis* which affects sheep and goats, *S. pullorum* and *S. gallinarum* which affects poultry, *S. choleraesuis* which affects pigs, *S. dublin* which affects cattle and *S. arizonae* which has been isolated from reptiles, are the most highly adapted to specific animal species, but occasionally infect humans (Chiu, et al. [5,2]). The intestinal tract of almost all animal species is the main reservoir of *Salmonella enterica* and has been isolated from feces, water, food and products of animal origin (Silva, et al [2]). The bacteria can remain viable in feces for up to months (Ochoa, et al. [6]). They are bacteria classified as Gram negative, they essentially have 3 layers: the cytoplasmic membrane (inner membrane), the peptidoglycan cell wall (murein) and an outer membrane. The space between the two membranes is called the periplasmic space and comprises the cell wall (Wray, et al. [7]).

The cytoplasmic membrane is composed of phospholipids and proteins, where the synthesis of phospholipids, peptidoglycan and lipopolysaccharides takes place, it is also the anchoring site of DNA during replication and has an important role in cell division (Wray, et al. [7]). The presence of this pathogen can be confirmed by different methods, being the bacterial isolating the most effective, but with a lot of time investment. There are other insensitive methods such as serological ones, but they are rapid diagnostic methods. In general, the identification of microorganisms is done through stool samples and in the case of disseminated disease, through blood. Biochemical tests help us to identify the presence of *Salmonella* and distinguish between serotypes, using serological tests for somatic, flagellar and capsular antigens (Levy et al. [8]). Serological tests include agglutination tests and enzyme-linked immunosorbent assays (ELISA).

ELISA tests can be used to screen for pathogens in bulk milk or in pig muscle tissue samples. Serological tests detect only a limited number of serotypes, serology is often of limited use in individual animals, since antibodies do not appear until a few weeks after infection and may also be present in uninfected animals (Chen, et al. [9]). On the other hand, molecular methodologies are more precise and faster, although their use is rare, the case of PCR is a technique that has contributed to the development of medicine, in such a way that treatments are effective when there is identified the presence of the pathogen.

Methodology

A complete cycle farm was located with frequent diarrhea problems in piglets and which also presented decay. Subsequently, stool samples were collected in different places, a part was sent to the Microbiology and Public Health laboratory of the FMVZ of the BUAP in the Zootechnical Post for bacterial isolation. The other part was used to obtain genomic DNA using a commercial Axygen® multisample kit following the manufacturer's protocol, later the DNA was quantified by spectrophotmetry and its integrity was observed in 1% agarose gels with 1X TBE as a running buffer. For the design of the oligonucleotides, the Perlprimer v1.1.21 program was used and the conserved regions of two specific flagellar genes (FliA and FljB) were considered as a reference, the specificity of the oligonucleotides was corroborated by an alignment with all the sequences of the genes reported for all organisms in GenBank. The following table (Table 1) shows some characteristics of the designed oligonucleotides.

GenBank Access	Sense	Antisense	Amplicon
U17177.1	TGAACAAATCCCAGTC-CGCA	TCCTGTCGCTTCATCG-TAATCTG	1003 pb
AB010947.1	CTGGACGCTAAACG-TATTGATGTG	ACGTAGCCACTCTT-CATAGTTAGTC	209 pb

The standardization of the mean temperature (TM) for the alignment of the oligonucleotides was carried out using an end-point thermocycler with Thermo® brand gradients, which served to establish an amplification protocol for multiplex PCR, in which they were included the two pairs of primers for the specific sequences of interest and DNA samples of *Salmonella typhimurium*, *Salmonella choleraesuis* and *Salmonella senftenberg* already identified and reconfirmed by means of bacterial cultures. The first two bacteria present flagella (the oligonucleotides were specifically designed for these serotypes, the third was used as an internal control since this serotype does not have a flagella and ensures the correct and adequate amplification of the PCR products). The following are the thermal cycler conditions for PCR (Table 2). Subsequently, the amplicons were subjected to horizontal electrophoresis in 1.5% agarose gels with 1X TBE and a molecular weight marker as a reference standard on a 100 bp scale.

Temperature	Time	Cycles
94° C	3 minutes	1
94° C	30 seconds	25
60° C	30 seconds	
72° C	1 minute	
72° C	10 minutes	1

The expected size of the bands for the classification of the genes was 1003 bp for FljB and 209 bp for FliA. *Salmonella tiphymurium* only expresses the FliA gene, so only a 209 bp band was expected, while *Salmonella choleraesuis* expresses both FliA and FljB and would amplify two bands, one of 209 bp and the other of 1003 bp. *Salmonella senftenberg* is a bacterium that does not have a flagellum, therefore, it does not express either of the two genes and therefore does not amplify any band, so it was used as an internal negative control.

Results

The expected fragment sizes for the corresponding amplified regions were 1003 and 209 base pairs (Figure 1). In lane M, the Molecular Weight Marker is shown in a ladder from 100 to 3000 bp. The ST lane shows the amplicon for *Salmonella thyphimurium*, the SCS lane the one corresponding to *Salmonella choleraesuis*, and SS the amplified one for *Salmonella senftenberg*. N represents the negative control (no DNA). Figure 1 shows that *Salmonella typhimurium* and *Salmonella choleraesuis* amplify a 209 bp fragment and only *Salmonella choleraesuis* amplifies the 1003 bp fragment and while *Salmonella senftenberg* used as internal negative control does not amplify either of the two fragments, this because the oligonucleotides are specific for flagellar genes that *Salmonella senftenberg* does not possess. From the stool samples that were collected on the farm, the DNA was isolated and PCR was performed for diagnostic purposes, two samples were identified as positive that were also corroborated by bacterial isolation, which confirmed the efficacy of our diagnosis, in addition to be able to do it in a short time (approximately 7 hours).

Figure 2 shows an image of an agarose gel with the banding patterns that served for the diagnosis. All the tests that were carried out by PCR were corroborated by bacterial isolates for each of the samples, and Escherichia coli strains were also found in these isolates.

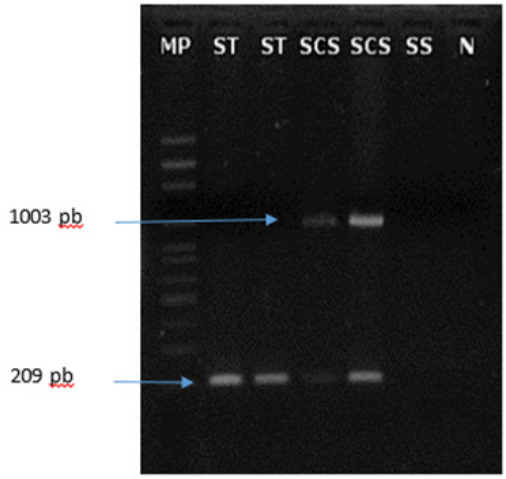

Figure 1: Digital image of a 1.2% agarose gel with 1X TAE, the amplification pattern for the three Salmonella serotypes is shown; ST, S. typhimurium, SCS, S. *cholerasuis* and SS, S. *senftenberg*. MP, molecular weight marker and N, negative control.

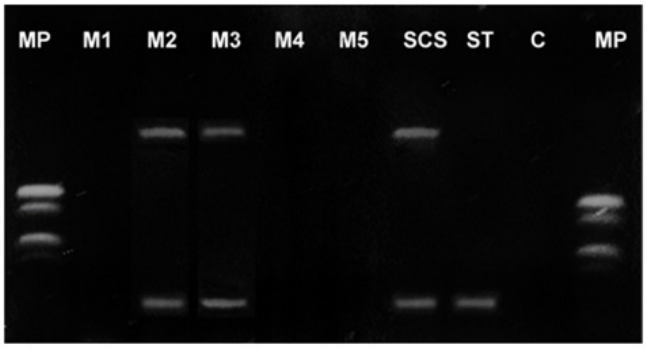

Figure 2: Digital image of a 1.5% agarose gel, 1X TBE. The first lane shows the molecular weight marker (MP), from lanes 2 to 6 (M1, M2, M3, M4, M5) samples of animals suspected of salmonellosis, samples M2 and M3 were identified as positive for *Salmonella choleraesuis* (the two bands are observed; one of 1003 and the other of 209 bp), in lane 7 a typified sample of *Salmonella choleraesuis* (SCS) used as positive control is presented, in lane 8 *Salmonella typhimurium* (ST) as control of *Salmonella* with flagella, in lane 9 the negative control (C) and in lane 10 molecular weight marker (MP).

Discussion

Molecular techniques such as PCR offer alternatives to improve detection quickly in the diagnosis of diseases, also offering high sensitivity and specificity. The identification of *salmonellae* represents a major problem for those engaged in microbiological diagnostics, due to the large number of existing serotypes. Most diagnoses are made through serological tests such as ELISA and Dot-blot tests that use antibodies for detection, these tests are of great help especially when the bacteria cannot be isolated due to the existence of a treatment with antibiotics or due to the lack of implements to carry out a microbiological diagnosis. However, these tests are very slow to perform and not very sensitive compared to molecular methodologies Some authors such as (Haque, et al. [10]) developed a nested PCR using the oligonucleotides reported by (Song, et al. [11]) and by (Frankel [12]). However, the methodology used for a nested PCR requires two amplification processes, which increases the time and costs of the diagnosis.

The oligonucleotides that we use in this work were designed with the objective of detecting two specific genes of the flagellum (multiple PCR). Several pairs of oligonucleotides have been proposed based on non-flagellar gene sequences such as hilA and invA, but which detect many serotypes such as; S. typhi, S. typhimurium, S. choleraesuis, S. paratyphi A, S. paratyphi B, S. enteritidis, S. gallinarum and S. pullorum (Cardona-Castro, et al. [13,14]). The existence of the large number of *Salmonella* serotypes complicates the specificity of the proposed oligonucleotides. In the tests that were carried out in this work, the effectiveness of the designed oligonucleotides could be verified, since serotypes that have a flagellum can be discriminated from those that do not, and in turn, separate different serotypes that occur very frequently. As contaminants in products of animal origin and that are not always pathogenic [15,16].

Conclusion

During the study it was determined that the samples collected and analyzed by PCR were very useful to corroborate the effectiveness of the oligonucleotides. In the tests carried out, some samples, both positive and negative, could be diagnosed with great effectiveness. It is worth mentioning that all this was confirmed by bacterial isolates, showing similarity in the results, so the effectiveness of the methodology is high to identify *Salmonella* choleraesuis in pigs.

Acknowledgments

We greatly appreciate the support to Dr. Jesús Hernández, QB Mónica G. Reséndiz, MVZ Elvira Carrera Salas and M en C. Guadalupe Socci.

References

1. Boyen F, Haesebouck F, Maes D, Van Immerseel, F Ducatelle, et al. (2008) Non-typhoidal Salmonella infections in pigs: A closer look at epidemiology, pathogenesis and control. Veterinary microbiology Epub 130(1-2):1-19.
2. Silva C, Calva E, Maloy S (2014) One Health and food-borne disease: Salmonella transmission between humans, animals, and plants. Microbiol Spectrum 2(1): OH-0020-2013.
3. EFSA (2011) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. EFSA J 9(3): 2090.
4. Bugarel M, Vignaud ML, Moury F, Fach P, Brisabois A (2012) Molecular identification in monophasic and nonmotile variants of Salmonella enterica serovar Typhimurium. Microbiology Open 1(4): 481-489.
5. Chiu C, Su L, Chu C (2004) Salmonella entérica serotype Choleraesuis: Epidemiology, pathogenesis, clinical disease, and treatment. Clin Microbiol Rev 17(2): 311-322.
6. Ochoa I, Rodríguez A (2005) Mecanismos moleculares de patogenicidad de Salmonella spp. Revista Latinoamericana de Microbiología 47(1-2): 25-42.
7. Wray C, Wray A (2000) Salmonella in domestic animals. CABI Publishing, CAB International. London, UK, p. 19-33.
8. Levy H, Diallo S, Tennant SM, Livio S, Sow SO (2008) PCR method to identify Salmonella enterica serovars Typhi, Paratyphi A, and Paratyphi B among Salmonella Isolates from the blood of patients with clinical enteric fever. J Clin Microbiol, 46(5): 1861-1866.
9. Chen W, G Martinez, A Mulchandani (2000) Molecular Beacons: A Real-Time Polymerase Chain Reaction Assay for detecting salmonella. Analytical Biochemistry 280(1): 166-172.
10. Haque A, Ahmed J, Qureshi J (1999) early detection of typhoid by polimerase chain reaction. Ann Saudi Med 19(4): 337-340.
11. Song JA, Cho H, Park MY, Na DS, Moon HB (1993) Detection of Salmonella Typhi in the blood of patients with typhoid fever by polymerase chain reaction. J Clin Microbiol 31(6): 1439-1443.
12. Frankel G (1994) Detection of Salmonella typhi by PCR. J Clin Microbiol 32: 1415.
13. Cardona Castro N, Restrepo Pineda E, Correa Ochoa M (2002) Detection of hilA gene sequences in serovars of Salmonella enterica subspecies Enterica. Mem Inst Oswaldo Cruz 97(8): 1153-1156.

14. Soria MC, Soria MA, Bueno DJ (2012) Comparison of 2 culture methods and PCR assays for Salmonella detection in poultry feces. Poultry Science 91(3): 616-626.

15. Joklik W, Willett H, Amos B, Wilfert C (1996) Zinsser Microbiologia. Editorial Médica Panamericana. Buenos Aires.

16. Frank P, Peter H, Jeroen D, Freddy H (2002) Pathogenesis of infections with Salmonella enteric subps. Enteric serovar Muenchen in the turtle Trachemys scripta scripta. Veterinary Microbiology 87(4): 315-325.