UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE CIÊNCIA E TECNOLOGIA DE ALIMENTOS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

Avaliação quantitativa do risco de *Salmonella* spp. em frango e em ovos produzidos sob inspeção oficial no Brasil

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Mestre em Ciência e Tecnologia dos Alimentos

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciencia e Tecnologia dos Alimentos da Universidade Federal do Rio Grande do Sul, como parte dos requisitos necessários à obtenção do título de Doutor em Ciência e Tecnologia dos Alimentos.

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Orientador: Prof. Dr. Eduardo Cesar Tondo
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"O sucesso da nossa vida e do nosso futuro depende da nossa motivação e determinação ou confiança em nós mesmos. Através das experiências difíceis, a vida, às vezes, ganha maior significado"

Dalai Lama

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RESUMO

Atualmente, o Brasil é líder na exportação mundial de frango e alcançou uma produção recorde de frango e ovos, nos últimos anos. *Salmonella* spp. é um dos principais patógenos de alimentos em nível mundial, sendo frequentemente transmitida por carne de frango e ovos. No presente estudo, foram realizadas avaliações quantitativas de risco de salmonelose, devido ao consumo de frango e ovos produzidos sob inspeção oficial no Brasil. Para tanto, informações provenientes de uma revisão sistemática de bibliografias científicas, dados obtidos do Ministério da Agricultura, Pecuária e Abastecimento (MAPA), informações vindas de indústrias e outros setores da cadeia produtiva e da população brasileira foram considerados. Modelos de microbiologia preditiva foram elaborados, considerando a multiplicação de alguns dos princiapais sorovares de *Salmonella* spp. na carne de frango brasileira. A multiplicação de *Salmonella* em ovos foi modelada através de *software* de microbiologia preditiva. As informações foram utilizadas para construir um modelo matemático para calcular o risco de salmonelose através de carne de frango, o qual considerou 21 módulos, desde o abate até o consumo nas residências e 20 módulos, desde o abate até o consumo em serviços de alimentação brasileiros. Na avaliação de risco para ovos, foram identificados 13 módulos, desde a produção até o consumo em residências, e 10 módulos desde a produção até o consumo em serviços de alimentação. As modelagens matemáticas foram realizadas no programa @RISK, utilizando o modelo de Monte Carlo, com 100.000 iterações para cada modelagem. A revisão sistemática demostrou que a prevalência de *Salmonella* spp. em carne de frango no Brasil foi de 14,96% e em ovos foi de 2,10%. Foram coletados 60.166 dados de tempo e temperatura na cadeia de carne de frango brasileira, os quais demonstraram adequação das temperaturas refrigeradas e congeladas. Também foram coletados 14.159 dados de tempo e temperatura na cadeia de ovos, demonstrando que a produção e distribuição ocorreram em temperatura ambiente. Esses dados foram utilizados para modelar os 15 cenários da cadeia produtiva de frangos e 10 cenários da cadeia de ovos, objetivando identificar estratégias de mitigação de risco de salmonelose. Um trabalho foi publicado, abordando as boas práticas e hábitos de consumo na população brasileira e demonstrou que 96,79% dos respondentes consumiam carne de frango e 97,54% consumiam ovos, pelo menos, 2 vezes na semana, em uma refeição diária. Considerando a dose infectante de apenas 1 UFC de *Salmonella*, o risco de infecção devido ao consumo de carne de frango nas residências foi de 8,092 em 1.000 exposições e, nos serviços de alimentação, foi de 7,95 casos em 1.000 exposições. O risco inicial de infecção devido ao consumo de ovos em casa ou em serviços de alimentação foi de 6 casos em 100 exposições. Os cenários modelados demonstraram que a redução de contaminação cruzada dentro das cozinhas de residências e serviços de alimentação, após cocção adequada da carne de frango e a redução na prevalência inicial de *Salmonella* spp. foram as estratégias mais eficazes para redução do risco, sendo que a redução das concentrações desse microrganismo não afetaram o risco. Métodos de redução da contaminação dentro da indústria, como lavagem de carcaças, ausência de contaminação cruzada na depenagem e evisceração, não reduziram o risco de salmoneloses na população, porém foram considerados importantes para reduzir a concentração e possivelmente a prevalência de *Salmonella* spp. das carcaças de frango e ovos liberadas para o comércio interno e exportação. As avaliações de risco desenvolvidas nessa Tese podem auxiliar no desenvolvimento de estratégias de intervenção e gestão para mitigar os riscos de salmonelose pelo consumo de frangos e ovos no Brasil.

ABSTRACT

Currently, Brazil is the world leader in chicken exports and, in recent years, has achieved the record production of chicken and eggs. *Salmonella* spp. is one of the main food pathogens in the world, being frequently transmitted by chicken meat and eggs. In the present study, quantitative risk assessments of salmonellosis were performed, due to the consumption of chicken meat and eggs produced under official inspection in Brazil. For this, information from a systematic review of scientific literature, data obtained from the Ministry of Agriculture, Livestock and Supply (MAPA), information from industries and other sectors of the production chain and the Brazilian population were considered. Predictive microbiology models were developed, considering the multiplication of some of the main serovars of *Salmonella* spp. in Brazilian chicken meat. The multiplication of *Salmonella* in eggs was modeled using predictive microbiology software. The information was used to build a mathematical model to calculate the risk of salmonellosis through chicken meat, which considered 21 modules, from slaughter to consumption in homes and 20 modules, from slaughter to consumption in Brazilian food services. In the risk assessment for eggs, 13 modules were identified, from production to consumption in homes, and 10 modules from production to consumption in food services. The mathematical models were performed using the @RISK program, by the Monte Carlo model, with 100,000 iterations for each model. The systematic review showed that the prevalence of *Salmonella* spp. in chicken meat in Brazil it was 14.96% and in eggs it was 2.10%. 60,166 time and temperature data were collected in the Brazilian chicken meat chain, which demonstrated the adequacy of chilled and frozen temperatures. 14,159 time and temperature data were also collected in the egg chain, demonstrating that production and distribution occurred at room temperature. These data were used to model the 15 scenarios of the chicken production chain and 10 scenarios of the egg chain, aiming to identify risk mitigation strategies for salmonellosis. A study was published, addressing good practices and consumption habits in the Brazilian population and showed that 96.79% of respondents consumed chicken meat and 97.54% consumed eggs at least twice a week, in a daily meal. Considering the infective dose of only 1 CFU of *Salmonella*, the risk of infection due to the consumption of chicken meat in homes was 8.092 in 1,000 exposures and, in food services, it was 7.95 cases in 1,000 exposures. The initial risk of infection due to the consumption of eggs at home or in food services was 6 cases per 100 exposures. The modeled scenarios demonstrated that the reduction in cross-contamination inside home kichen and food services, after adequate chicken meat cooking, and reduction in the initial prevalence of *Salmonella* spp. it was the most effective strategy for reducing risk, and reducing the concentrations of this microorganism did not affect the risk. Methods for reducing contamination within the industry, such as carcass washing, absence of cross contamination in plucking and evisceration, did not reduce the risk of salmonellosis in the population, but were considered important to reduce the concentration and possibly the prevalence of *Salmonella* spp. of chicken and egg carcasses released for domestic and export trade. The risk assessments developed in this Thesis can assist in the development of intervention and management strategies to mitigate the risks of salmonellosis due to the consumption of chickens and eggs in Brazil.

SUMÁRIO

1. INTRODUÇÃO

O Brasil se destaca no cenário internacional como grande produtor e exportador de produtos agropecuários. Atualmente, o país é o segundo maior produtor de carne de frango e ocupa o primeiro lugar nas exportações mundiais, sendo responsável por 37% delas. Em relação à produção e ao consumo brasileiro de ovos, esses também têm crescido. Levantamentos reaizados pela ABPA demonstraram que a produção brasileira de ovos totalizou, no ano de 2017, 39,9 bilhões de unidades, um recorde histórico (ABPA, 2018). Além disso, o volume em toneladas das exportações de ovos férteis de galinha cresceu 27,02%, de 2016 para 2017 (MAPA 2018; ABPA 2018).

Atualmente, *Salmonella* spp. é um dos principais patógenos de alimentos em nível mundial, sendo responsável por cerca de 15% das DTA registradas no mundo (FAO/WHO, 2002; BRADEN; TAUXE, 2013; WHO, 2013; WHO, 2015; JARVIS et al., 2016; WHO, 2017). No Brasil, este microrganismo também tem sido apontado como um dos principais agentes etiológicos identificados nas investigações de DTA, desde 2000 (BRASIL, 2019). Tradicionalmente, salmoneloses alimentares têm sido associadas ao consumo de aves e ovos (FAO/WHO, 2002; GEIMBA et al., 2004; FSIS, 2015; MATTIELLO et al., 2015; NAIR et al., 2015; WHO, 2015; JARVIS et al., 2016; ROUGER et al., 2017; EFSA, 2018), logo, a presença de *Salmonella* spp. não é desejável, uma vez que pode provocar graves prejuízos à saúde pública brasileira e a economia (NAZIR et al, 2012; BERSOT et al., 2019).

Neste contexto, a fim preservar a saúde pública e não prejudicar o desenvolvimento do comércio nacional e internacional, ferramentas de gestão da segurança dos alimentos tornam-se muito importantes.

A Análise de Risco (AR) tem sido utilizada para avaliar os riscos de perigos específicos em alimentos de grande relevância para um país ou região. O objetivo principal da AR é prevenir a ocorrência de DTA, através de medidas de controle factíveis em situações reais. Este é um método sistemático e altamente estruturado para a segurança de alimentos, o qual se baseia em três componentes: gestão de riscos, avaliação de riscos e comunicação de riscos (FAO/WHO, 2006; TONDO; BARTZ, 2019) Na AR, um perigo significativo de segurança de alimentos é identificado, como a *Salmonella* spp. em carne de frango e ovos, e as opiniões de

especialistas, de governos, das indústrias, das universidades e da comunidade em geral são consideradas para a tomada de decisões de modo consciente e adequado.

A etapa de avaliação de riscos, é considerada a etapa científica da AR. Nesta etapa pode-se utilizar diferentes métodos de análise, modelagem matemática e microbiologia preditiva para avaliar quantitativamente os riscos de contaminação e ocorrência de DTA, permitindo também identificar medidas de controle efetivas (OPAS, 1999; FAO/WHO, 2006, TONDO; BARTZ, 2019). Uma avaliação de riscos bem desenvolvida permite identificar as melhores estratégias de controle do perigo investigado, as quais podem ser comunicadas à população e/ou setores produtivos, geralmente pelos órgãos reguladores, os quais frequentemente são responsáveis pela gestão de riscos.

Com base nesses aspectos, o presente estudo realizou avaliações de riscos quantitavas, considerando cenários reais da cadeia produtiva de frangos e ovos, no Brasil. Para tanto, dados de prevalência e concentração de *Salmonella* spp., de tempo e temperaturas de indústrias, supermercados, centro de distribuição e hábitos de consumo foram utilizados. Além disso, foi construído um modelo para predizer a multiplicação de *Salmonella* spp. na carne de frango produzido no Brasil. Esses dados foram utilizados para calcular o risco de salmonelose, devido ao consumo de carne de frango e ovos produzidos sob inspeção oficial no Brasil, assim como sugerir estratégias de controle desse microrganismo, nesses alimentos.

2. OBJETIVOS

2.1. Objetivo Geral

Realizar avaliações de risco quantitativas para cálculo do risco de salmonelose a partir do consumo de frangos e ovos produzidos sob inspeção oficial no Brasil.

2.2. Objetivos Específicos

1) Identificar e caracterizar o perigo *Salmonella* spp. veiculado por carne de frango e ovos produzidos sob inspeção oficial no Brasil.
2) Construir cenários de tempos e temperaturas relacionados às etapas da cadeia produtiva de frango e ovos no Brasil.
3) Avaliar a exposição ao perigo *Salmonella* spp. pelo consumo de carne de frango e ovos produzidos sob inspeção oficial no Brasil.
4) Estimar a probabilidade de ocorrência de salmonelose a partir do consumo de frango e de ovos produzidos sob inspeção oficial no Brasil.

3. REVISÃO BIBLIOGRÁFICA

A Organização Mundial de Saúde (OMS) estimou que aproximadamente 75% das doenças que afetaram os humanos, nos últimos 10 anos, foram ocasionadas por patógenos presentes em animais ou em produtos de origem animal (WHO, 2015). Este dado reflete a importância de produtos de origem animal, os quais podem se tornar contaminados por microrganismos e também promover a sua multiplicação se não forem devidamente tratados, processados, preservados e cozidos, o que pode resultar em uma ameaça importante para à saúde pública (WHEELER et al., 2014).

Os patógenos contaminantes de produtos avícolas são oriundos da microbiota natural dos animais, do ambiente do abate, armazenamento, transporte e manipuladores (KIMURA et al., 2004; MEAD et al., 2010; CARRASCO; MORALES-RUEDA; GARCÍA-GIMENO, 2012; RAJAN; RICKE, 2017). Em recente estudo realizado por Gonçalves-Tenório et al. (2018), em 21 países avaliados, os principais patógenos encontrados em frangos e produtos avícolas foram *Salmonella* spp., *S. aureus*, *Campylobacter* spp. e *L. monocytogenes*. Considerando que os produtos avícolas são as fontes principais de casos de salmonelose, na Europa, a EFSA (2019) determinou como prioritário o controle de *Salmonella* spp. na produção de aves e ovos.

A principal forma de transmissão de *Salmonella* spp. aos humanos ocorre através do consumo de carne de frango e ovos (CARRASCO; MORALES-RUEDA; GARCÍA-GIMENO, 2012). A contaminação da carne de frango acontece, principalmente, devido a presença deste microrganismo no ambiente de criação das aves e posterior disseminação nas carcaças, durante as operações de abate e processo (CARRASCO; MORALES-RUEDA; GARCÍA-GIMENO, 2012). Segundo Rajan et al. (2016), após o transporte do aviário a indústria, as aves e posteriormente suas carcaças são submetidas a muitos processos, que vão desde a pendura do frango (pré sangria) ao corte e embalamento (Figura 1). Dentre estes processos (demonstrados através de fluxograma no item 4.1.1), os principais pontos de contaminação em carne de frango por *Salmonella* spp. em indústrias são as etapas de escaldagem, evisceração e refrigeração (GONÇALVES-TENÓRIO et al. 2018, ROUGER et al. 2017, RAJAN et al. 2016). Outros autores relatam que oas etapas de depenagem e corte, assim como equipamentos sujos em qualquer etapa

de processo, também podem contribuir com a contaminação das carcaças (RAJAN et al., 2016; ROUGER et al., 2017).

Nos ovos, os processos de contaminação de *Salmonella* spp. ocorrem de 2 formas principais: transmissão vertical e transmissão horizontal. A transmissão vertical acontece quando os ovários e ovidutos das aves estão contaminadas com o patógeno, contaminando o interior dos ovos, antes da casca ser formada. Já a transmissão horizontal ocorre depois que a casca do ovo foi formada, durante a passagem do ovo pela coacla, devido a presença de *Salmonella* spp. nas fezes da ave (RAJAN et al., 2016; TONDO; BARTZ, 2019).

3.1. Segurança dos alimentos na produção de carne de frango

A avicultura brasileira é reconhecida hoje como uma das mais desenvolvidas do mundo, com expressivos índices de produtividade. Este patamar foi atingido, devido aos programas de qualidade implementados em todos os elos da cadeia, nos últimos anos, com destaque para genética, nutrição, manejo, biosseguridade, boas práticas de produção, rastreabilidade, programas de bem-estar animal e de preservação do ambiente (ABPA, 2008).

Segundo o ultimo relatório anual da ABPA, o Brasil é o segundo maior produtor de frango do mundo, sendo que 66,9% da produção atende ao mercado interno (ABPA, 2018). O consumidor brasileiro tem a sua disposição um produto mais acessível que a carne vermelha, e de excelente qualidade sanitária e nutricional, com uma gama elevada de produtos *in natura* e processados, como frango inteiro e cortes congelados, resfriados e industrializados, na forma de empanados, marinados, temperados, cozidos, entre outros.

Antigamente, a legislação sanitária nacional atuava majoritariamente sobre desvios detectados e as empresas não eram protagonistas nos controles de qualidade considerados necessários pelos órgãos reguladores. Atualmente, as modernas legislações internacionais e nacionais preconizam os Programas de Autocontrole como requisitos básicos para a garantia da inocuidade dos produtos alimentícios (MAPA, 2005). Considerando a importância epidemiológica que *Salmonella* spp. possui em frangos, desde 2003, o Ministério da Agricultura Pecuária

e Abastecimento (MAPA) monitora a presença deste patógeno nas carcaças destes animais por meio do “Programa de Redução de Patógenos” (PRP). Este programa foi instituído pela Instrução Normativa Nº 70 (IN-Nº70/03) e substituído em 2016 pela Instrução Normativa Nº 20 (IN-Nº20/16), com o objetivo de garantir uma redução gradual da ocorrência de *Salmonella* spp. nos produtos avícolas, através do monitoramento constante dos ciclos de amostragem e para estabelecer um nível adequado de proteção ao consumidor (BRASIL, 2003; BRASIL, 2016).

De acordo com a IN-Nº20, estabelecimentos de abates de frangos (registradas no SIF) deverão fazer ciclos de amostragem anuais para monitoramento de *Salmonella* spp., desde a obtenção da matéria-prima até o produto final. Para determinação dos ciclos de amostragem, os estabelecimentos são classificados de acordo com o volume de abate. Por exemplo, para estabelecimentos com volume de abate superior a 100.000 aves/dia (tamanho G), a Normativa estabelece limites aceitáveis de 12 amostras positivas para *Salmonella sp*p. em um ciclo de amostragem de 51 carcaças (BRASIL, 2016). Através desse monitoramento, é possível identificar às indústrias com alta incidência de *Salmonella* e realizar ações que objetivam garantir limites aceitáveis de contaminação (BERSOT et al., 2019).

Como citado anteriormente, o processamento da carne de frango passa por diversas etapas, sendo algumas consideradas críticas no que se refere a contaminação por *Salmonella* spp. Segundo Rouger et al. (2017), embora existam algumas diferenças entre indústrias de grande e pequena escala, as principais etapas do abate de frangos são semelhantes. Segundo Rajan et al. (2016) cada uma das etapas do processamento primário pode atuar como fonte de contaminação por *Salmonella* spp., sendo que o manuseio não higiênico das carcaças e os equipamentos de abate sujos são as principais fontes de contaminação nas plantas de processamento de aves. Rouger et al. (2017) relataram que a contaminação bacteriana na carne de frango também pode ocorrer nas etapas do processamento que empregam ar e água (Figura 1), sendo que na carne fresca as bactérias permanecem presentes na superfície (ex. carcaças), diferentemente do que ocorre nos produtos processados, como os marinados.

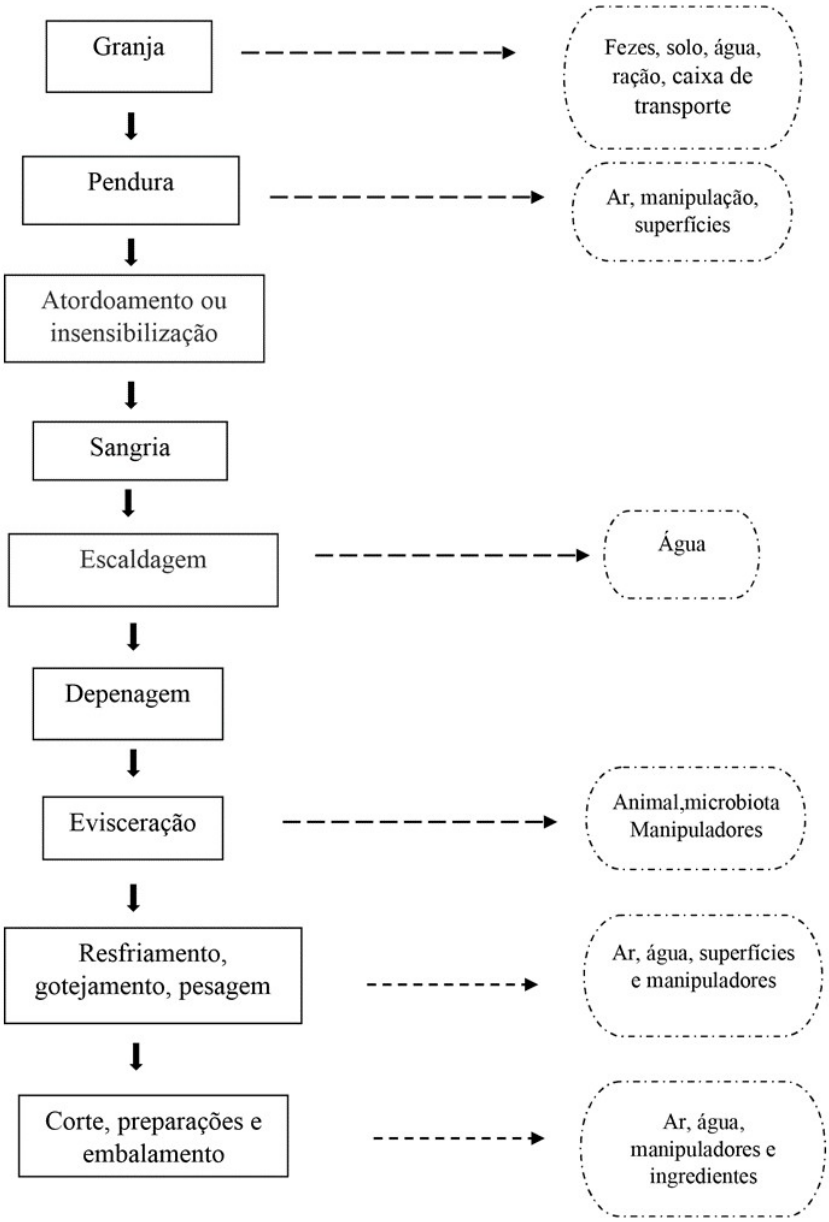

Figura 1: Fluxograma produtivo do frango com respectivas etapas de risco para contaminação por *Salmonella* spp.

3.2. Segurança dos alimentos na produção de ovos

Ovos compreendem um grupo grande de *commodities*, podendo ser consumidos como ovos ou como ingredientes, em muitos produtos processados. Estes alimentos são comercializados principalmente como ovos em casca ou como produtos de ovos (líquido, congelado ou desidratado; integral, clara ou gemas) ou produtos cozidos de ovos (refrigerados ou congelados) (ICMSF, 2015).

Levantamentos realizados pela ABPA demonstraram que a produção brasileira de ovos totalizou, no ano de 2017, 39,9 bilhões de unidades, um recorde histórico (ABPA, 2018). Além disso, o volume em toneladas das exportações de ovos férteis de galinha cresceu 27,02%, de 2016 para 2017 (ABPA, 2018). Apesar do crescimento expressivo da exportação de ovos no Brasil, 99,74% de toda produção nacional é destinada ao consumo interno (ABPA, 2018).

Os ovos ou produtos de ovos estão associados a um número significativo de surtos de DTA e têm *Salmonella* spp. como agente etiológico mais comumente envolvido nos Estados Unidos, na Europa e no Brasil (EFSA, 2007; GANTOIS et al., 2008; AYRES et al., 2009; WALES et al., 2011; MOFFATT et al., 2013; DENAGAMAGE et al., 2015; WHILEY; ROSS, 2015; BRASIL, 2016). Surtos recentes indicaram que as estratégias atuais para o controle de *Salmonella* spp. precisam ser melhoradas para minimizar ainda mais a contaminação dos ovos comerciais (SEOCKMO et al., 2016).

A contaminação de ovos por *Salmonella* spp. é uma questão complexa, influenciada por muitas variáveis (GAST et al., 2014; DENAGAMAGE et al., 2015; ICMSF, 2015; SEOCKMO et al., 2016). Os ovos tornam-se contaminados por *Salmonella* spp. de duas maneiras: por infecção transovariana ou penetração pela casca. Estas vias de contaminação podem ser influenciadas por inúmeras variáveis, como por exemplo, a dimensão do bando, a idade dos animais, estresse, alimentação, vacinação e rotinas de limpeza (WHILEY; ROSS, 2015). Além disso, podem influenciar também o processo de produção de ovos, sua preparação, armazenamento e manuseio (DAVIES; BRESLIN 2003; DENAGAMAGE et al., 2015).

Segundo Seockmo et al. (2016), as causas de contaminação dos ovos são classificadas como fatores intrínsecos, como porosidade e espessura da casca; e fatores extrínsecos, como lavagem incorreta da casca, condições de armazenamento dos ovos e tipo de sorovar contaminamente. As etapas de risco para contaminação dos ovos estão descritas na Figura 2, bem como as etapas de maior risco de contaminação dos ovos no processamento primário (FOOD STANDARDS AUSTRALIA NEW ZEALAND, 2009).

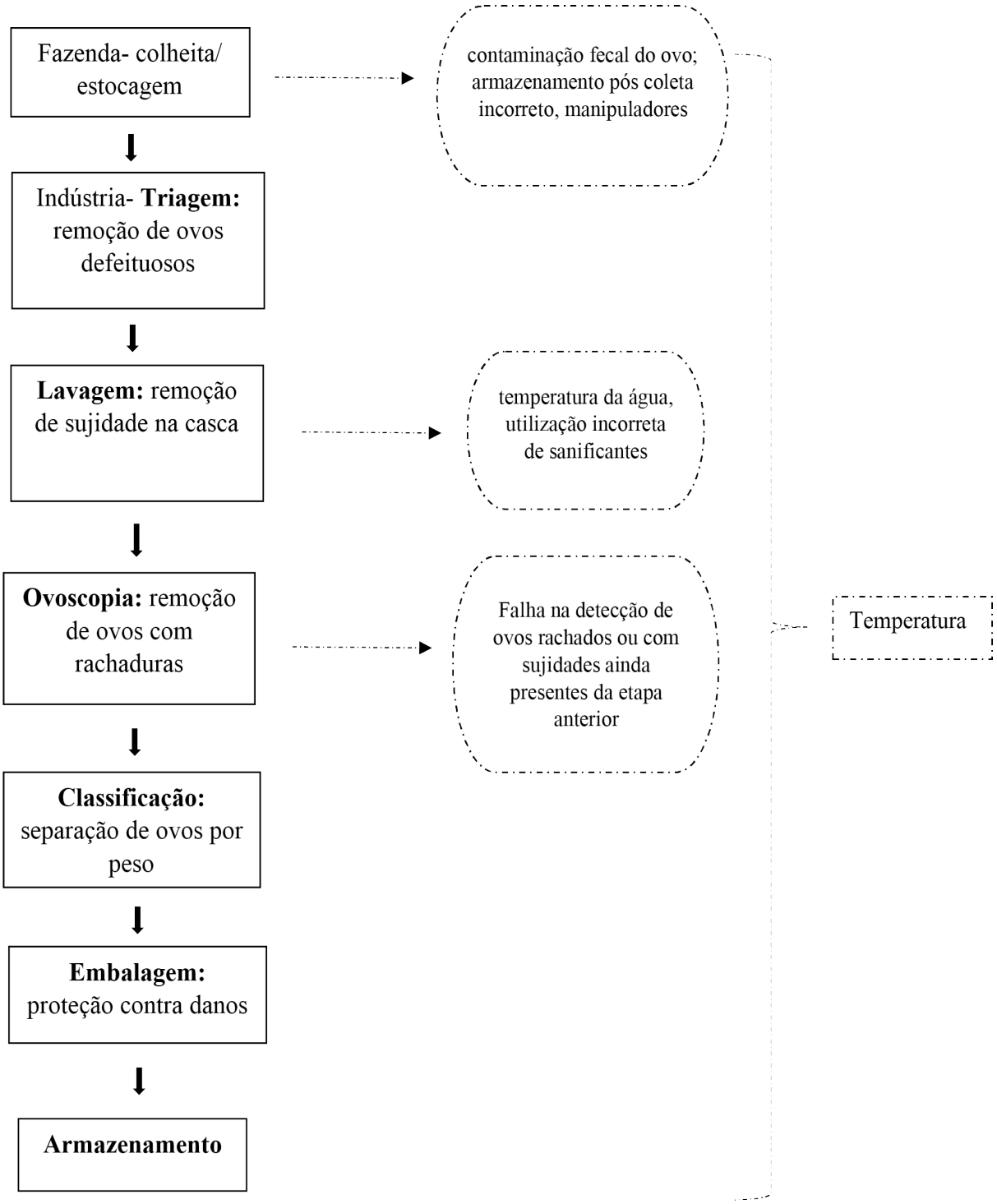

Figura 2: Fluxograma produtivo de ovos com respectivas etapas de risco para contaminação por *Salmonella* spp.

Tendo em vista os riscos identificados, a prevenção de *Salmonella* spp. em plantéis de aves poedeiras exige a realização de análises e implementação de medidas de controle, desde o incubatório que fornece os animais até os próprios animais (CODEX ALIMENTARIUS, 2007; GAST et al., 2014; DENAGAMAGE et al.,

2015). Assim, as principais medidas de controle recomendadas são às práticas de biossegurança na granja, resfriamento dos ovos após a coleta e durante o transporte, retirada dos ovos rachados do comércio, evitar água livre no ovo e condenação, devido à mudanças na temperatura e lavagem dos ovos com biocida, quando permitido (ICMSF, 2015).

Geralmente, os derivados de ovos utilizados em alimentos são cozidos ou processados de tal forma que células de *Salmonella* spp. são destruídas. Entretanto, ingredientes à base de ovos contaminados que entram na indústria apresentam o perigo potencial de contaminar produtos alimentícios (ICMSF, 2015).

3.3. Contaminação de carne de frango e ovos por *Salmonella* spp.

Microrganismos do gênero *Salmonella* spp. representam um dos mais importantes agentes patogênicos de origem alimentar no mundo (FAO/WHO 2002; BRADEN; TAUXE, 2013; WHO, 2013; JARVIS et al., 2016; WHO, 2018; RITTER et al., 2019). A *Salmonella* spp. é uma bactéria Gram-negativa, anaeróbia facultativa, não formadora de esporos e com formato de bastonete. Possui como temperatura mínima de multiplicação 5 ºC e temperatura ótima de aproximadamente 35 a 37ºC. São microrganismos mesófilos, podendo ser destruídas a 60 ºC por 15 a 20 minutos. Este gênero é pertencente à família *Enterobacteriaceae* e é composto por apenas duas espécies, *enterica* e *bongori*. O gênero enterica se divide em seis subespécies, sendo um de seus representantes *Salmonella* enterica subespécie enterica (TONDO; BARTZ, 2019). A subespécie *enterica* abrange 1586 sorovares, sendo a única subespécie reconhecida como patogênica a humanos e/ou animais (ISSENHUTH-JEANJEAN et al., 2014). Mais de 2.600 sorovares já foram descritos para *Salmonella* spp., no entanto menos de 100 sorovares são os responsáveis pela maioria das infecções humanas (FORSYTHE, 2013). Geralmente os sorovares causadores de infecção alimentar fazem parte da espécie *enterica*, como é o caso da *S*. Enteritidis e *S*. Typhimurium (WHO, 2013; CDC, 2016).

Salmonella spp. causam aproximadamente 94 milhões de casos de gastrenterite aguda e 155.000 mortes por ano, sendo responsável por um em cada quatro casos de diarreia no mundo (WHO, 2018; TONDO; BARTZ, 2019). Esta alta

prevalência se mantém desse os anos 1990 (BRADEN; TAUXE, 2013; WHO, 2013; WHO, 2018).

No Brasil, de 2009 a 2018, foram notificados 2.431 surtos de DTA e a *Salmonella* spp. foi responsável por 11,3% dos casos (BRASIL, 2019). Além disso, uma cepa específica de *Salmonella* spp., *S.* Enteritidis SE86 foi o principal causador de surtos, desde 1993 a 2012, no Estado do Rio Grande do Sul, sendo provavelmente, o patógeno de origem alimentar mais estudado do sul do Brasil (TONDO; RITTER, 2012; TONDO et al., 2015; RITTER et al., 2019).

Ao contrário de outros agentes patogênicos de origem alimentar, *Salmonella* spp. tem sido implicada em surtos veiculados por uma grande variedade de alimentos, pois eles ou seus ingredientes podem ser contaminados em nível de campo (nos reservatórios) e ao longo da cadeia de produção, abate e processamento (BRADEN; TAUXE, 2013). Assim, podem ser citados como alimentos envolvidos em DTA causadas por *Salmonella* spp. as carne de aves, suínos, ovos, leite e produtos lácteos, vegetais frescos, nozes e chocolate (FORSYTHE 2010; CDC 2014). No entanto, tradicionalmente, DTA por *Salmonella* spp. têm sido associada ao consumo de aves e ovos (FAO/WHO 2002; GEIMBA et al., 2004; FSIS, 2015; MATTIELLO et al., 2015; NAIR et al., 2015; WHO 2015; JARVIS et al., 2016; ROUGER et al., 2017; EFSA, 2018). Também há relatos de contaminação cruzada a partir de manipuladores de alimentos, água ou contato com animais (BRADEN; TAUXE, 2013; CDC, 2016).

Apesar das infecções por *Salmonella* spp. não terem diminuído ao longo dos últimos anos, a incidência de sorovares envolvidos nessas doenças mudou (WHO, 2013). Em nível mundial, o sorovar *S.* Typhimurium diminuiu significativamente e o sorovar *S.* Enteritidis inicialmente diminuiu e depois aumentou novamente. Este comportamento iniciou na década de 1990, devido aos esforços das indústrias de ovos para reduzir a contaminação por *S.* Enteritidis e a utilização crescente de ovos pasteurizados (BRADEN; TAUXE, 2013; JARVIS et al., 2016).

Outros fatores que aumentam a preocupação de DTA causadas por *Salmonella* spp. é a resistência ácida, térmica, a antimicrobianos e sanitizantes comumente utilizados na produção de alimentos demonstrada por cepas desse microrganismo (OLIVEIRA et al., 2005; MALHEIROS et al., 2009; PEREZ et al., 2010; ÁLVAREZ-ORDÓÑEZ et al., 2012; HUR et al., 2012; SPECTOR; KENYON, 2012; WHO, 2013; CDC, 2014; PARK et al., 2014; COSBY et al., 2015; NAIR et al., 2015; JARVIS et al.,

2016; ZIECH et al., 2016; RITTER et al., 2019). Especialmente, em relação à resistência antimicrobiana, a resistência de isolados de *Salmonella* spp. em aves de granjas é aparentemente maior quando comparado a isolados de alimentos (OLIVEIRA et al., 2005; MATTIELLO et al., 2015; EFSA, 2018).

A presença de *Salmonella* spp. em frangos de corte comerciais, além da preocupação com a saúde pública, provoca graves prejuízos econômicos, constituindo um obstáculo para a indústria avícola em todo o mundo (NAZIR et al., 2012; BERSOT et al., 2019). Por exemplo, entre março de 2013 e julho de 2014, mais de 600 pessoas, em 29 estados americanos, e em Porto Rico foram infectadas por *S.* Heidelberg. Este surto foi causado por um produto de frango, que levou a empresa a realizar um *recall* de mais de 15 mil produtos. Além do prejuízo econômico sofrido pela empresa, o órgão de regulamentação nacional determinou que a empresa implementasse medidas de controle de processo para minimizar consistentemente a contaminação de *Salmonella* spp. nas carnes de frango (CDC, 2014). No que se refere aos ovos, Seockmo et al. (2016) relatam que a contaminação por *S.* Enteritidis levou à retirada de mais de 500 milhões de ovos proveniente do estado de Iowa (EUA), entre maio e novembro de 2010, sendo que houve 1.939 casos de salmonelose relacionadas a esse surto. Assim, a prevenção de infecções por *Salmonella* spp. depende de ações tomadas por agências reguladoras, produtores, indústrias de alimentos e consumidores, bem como as medidas tomadas para a detecção e resposta aos surtos quando ocorrem (FSIS, 2015).

3.4. Análise de Risco como ferramenta de Gestão da Segurança de Alimentos

A análise de riscos (AR) é um método sistemático e altamente estruturado que se baseia em avaliações científicas, opiniões de especialistas de governos, das indústrias, das universidades e da comunidade em geral para possibilitar a tomada de decisões de modo consciente e adequado (FAO/WHO, 2006). Dentre os principais objetivos da AR estão: reduzir os níveis de DTA e melhorar a segurança de alimentos. Esta ferramenta atua através de um processo transparente e participativo, onde análises científicas podem ser essenciais para alcançar soluções sólidas e consistentes para os problemas de segurança dos alimentos (TONDO; BARTZ, 2019). De forma geral, a AR que trata de problemas microbiológicos pode

permitir identificar e avaliar os possíveis riscos relacionados a microrganismos específicos em determinados alimentos e, na sua melhor versão, fornecendo, base científica para o estabelecimento de medidas de controle, quando necessário.

A AR possui três componentes básicos: gestão ou gerenciamento de risco, avaliação de risco e comunicação de risco (Figura 1). No desenvolvimento de uma AR devem ocorrer interações frequentes entre os gestores de risco e os avaliadores de risco, em um ambiente caracterizado pela troca de informações frequentes, o que é peculiar e desejável na comunicação de risco.

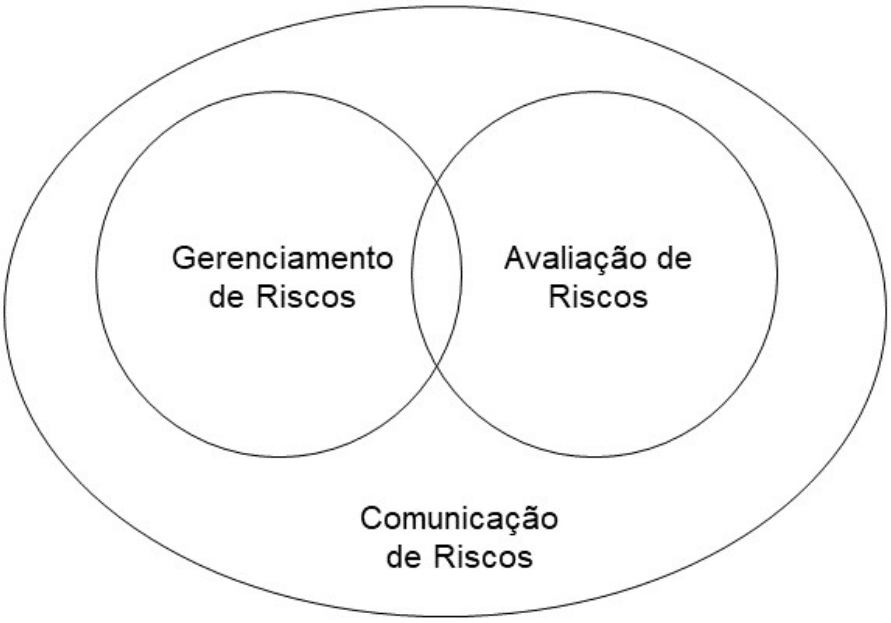

Figura 3: Componentes da Análise de Risco (Elaborado pela autora. Adaptado de FAO/WHO, 2006).

Na gestão de riscos os interesses das diversas partes envolvidas com o problema de segurança de alimentos são considerados, devendo, de forma ideal, abordar toda a cadeia de produção do alimento. Nessa etapa, as diferentes partes da sociedade ou entidades envolvidas são consultadas para garantir que o problema seja abordado de forma integral e que a tomada de decisões seja assertiva (FAO/WHO, 2006). A gestão de riscos, um problema de segurança de alimentos é claramente definido, o qual deve envolver um microrganismo específico e um alimento determinado. O gestor de riscos, encarregado da gestão de riscos, deve levantar o máximo de informações sobre esse problema e, tendo informações

suficientes para resolvê-lo, assim o faz, sem maiores gastos de recursos econômicos ou tempo. Caso a resolução do problema não seja possível com as informações levantadas pelo gestor, ele solicita uma avaliação de risco.

A avaliação de riscos é a etapa que corresponde ao embasamento científico da AR, sendo realizada por institutos de pesquisa e universidades (Figura 3). Essa avaliação pode gerar estimativas de riscos, podendo ser qualitativa ou quantitativa. A avaliação de risco qualitativa é utilizada quando não há dados quantitativos disponíveis e, usualmente, utilizada como avaliação inicial. Já na avaliação de risco quantitativa, o risco é expresso em valores numéricos em termos de probabilidade, o que permite uma noção mais precisa da ocorrência de um evento adverso. Por este motivo, a avaliação de risco quantitativa oferece uma base mais sólida para a tomada de decisões, já que há uma mensuração dos valores de probabilidade quanto aos riscos, possibilitando melhores medidas de controle. Geralmente, a avaliação de riscos quantitativa utiliza modelagem matemática, por meio de cálculos ou *softwares* específicos, além de microbiologia preditiva, identificando diferentes estratégias de intervenção (FAO/WHO 2006; ILSI, 2007; JACXSENS; UYTTENDAELE; DE MEULENAE, 2016).

Por fim, o último componente da AR é a comunicação de riscos (Figura 3). O objetivo desta etapa é divulgar as conclusões da AR, como as ameaças à saúde identificadas, à segurança ou ao ambiente, com o propósito de ampliar o conhecimento sobre a natureza e os efeitos de alguns riscos e promover o trabalho colaborativo em busca das soluções (FAO/WHO, 2005). Nesta etapa ocorre a troca de informações interativa e de opiniões de todas as partes envolvidas na AR, como membros da equipe de AR, setores da cadeia produtiva, órgãos reguladores e a população, ao longo de todo o processo (ILSI, 2007). Tanto os riscos quanto as medidas de controle devem ser claramente informados, evitando exageros, pânico e má interpretação (TONDO; BARTZ, 2019). Em nível governamental, essa comunicação pode ocorrer através de campanhas, publicação de legislações e normatização de parâmetros e procedimentos ou demais medidas de controle que forem consideradas necessárias pelo gestor de riscos, à luz das informações obtidas pela AR (SILVA, 2016).

De forma ideal, no processo de AR, são consideradas todas as etapas da cadeia produtiva do alimento em que as medidas de controle podem ser potencialmente aplicadas. Isto é particularmente importante quando são

identificadas diferenças em sistemas de produção e processamento primário entre os países e os gestores de risco precisam ter flexibilidade para escolher as opções de gestão de risco que são apropriados em seu contexto nacional (CODEX ALIMENTARIUS, 2007; FAO/WHO, 2012).

Uma das medidas de incentivo para a utilização institucional da AR pode ser observada no Acordo de Medidas Sanitárias e Fitossanitárias (SPS *Agreement*) da Organização Mundial do Comércio (OMC), que determinou que os países signatários, entre eles o Brasil, devem garantir que seus produtos não causarão danos aos países importadores e, que se houver dúvida, uma AR seja desenvolvida para esclarecê-las. A AR deve ser desenvolvida por órgãos e instituições reconhecidos e que sejam fundamentadas em dados científicos (FAO/WHO, 2006).

Muitas exigências sanitárias internacionais são decorrentes da atual política alimentar global, que se baseia nas diretrizes internacionais do *Codex Alimentarius*. Tal política abrange as matérias-primas, as práticas agrícolas e as atividades de processamento dos alimentos, visando reduzir o número de DTA, em um determinado local, região ou país.

Embora os princípios desse método possam ser utilizados em qualquer indústria de alimentos ou serviço de alimentação, a AR tem sido mais amplamente utilizada pelos governos nacionais e internacionais para avaliar possíveis perigos e riscos presentes nos alimentos e estabelecer (ou não) medidas de controle (TONDO; BARTZ, 2019).

Seguindo os princípios da AR, o MAPA, como uma instituição de regulação sanitária nacional, pode ser considerado o gestor de riscos dos produtos cárneos brasileiros. Diante disso, o MAPA tem utilizado sistematicamente os princípios da AR, a fim de ampliar seu escopo de informações e fundamentar o aperfeiçoamento de suas normas e práticas institucionais (LIMA, 2016).

A utilização da AR pode promover melhorias contínuas na saúde pública e proporcionar uma base para a expansão do comércio nacional e internacional de alimentos. Através do conhecimento científico sobre os perigos que causam DTA, os riscos que estes perigos representam para os consumidores e a capacidade de tomar as intervenções apropriadas, governo e indústrias podem reduzir significativamente os riscos relacionados aos alimentos. Além de melhorar a saúde pública, sistemas de segurança dos alimentos eficazes mantêm a confiança do

consumidor e fornecem uma base sólida para a regulamentação nacional e para o comércio internacional de alimentos.

3.4.1. Avaliação de risco

A avaliação de riscos, fundamentalmente, serve para suprir a necessidade de informações científicas para compreender a natureza e extensão do risco à segurança de alimentos de um perigo identificado. Ela também serve para a identificação e planejamento de ações de mitigação, controle ou prevenção, quando necessário (OPAS/OMS, 2008). De a cordo com a natureza dos dados disponíveis e das perguntas a serem respondidas, o processo de avaliação de risco pode ser realizado de forma qualitativa (classificação de risco) ou quantitativo (determinístico ou probabilística) (CAC, 1999).

A Organização Mundial de Saúde e a Comissão do *Codex Alimentarius* da FAO (OMS/CAC, 1999) definem a avaliação de riscos como um processo formado por quatro etapas. Estas etapas estão descritas abaixo:

1. Identificação de perigo: Nesta etapa ocorre a identificação do agente biológico (químico ou físico) que pode estar presente em um determinado alimento, ou grupo de alimentos, o qual é capaz de causar efeitos adversos à saúde.

2. Caracterização do perigo: é a avaliação qualitativa e/ou quantitativa dos efeitos adversos à saúde associados ao perigo que pode estar presente no alimento. Nesta etapa, desenvolve-se uma avaliação de dose-resposta para obtenção dos dados necessários, ou seja, avaliar quantas células (ou a concentração do perigo químico ou físico) são necessárias para o patógeno em questão causar o efeito adverso à saúde.

3. Avaliação da exposição: é a avaliação qualitativa e/ou quantitativa da probabilidade de ingestão do perigo, seja ele biológico, químico ou físico, através dos alimentos, assim como através da exposição a outras fontes. Nessa fase as quantidades de alimento ingeridas, conforme os hábitos de consumo da população de interesse, são avaliados.

4. Caracterização do risco: é a estimativa qualitativa e/ou quantitativa, que considera as probabilidades decorrentes da situação, da probabilidade e da ocorrência e gravidade dos efeitos adversos à saúde conhecidos ou potenciais em

uma determinada população, com base nos três passos precedentes da avaliação de risco, ou seja, da identificação do perigo, caracterização do perigo e avaliação da exposição (FAO/WHO, 2012).

3.4.1.1. Avaliação de risco qualitativa

A avaliação de risco qualitativa é utilizada quando não há dados quantitativos disponíveis. Nesta abordagem realiza-se uma classificação de risco através da utilização de uma matriz de risco. Nesta matriz, a severidade e probabilidade de ocorrência do perigos é determinada, de modo que o resultado é expresso em escalas descritivas: “alto”, “médio” ou “baixo” (Figura 4) (EFSA, 2011; JACXSENS; UYTTENDAELE; DE MEULENAE, 2016).

		Probabilidade de consumo		
		Baixo	**Médio**	**Alto**
Probabilidade	**Baixo**	Baixo	Médio	Alto
de	**Médio**	Médio	Médio	Alto
contaminação	**Alto**	Alto	Alto	Muito alto

Figura 4: Matriz de risco qualitativo (Elaborado pela autora. Adaptado de FAO/WHO, 2006).

Usualmente, a avaliação de risco qualitativa é utilizada quando não há informações suficientes para quantificar o risco ou em casos que o risco identificado não justifica o tempo e esforço necessário para uma análise quantititativa, muito mais detalhada. Esta análise também pode ser utilizada como avaliação inicial de uma avaliação de risco quantitativa (OIE, 2006).

3.4.1.2 Avaliação de risco quantitativa

A avaliação de risco quantitativa é considerada a base mais sólida para a tomada de decisões, pois, através desta análise, a ocorrência de um evento adverso é expresso em termos numéricos. Os dados numéricos em avaliações de risco quantitativas podem ser expressos em valores fixos ou em distribuições de probabilidade. Quando utilizados valores fixos, como médias, os modelos

matemáticos aplicáveis são modelos determinísticos e o resultado expresso é um valor fixo. Já, quando são utilizados muitos dados os quais podem ser agrupados em distribuições, modelos estocásticos ou probabilísticos são necessários. Por exemplo, a multiplicação microbiana pode ser expressa através de médias das contagens finais de microrganismos em um alimento, contudo, de fato, ela pode ser melhor considerda como um processo estocástico, devido a variação da dinâmica microbiana, ao longo do tempo e em cada amostra e alimento. Desse modo, orienta-se a utilização de distribuição de dados, analisados por modelos estocásticos, em avaliações quantitativas de risco, a fim de contemplar de forma mais realista a complexidade dos riscos possíveis (COLEMAN; MARKS, 1999).

Na avaliação de risco quantitativa estocástica, a aleatoriedade dos eventos é determinada através da aplicação de distribuições de probabilidades. Para isso utilizam-se cálculos e *softwares* específicos com simulações de Monte Carlo, que resultam em dados expressos em intervalos de probabilidade. A partir da simulação de Monte Carlo pode-se expressar o risco, assim como a incerteza e a variabilidade, envolvidas em sua determinação (JAYKUS, 1996).

Existem diversas limitações na realização de avaliação de riscos quantitativa estocástica, onde a principal dificuldade é elaborar e entender os componentes da avaliação de riscos e traduzir informações biológicas em uma estrutura matemática (JACXSENS; UYTTENDAELE; DE MEULENAE, 2016). As principais dificuldades são:

1) Alteração na incidência e patógenos causadores de DTA e surgimento de novos microrganismos associados à causas de DTA (patógenos emergentes);
2) Alterações na produção e processamento de alimentos;
3) Limitação nas investigações epidemiológicas que permitam identificar os patógenos e os alimentos envolvidos em DTA;
4) Limitação de estudos de dose-resposta de patógenos em humanos;
5) Limitações metodológicas para detecção de baixos níveis de contaminação de patógenos;
6) Limitação de estudos de contaminação cruzada em etapas do processamento de alimentos do campo à mesa;
7) Dificuldades no acesso à dados do processamento de alimentos do campo à mesa;

8) Ausência de dados de concentração microbiológica em etapas do processamento de alimentos do campo à mesa;
9) Dificuldades no acesso às práticas e hábitos do consumidor;
10) Limitação de *softwares* e métodos de modelagem matemática.

4. METODOLOGIA

A metodologia deste trabalho foi realizada na forma sequencial de uma Avaliação de Riscos Quantitativa, conforme preconizado pela FAO/WHO (2006). Deste modo, foram realizadas as etapas abaixo descritas:

4.1. Identificação do perigo

Para identificação do perigo foram utilizadas informações provenientes de artigos científicos, assim como informações epidemiológicas sobre as cepas de *Salmonella* spp. envolvidas em surtos e contaminações de frango e ovos no Brasil (TONDO; RITTER, 2012; WAGNER; SILVEIRA; TONDO, 2013; TONDO; RITTER; CASARIN, 2015; BRASIL, 2019; RITTER et al., 2019). Nessa etapa foram identificadas as informações sobre doses infectantes e os principais alimentos contaminados e envolvidos com os surtos ocorridos no Brasil.

4.2. Caracterização do Perigo

A caracterização dos perigos consistiu na avaliação qualitativa dos efeitos adversos à saúde humana, devido a *Salmonella* spp. associada a frango e ovos produzidos sob inspeção oficial no Brasil. Nesta etapa, devido à ausência de informações de correlação entre doses-respostas e diferentes níveis de exposição de *Salmonella* em frangos e ovos com base na literatura científica brasileira, foi adotado o enfoque de modelo de dose-resposta de Beta-Poisson (FAO/WHO, 2003). O modelo Beta-Poisson, assume a relação dose-resposta onde uma única célula de *Salmonella* (1 Unidade Formadora de Colônia, 1 UFC) é capaz de infectar e causar doenças *(single hit*). Este modelo tem sido utilizado em outras avaliações de risco quantitativas de *Salmonella* (HOLCOMB et al., 1999; WHO, 2002; SMADI; SARGENT, 2012; HAAS; ROSE; GERBA, 2014).

4.3. Avaliação da exposição

A avaliação da exposição consistiu na caracterização da quantidade de frango e ovo consumida pela população brasileira. Essa análise considerou a quantidade do perigo existente nos alimentos (frango e ovo) e se eles foram eliminados ou controlados ao longo do processamento. Para tanto, inicialmente, foi elaborado um fluxograma da cadeia produtiva de frango e ovos a partir do documento publicado pelo *Codex Alimentarius*, "*Guidelines for the control of Campylobacter and Salmonella in chicken meat* - CAC/GL 78-2011 e pela FAO/WHO "*Risk assessment of Salmonella in eggs and broiler chicken*". O fluxograma foi validado com dados fornecidos pelo MAPA e setores produtivos regulados por inspeção oficial (aviários e abatedouros-frigoríficos) e por serviços de alimentação (supermercados e restaurantes), assim como por profissionais da área de produção avícola e segurança de alimentos.

A partir do fluxograma validado, informações de tempo e temperatura referentes a cada etapa da cadeia produtiva e do consumo capaz de propiciar a multiplicação, sobrevivência ou inativação do perigo *Salmonella* spp. foram identificados. A prevalência e concentração do perigo *Salmonella* spp. foi identificada, sempre que possível, a partir de dados científicos ou oficiais fornecidos pelo MAPA. Essas informações serviram como embasamento para a modelagem matemática e experimentos de microbiologia preditiva, os quais foram realizados na etapa de caracterização de risco. Ainda, na etapa de avaliação da exposição, ocorreu a avaliação da quantidade de frango e ovos ingeridos pela população brasileira, o que ocorreu através de um questionário *on-line,* distribuído através do programa *GoogleDocs* (Google®). O questionário foi composto de 61 questões sobre hábitos de consumo, boas práticas de manipulação de alimentos e percepção de risco dos consumidores brasileiros. Os resultados desse questionário foram publicados em um artigo da revista *Food Research International*.

4.4. Caracterização de risco

A caracterização de riscos integrou as informações das três partes anteriores da avaliação de riscos, ou seja, a identificação do perigo *Salmonella* spp. e seus sorovares, a caracterização dos perigos e a avaliação da exposição. Nesta etapa foi realizada a quantificação dos riscos de ocorrência de salmonelose, através do

consumo de carne de frango e ovos produzidos por inspeção oficial no Brasil. Na quantificação do risco foram consideradas as incertezas e variabilidades das probabilidades de ocorrência de sorovares de *Salmonella* spp. em carne de frango e ovos. Para tanto, informações referentes à distribuição das quantidades de alimento consumido e da distribuição das possíveis quantidades e prevalências de *Salmonella* spp. foram avaliadas pelo Programa @RISK (Palisade, Newfield, NY, USA), utilizando a simulação de Monte Carlo. Em cada modelagem, 100.000 iterações foram realizadas, a fim de calcular a probabilidade de doença.

5. RESULTADOS

Os resultados e discussões da presente Tese serão apresentados a seguir na forma de artigos científicos. Posteriormente aos artigos, a Tese apresenta uma discussão geral e as conclusões provenientes de todos artigos científicos.

- Artigo científico 1: *Systematic review about Salmonella spp. prevalence and levels on raw chicken meat.*
- Artigo científico 2: *Assessment of time and temperatures in the chicken meat chain and predicted pathogen growth under different scenarios.*
- Artigo científico 3: *Food safety behavior and handling practices during purchase, preparation, storage and consumption of chicken meat and eggs.*
- Artigo científico 4: *Quantitative Risk Assessment of human salmonellosis linked to Brazilian chicken meat.*
- Artigo científico 5: *Quantitative Microbial Risk Assessment of Salmonella in eggs in Brazil.*

5.1. Artigo científico 1:

Systematic review about *Salmonella* spp. prevalence and levels on raw chicken meat

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Abstract

Chicken meat and related products are foods frequently involved with salmonellosis, thus proper and effective measures should be taken to control *Salmonella* during broiler production. Information about prevalence and concentration of this pathogen on raw chicken meat are important to control *Salmonella* using modern food safety tools as modeling and predictive microbiology, however this information are variable or difficult to find. This systematic review aims to estimate the prevalence and levels of *Salmonella* spp. on raw chicken meat using the most reliable available data. We searched for the terms "*Salmonella*" AND "chicken" AND "poultry" in Pubmed and in Web of Science. After deleting duplicates, abstract/title verification, processed meat, cooked meat, drag swabs, cloacal swabs in live animals, feet, giblets, intestines and feces, 100 studies about raw chicken contamination were included in analysis. The overall prevalence of *Salmonella* spp. in samples collected from markets/stores was 23.1%, and contamination in slaughterhouses/processing plants was 23.2%. Carcasses samples had lower prevalence (19.7%) when compared with the *Salmonella* prevalence in chicken meat cuts (23%). There was a small difference in *Salmonella* prevalence of frozen (21.1%)

and chilled/fresh (20.9%) meat samples. Brazilian studies counted for 17% of the analyzed data and *Salmonella* spp. prevalence average was 15% (range from 0 to 44.6%), similar to the overall prevalence in Latin America (15.2%, value range from 0 to 44.6%) and Africa (15.3%, value range from 4.44% to 50%). Data from USA and Canada had the highest *Salmonella* prevalence with 38.98% (values ranging from 21% to 85%). Studies from European Union (EU) were more numerous than other parts of the world (31%) and its data shows *Salmonella* prevalence of 18.82%, which was lower than the prevalence found in Asian studies (25.9%, values ranging from 0 to 93%). The overall *Salmonella* spp. prevalence in the world was 21.02% (values ranging from 0 to 93%). Different serotypes were reported in the studies, from which *S.* Enteritidis was the most identified in Brazil, Latin America, Asia and EU. Only three studies verified the concentration per gram (1,6-110 cfu/g), per carcass (2,1-2,5 log MPN) or per carcass over the production line (2,11 log MPN after plucking to <1,08 log MPN after chilling). All these data reinforce the importance of monitoring and overall good hygienic practices in the microbiological control in broiler producers worldwide, therefore the aim of this systematic review is to compile data on prevalence and concentration of *Salmonella* in broiler meat.

Keywords: *Salmonella,* prevalence, concentration, foodborne pathogen, raw chicken meat.

1. Introduction

Pathogens that cause foodborne diseases are a great public health issue and an important cause of morbidity mostly in the developing world (Vinueza-Burgos et al., 2016). Among the most important foodborne pathogens, *Salmonella* raises as the one frequently linked to poultry products, such as chicken meat (EFSA, 2018; Rajan, Shi, & Ricke, 2017; WHO, 2018). These chicken products are the main vehicle of dissemination of *Salmonella* and susceptible patients like children, elderly or immunocompromised people are the most affected (Vinueza-Burgos et al., 2016). In 2010, Brazil was one of the biggest producers of chicken meat in the world and was the country that exported the most, making the control of *Salmonella* contamination an essential issue for economics and public health (Cossi et al. 2012). Even with all the efforts from industries and from the government to control *Salmonella*, salmonellosis still be one of the most frequent foodborne diseases and chicken

products have been identified as one of the major responsible for the dissemination of this disease (BRASIL, 2019; Elias, Tomasco, Alvarenga, Sant'Ana, & Tondo, 2015).

It is well know that contamination of raw broiler meat can be caused by inadequate hygienic practices in industry processing, inappropriate storage or undercooking of meat. These conditions provide a conducive environment to contamination by *Salmonella* spp. and other pathogens (Oh & Park, 2017; Xavier et al. 2013). *Salmonella* spp. can be found in water, soil, fecal matter, gastrointestinal tract of livestock animals (Elias, Noronha & Tondo, 2019; Xavier et al., 2013). Even asymptomatically colonised individuals in a broiler flock are active transmission vehicles of this microorganism and the contamination normally happen as result of producers' lack of good habits and/or careless processing as well as direct infection of offspring (Chlebicz & Slizewska, 2018). *Salmonella* spp. is a bacterium that can survive in a wide range of conditions like temperatures ranging between 5 and 40ºC and a wide range of environment pH from 4.0 to 9.5 due to defense systems know as acid tolerance response (Chlebicz & Slizewska, 2018; Dunkley et al. 2009). With such ability to survive and adapt, *Salmonella* represents a great difficulty and risk in broiler meat production and consumption especially when considering non-isothermal conditions. Several studies emphasize the importance of maintaining these parameters, since an environment with a temperature above the recommended and/or non-isothermal conditions will increase bacteria growth rates and reduce safe shelf life of products (Veys, et al., 2016; Chlebicz & Slizewska, 2018; Elias et al., 2019).

Currently, the genus is divided into two species (*S. bongori* and *S. enterica*) and seven subspecies: I, II, IIIa, IIIb, IV, V, and VI and there are over 2500 reported serovars (Oh & Park, 2017). Most serotypes are classified as *S. enterica* subsp. *enterica* (subspecies I) and they are responsible for 99% of the salmonellosis cases. The most related serotypes to food poisoning cases worldwide are *S. typhimurium, S.* Enteritidis*, S. Javiana, S. newport*, and *S. heidelberg* (Chlebicz & Slizewska, 2018; Oh & Park, 2017). It is estimated that food contamination by *Salmonella* spp. cause 85% of over 90 million cases of gastroenteritis worldwide, plus 155,000 deaths per year (Chlebicz & Slizewska, 2018; Vinueza-Burgos et al., 2016; Xavier et al., 2013).

Three kinds of salmonellosis can occur in human infections: noninvasive and nontyphoid; invasive and nontyphoid; and typhoid or paratyphoid fever (*S. typhi* and

S. *paratyphi*) (Chlebicz & Slizewska, 2018). The high fatality rate expected in diarrhea-associated diseases is over 150.000 all over the world been children below 4 years the most affected, mainly when the *S.* Enteritidis or *S. typhimurium* serotypes are involved in the infection (Chlebicz & Slizewska, 2018).

Although many studies have analyzed the presence of *Salmonella* spp. on chicken meat, prevalence data are variable in different regions and information about concentration of *Salmonella* are scarce. These data are important to carry out quantitative microbial risk assessments and predictive microbiology studies, which are necessary for quantifying the potential risk of salmonellosis associated with chicken consumption, being possible to improve control measures in primary production and inside food industries. Taking that into account, this systematic review was carried out to estimate and summarize prevalence and concentration of *Salmonella* spp. on raw chicken meat at a global scale.

2. Materials and methods

2.1 Search strategy and criteria selection of the systematic review

A reference search for references was carried out using the terms ‘‘chicken’’ OR ‘‘poultry*”* AND “*Salmonella.*” in the PubMed and Web of Science platforms. No data restrictions were used. Endnote version X6 (Thomson Reuters) was used to collect publications and the strategies for research databases are demonstrated in Table 1.

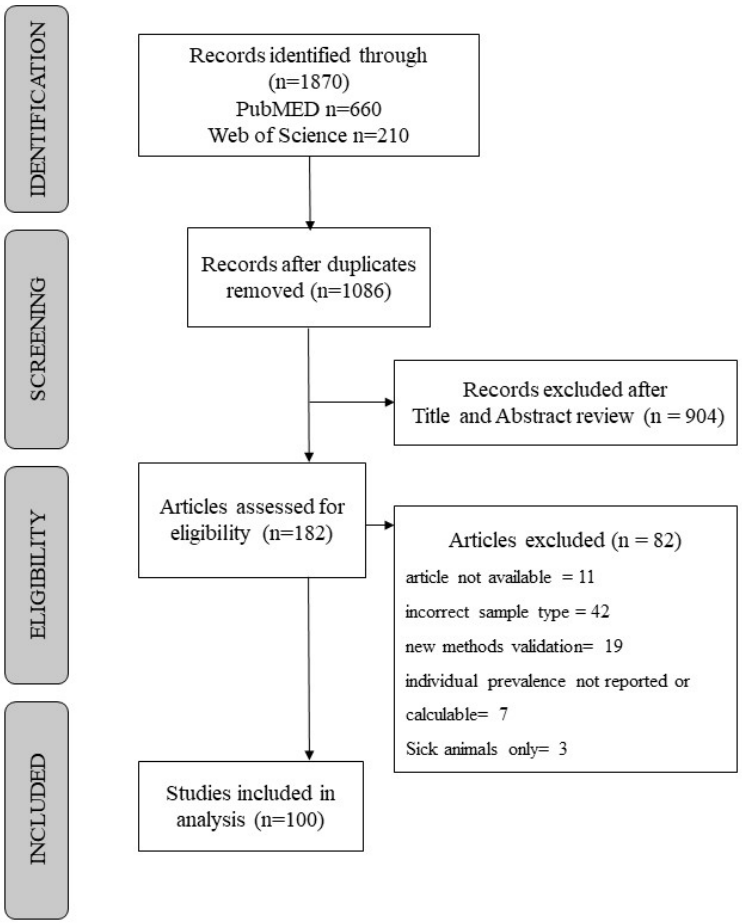
Table 1: Strategies for electronic search in databases and search results of systematic review about *Salmonella* on chicken meat.

Database	*Salmonella* spp. and chicken	*Salmonella* spp. and poultry	Search strategy
PubMed MEDLINE	248	412	Abstract
Web of Science (Science Citation Index)	527	683	title/keywords/abstract
Total	775	1095	

All titles found were checked for duplicates, using Endnote and Mendeley (https://www.mendeley.com/). Manuscripts were collected and included when they were published in English, Spanish or Portuguese and relevant search terms appeared in the title, abstract or key words. Publications were excluded if they were

review articles and book chapters; had incomplete information on the prevalence and concentration of *Salmonella* on chicken, had information only about processed meat, cooked meat, drag swabs, cloacal swabs in live animals, feet, giblets, intestines and feces; had data only about turkey, quail or duck meat (Figure 1).

Figure 1: Flow diagram of the literature search and selection of eligible studies.



Full-text articles were accessed and when these were not available, abstracts and titles were evaluated for relevance. When full text was not available and abstracts did not provide complete information, additional efforts were done in order to access full articles, otherwise articles were excluded.

2.2 Data extraction

Data related to *Salmonella* spp. prevalence and concentration on chicken meat were extracted from the studies selected through the systematic review of the literature and included in the study database independently by a single trained

reviewer and validated by a second reviewer. Data extracted included country where samples were taken; *Salmonella* spp. prevalence, sample size, level of contamination in CFU/g or MPN/g, *Salmonella* serovars prevalence, sample origin and chicken parts sampled.

3. Results and discussion

This study is based on available data and was conducted to identify study references that estimate the prevalence and concentration of *Salmonella* spp. on raw chicken meat worldwide. The search for the terms "*Salmonella*" AND "chicken" resulted in 248 manuscripts in Pubmed and 527 in Web of Science, while the terms " *Salmonella*" AND "poultry" resulted in 412 manuscripts in Pubmed and 683 in Web of Science. A total of 1870 manuscripts were identified and, after duplicates removal, 1086 manuscripts were selected. Several studies analyzed unsuitable due to sample types analyzed (processed meat, cooked meat, drag swabs, cloacal swabs in live animals, feet, giblets, intestines and feces) and were excluded. After the evaluation of titles and abstracts, 182 manuscripts were assessed for eligibility and after analysis, 100 studies, published between 1981 and 2018, were included in the present meta-analysis (Fig. 1).

The methods of analyses of all manuscripts were based on microbiological and/or molecular detection. Most of the samples were collected from markets and butcheries (47%), while slaughterhouses, farms, and processing plants represented 34% of the collection points. The overall prevalence of chicken contamination sold in markets was similar to the ones found in industries (23.1% and 23.2% respectively). More than half of the selected manuscripts analyzed whole carcasses (58%) and the prevalence of contamination was lower than that found on chicken cuts (19.7% and 23% respectively). Studies evaluating chicken cuts and whole carcasses demonstrated varied results (0-93% in cuts and 0-88% in carcasses), this may indicate that the chicken meat cutting process does not necessarily increase *Salmonella* spp. contamination (Dias et al., 2016; Khan et al.,2018; Siriken et al., 2015; Vural et al., 2006). However, there are data indicating higher contamination of chicken breast compared to other parts of chicken meat or whole carcasses, probably due to the larger contact area of these kind of samples (Vural et al., 2006).

Comparing frozen or chilled/*in natura* samples, the prevalence was also similar. Only 10 manuscripts analyzed frozen samples and showed a contamination prevalence of

21.1%, while the prevalence of chilled/*in natura* samples was 20.9%. The studies that analyzed both types of samples simultaneously (n=8) also presented varied results which ranged from 1.1% to 57.8%. Freezing the chicken meat is expected to reduce the prevalence of *Salmonella* spp. (Fernandes et al., 2016; Khan et al., 2018; Li et al., 2017; Scheinberg et al.,2013), but in some investigations the data show the opposite (Adeyanju & Ishola, 2014; Myskova & Karpiskova, 2017). Is was observed that, despite the wide variation of results among the manuscripts, the contamination with *Salmonella* spp. on chicken meat from industry, farms or markets are still a critical problem. The prevalence in big industries and processing plants are 28,9%, higher than the overall worldwide prevalence, despite applying a more developed contamination control system than small slaughterhouses. All these data reinforce the importance of monitoring and overall good hygienic practices by small and large capacity broiler producers considering the colonization and meat contamination with *Salmonella* spp..

Brazilian studies constitute 17% of the analyzed data and *Salmonella* spp. prevalence demonstrated by them was 15% (values ranging from 0 to 44.6%), similar to the overall prevalence in Latin America (15.2%, values ranging from 0 to 44.6%) and countries in Africa (15,3%, values ranging from 4.44% to 50%). The 7 articles from USA and Canada reported the highest prevalence 38.9%, and the values ranged from 21% to 85% of *Salmonella* spp (Table 2).

EU was the region with the largest number of selected manuscripts (31) and data shows prevalence of 19.2% (values ranging from 0 to 58%) lower than Asia where the average *Salmonella* prevalence was 25.9%, but values ranged from 0 to 93%. The overall prevalence of *Salmonella* spp. worldwide was 21.02% (values ranging from 0 to 93%). The highest prevalence was found in Thailand (62%, 87.7% and 93%) and in USA (85%) (Kotula & Pandya, 1994; Vindigni et al. 2007; Bodhidatta et al. 2013; Boonprasert et al. 2014) (Table 2).

Table 2: Variation in the results of *Salmonella* spp. concentration in chicken meat.

Region	Country	Nº. of papers	Prevalence found (n)[a]
Latin America	Chile	1	1,8% (280)
	Colombia	1	17,4% (270)
	Argentina	1	6% (108)
	Guatemala	1	34,3% (300)

	Brazil	17	0% (24), 0,3% (30), 2,1% (48), 2,2% (452), 2,7% (2679), 4,1% (1200), 6,6 (227), 8,1%/9,3%/11,9% (135)[b], 8,3% (60), 9,6% (260), 9,6% (240), 10,6% (8813), 20% (135), 21,6% (60), 30% (193), 31,7% (60), 38,5%/44,6%[b] (130), 42% (60)
	México	1	21% (1765)
North America	USA	5	21% (200), 29% (42), 35% (40), 35% (40), 85% (40),
	Canada	2	30% (185), 37,5% (1295)
Africa	Trinidad Tobago	1	14,2% (450)
	Zambia	1	20,5% (382)
	Ghana	1	5,7% (87)
	South Africa	1	19,2% (99)
	Senegal	1	7% (100)
	Nigeria	1	50% (106)
	Egypt	3	4,4% (45), 5% (100), 14% (50)
	Morocco	2	7,3% (288), 20,9% (86)
Asia	Malaysia	2	30% (120), 57,8% (102)
	Korea	6	0% (27), 0% (41), 9,2% (120), 15% (120), 25,9% (27), 33,8% (65)
	India	5	0% (144), 2,8% (175), 5,4% (240), 6,7% (324), 7% (200)
	Thailand	5	2% (30), 9,8% (498), 62% (50), 87,7% (49), 93% (40)
	Nepal	2	14,5% (55), 60% (15)
	China	2	19,2% (52), 33,75% (240)
	Iran	2	18% (134), 45,3% (190)
	Japan	1	24,1% (55)
	Vietnam	1	53,3% (30)
	Pakistan	1	2% (200)
European Union (EU)	Ireland	4	2,8% (18782), 5,3% (38), 23% (198), 26,4% (106)
	Northern Ireland.	1	1,4% (205)
	Spain	1	58% (90)
	Poland	3	6,5% (200), 29,6% (300), 30,6% (400)
	Turkey	6	0% (18), 8% (400), 15% (100), 34% (200), 42,7% (150), 51% (200)
	Italy	2	1,1% (180), 16,7% (1621)
	France	2	7,5% (425), 7,52% (425)
	Greece	3	21% (19), 37% (150), 39,5% (96)
	Germany	2	1,2% (426), 17% (500)
	Czech Republic	2	3,8% (160), 14,5% (152)
	Belgian	1	43,8% (466)

	Croatia	1	7,5% (67)
	Romania	1	23,9% (422)
	Swiss	1	0 (90)

[a] numbers of samples collected
[b] more than one laboratorial methodology

Serotype description were reported in 52% of the manuscripts, being that other articles reported *S. enteritidis* as the most widespread serovar identified by the Brazilian manuscripts, Latin America, Asia and EU (Table 3). Although this prevalence is recurrent in Brazil, it is quite worrying since this serovar can grow faster and be more acid-and-thermal-resistant than other serovars (Elias, et al. 2016). Almost half (48%) of the studies just made the detection of *Salmonella* spp. without identifying the serovar.

Table 3: Studies included in systematic review of the prevalence of *Salmonella* spp. in raw broiler meat by region, country, number of papers, number of samples, chicken part, collection point and prevalence.

Region	Country	N°. of papers	Sam-ples (n)	Chicken part	Collection point	Preva-lence (%)	References
Latin America	Chile	1	280	Cuts	Slaughterhouses	1,8%	Ulloa et al., 2019
	Colombia	1	270	Cuts	Stores/Markets	17,4%	Rodriguez et al., 2015
	Argentina	1	108	Carcasses	Slaughterhouses	6%	Jimenez et al. 2015
	Guatema-la	1	300	Carcasses	Stores/Markets	34,3%	Jarquin et al., 2015
	Brazil	17	730*	Carcasses (13) Cuts (2) Both(2)	Stores/Markets (4) Slaughterhouses (12) Slaughterhouses and Markets (1)	14,9%*	Lima et al. 2017; Duarte et al., 2019; Fuzihara et al., 2000; Cossi et al.,2012; Von Ruckert et al.,2009; Pires et al., 2009; Matias et al. 2017; Cossi et al., 2011; Medeiros et al., 2011; Simas et al., 2011; Possebon et al., 2012; Brizio & Prentice, 2015; Cintra et al., 2016; Dias et al., 2016; Fernandes et al., 2016.
	México	1	1765	Cuts	Slaughterhouses	21%	Zaidi et al. 2018
North	USA	5	72*	Carcasses	Stores/Markets	41%*	Kotula & Pandya,

America					(1) Slaughterhouses (4)		1994; McCrea et al., 2006; Kilonzo-Nthenge et al., 2008; Scheinberg et al., 2013; Lemonakis et al., 2017.
	Canada	2	740*	Carcasses (1) Cuts (1)	Stores/Markets (1) Slaughterhouses (1)	33,7%*	Bohaychuk et al., 2007; Ravel et al., 2010.
Africa	Trinidad Tobago	1	450	Carcasses and Cuts	Slaughterhouses and Markets	14,2%	Khan et al., 2018.
	Zambia	1	382	Carcasses	Slaughterhouses	20,5%	Hang'ombe et al., 1999.
	Ghana	1	87	Carcasses	Stores/Markets	5,7%	Sackey et al., 2001.
	South Africa	1	99	Carcasses	Stores/Markets	19,2%	van Nierop et al., 2005.
	Senegal	1	100	Cuts	Farms	7%	Missohou et al., 2011
	Nigeria	1	106	Cuts	Stores/Markets	50%	Adeyanju & Ishola, 2014.
	Egypt	3	65*	Cuts	Stores/Markets	7,8%*	Gharieb et al., 2015; Abdel-Aziz, 2016; Tarabees et al., 2017.
	Morocco	2	187*	Cuts	Stores/Markets (1) Slaughterhouses and Markets (1)	14,1%*	Amajoud et al. 2017
Asia	Malaysia	2	111*	Cuts (1) Carcasses and minced meat (1)	Stores/Markets	43,9%*	Thung et al., 2016; Shafini et al., 2017
	Korea	6	50*	Cuts (2) Carcasses (4)	Stores/Markets (3) Slaughterhouses (3)	14%*	Chang, 2000; Il Cho et al., 2012; Bae et al., 2013; Chon et al., 2015; Lee et al., 2016; Wu et al., 2016.
	India	5	217*	Cuts (3) Carcasses (2)	Stores/Markets (2) Slaughterhouses (1) Slaughterhouses and Markets (2)	8%*	Vaidya et al., 2005; Willayat et al., 2016; Vaidya et al., 2010; Naik et al., 2015; Rajashekhara et al., 2017.
	Thailand	5	133*	Cuts (2) Carcasses (3)	Stores/Markets (2) Slaughterhouses (1) Slaughterhous	50,9%*	Padungtod & Kaneene, 2006; Bodhidatta et al., 2013; Boonprasert et al., 2014;

					es and Markets (2)		Chotinun et al., 2014; Vindigni et al., 2007.
	Nepal	2	35*	Cuts	Stores/Markets	37,3%*	Bantawa et al., 2018; Maharjan et al., 2006.
	China	2	146*	Carcasses	Stores/Markets (1) Slaughterhouses (1)	26,5%*	Li et al., 2017; Wang et al., 2013.
	Iran	2	162*	Cuts	Stores/Markets	31,7%*	Jalali et al., 2008; Soltan Dallal et al., 2014.
	Japan	1	286	Cuts	Slaughterhouses and Markets	24,1%	Tokumaru et al., 1991.
	Vietnam	1	30	Cuts	Stores/Markets	53,3%	Van et al., 2007.
	Pakistan	1	200	Cuts	Undefined	2%	Afzal et al., 2015.
European Union (EU)	Ireland	4	4774*	Cuts (3) Carcasses (1)	Stores/Markets (2) Slaughterhouses (1) Laboratory (1)	14,4%*	Jordan et al., 2016; Duffy et al., 1999; Whyte et al., 2002; Catarame et al., 2006.
	Northern Ireland.	1	205	Cuts	Stores/Markets	1,4%	Soultos et al., 2003.
	Spain	1	90	Carcasses	Slaughterhouses	58%	Carraminana et al., 1997.
	Poland	3	200	Cuts (1) Carcasses (2)	Stores/Markets (1) Slaughterhouses (2)	22,2%*	Mikolajczyk & Radkowski, 2001; Mikolajczyk & Radkowski, 2002; Radkowski & Zdrodowska, 2016.
	Turkey	6	178*	Cuts (2) Carcasses (3) Carcasses and Cuts (1)	Stores/Markets	25,1%*	Kasimoglu et al., 2010; Yildirim et al., 2011; Siriken et al., 2015; Dumen et al., 2015; Abay et al., 2017; Bilge et al., 2018.
	Italy	2	171*	Cuts (1) Carcasses and Cuts (1)	Slaughterhouses (1) Farms (1)	8,9%*	Colmegna et al., 2009; Carraturo et al., 2016.
	France	2	425*	Carcasses	Slaughterhouses	7,5%*	Hue et al., 2011; Hue et al., 2011
	Greece	3	88*	Cuts (1) Carcasses (2)	Stores/Markets (2) Slaughterhouses (1)	32,5%*	Sakaridis et al., 2011; Gousia et al., 2011; Zdragas et al., 2012.
	Germa	2	463*	Cuts	Slaughterhous	9,1%*	Schwaiger et al.,

ny				es (1) Border inspection post (1)		2012; Jansen et al., 2018.
Czech Republic	2	156*	Cuts (1) Carcasses (1)	Stores/Markets (1) Slaughterhouses (1)	9,1%*	Svobodová et al., 2012; Myskova & Karpiskova, 2017.
Belgian	1	466	Carcasses and Cuts	Stores/Markets	43,8%	Uyttendaele et al., 1999.
Croatia	1	67	Cuts	Slaughterhouses	7,5%	Kozacinski et al., 2012.
Romania	1	422	Cuts	Slaughterhouses and Markets	23,9%	Mihaiu et al., 2014.
Swiss	1	90	Carcasses	Slaughterhouses	0	Althaus et al., 2017.

* Mean between studies

Only three manuscripts reported the concentration of *Salmonella* spp. on chicken samples (Hue et al., 2011; Jarquin et al., 2015; Svobodová et al., 2011) (Table 4). And, only one study analyzed the concentration of *Salmonella* spp. at various stages of chicken production, demonstrating reductions in counts of *Salmonella* while broilers advanced in production line (2.11 log MPN per carcass after plucking> 1.56 after evisceration > 1.53 after washing > 1.08 after chilling) (Svobodová et al., 2011) .

Table 4: Concentration of *Salmonella* on raw chicken meat.

Reference	Concentration of *Salmonella* spp.
Hue et al., 2011	1.6 cfu/g in three samples 110 cfu/g in one sample
Svobodová et al., 2011	2.11 log MPN per carcass after plucking 1.56 log MPN per carcass after evisceration < 1.53 log MPN per carcass after washing < 1.08 log MPN per carcass after chilling
Jarquin et al., 2015	2.3 ±0.2 log MPN per carcass

Prevalence and concentration data of *Salmonella* in chicken meat are essential to carry out quantitative microbial risk assessments, which are necessary for quantifying the potential risk associated with chicken meat consumption and to improve control measures in primary production and inside food industries.

According to the Guide for National Food Safety Authorities, published by Food and Agriculture Organization of the United Nations (FAO/WHO) (2007), in the context of Risk Analysis, Risk Assessment should be based on scientific data of

sufficient quality, detail and representativeness must be located from appropriate sources and assembled in a systematic manner (CAC/GL, 2007). However, this step can be tough, since great deep studied previously occur while the model is conducted. Among the scientific data necessary are: the level of control of a hazard at a step (or series of steps) in a food chain and the prevalence and concentrations of microbes present during each procedure related to food preparation and consumption. This data are applied in risk modeling to estimate the pathogen prevalence at any given point in the food supply chain. These estimations can be used in planning and enforcing food safety decisions at micro- and macro-levels (Whiting & Buchanan, 1997; CAC/GL, 2007; Katiyo, de Kock, Coorey, & Buys, 2019) To collect this information several studies are necessary, and, usually data are based in different web-bases (Rajan et al., 2017; Manfreda & De Cesare, 2014; Tuominen et al., 2007; van der Fels-Klerx et al., 2008).

4. Conclusion

The prevalence of *Salmonella* spp. on chicken meat from industry, farms or markets in different regions of the world ranged from 0% to 93% (Table 2). These data are extremely variable even within the same country and although there are several studies, this variation and the low number of samples collected in several of them, hamper a real view of the prevalence of *Salmonella*. On the other hand, information about concentration of *Salmonella* are still scarce.

We found an average worldwide prevalence of 21%, and the highest ones was in USA and Canada (39%) and Asia (26%). Data showed that the average prevalence of *Salmonella* in raw chicken meat in Brazil (15%) is similar to that of the rest of Latin America, but it is well below the world average. Considering the importance of chicken meat in food trade and the biological hazard *Salmonella*, the limited data on prevalence of *Salmonella* serovars and concentration in raw chicken meat indicate that further studies focusing on these themes are extremely necessary to develop microbiological risk assessments.

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Authors' Contributions

In this research, all authors contributed effectively. Claudia Titze Hessel, Roberta Taufer Boff and João Pedro Pessoa conducted the data collection, organization, analysis, interpretation and wrote the manuscript and Eduardo Cesar Tondo supervised the project and reviewed the manuscript.

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5.2. Artigo científico 2:

Artigo submetido na Revista Food Microbiology.

Assessment of time and temperatures in the chicken meat chain and predicted pathogen growth under different scenarios

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Abstract

Chicken products are among the major vehicles of food pathogens and the probability of foodborne occurrence increases when these microorganisms multiply during food production. The objective of this study was to investigate time and temperature data from slaughter to the consumption of chicken meat in Brazil and to predict foodborne pathogen growth under these scenarios. A total of 60,166 data points on time and temperature were compiled from the chicken production flowchart, and the behavior of *Clostridium perfringens, Escherichia coli, Listeria monocytogenes,* and *Salmonella* spp. was modeled under real scenarios. Results demonstrated that chilled chain temperatures were mostly <5 ºC, despite some observed temperature breaches. The temperature data were fitted to Beta General and Logistic distributions. Pathogen growth did not occur under slaughter industry and transport time and temperature scenarios, while slight microbial growth was predicted during storage in the retail to home storage scenario, depending on the model used. Considering the whole chilled chain, *L. monocytogenes* reached the

lowest counts when compared with the growth of other pathogens, although this microorganism was the first to start growing in storage under retail conditions. *C. perfringens* reached the highest counts in the chilled chain, while *E. coli* was the microorganism that multiplied better in the frozen chain scenario. *Salmonella* spp. growth was expressively different when different predictive models were used. The data from the present study can be used to estimate microbial growth, survival and the probability distributions of time and temperature in stochastic modeling, considering time and temperature scenarios of the chicken meat chain.

Keywords: Microbiological assessment; probability distribution; chicken meat; temperature abuses; cold chain; supply chain.

1. Introduction

Chicken products are important sources of meat at the global level, across diverse cultures, traditions, and religions. The demand for chicken meat is expected to continue increasing due to population growth and the rise in individual consumption (ABPA, 2019; FAO, 2019; USDA, 2019).

Brazil is the second largest producer and the first largest exporter of chicken meat in the world, distributing more than 4 million tons to more than 150 countries annually. In 2019, Brazilian chicken production is expected to increase by 2.3%, achieving 36% of world's exportation (ABPA, 2019; EMBRAPA, 2019; USDA, 2019).

Even though industrial chicken production is generally very well controlled, foodborne outbreaks involving chicken products are still cited as one of the major causes of foodborne disease in the world (BRASIL, 2019; Chai, Cole, Nisler, & Mahon, 2017; EFSA, 2018). Some foodborne pathogens of interest to chicken producers are *Clostridium perfringens*, Enteropathogenic *Escherichia coli*, *Listeria monocytogenes,* and *Salmonella* spp. (Chai et al., 2017; Chousalkar & Gole, 2016; Gonçalves-Tenório, Silva, Rodrigues, Cadavez, & Gonzales-Barron, 2018).

Several authors have demonstrated the presence of temperature fluctuations during the chicken meat cold chain (Gonçalves-Tenório et al., 2018; Ndraha, Hsiao, Vlajic, Yang, & Lin, 2018). The rise of a few degrees in temperature may result in microbial growth, leading to decreased quality, food spoilage, and an increased risk of food poisoning. However, if food products are stored and distributed at appropriate temperatures, the risk of foodborne disease to consumers is usually low, even when

pathogenic microorganisms are present (Aung & Chang, 2014; Daelman, Jacxsens, Devlieghere, & Uyttendaele, 2013; Kuo & Chen, 2010; Mercier, Villeneuve, Mondor, & Uysal, 2017; Morelli, Noel, Rosset, & Poumeyrol, 2012; Ndraha et al., 2018; Anna Roccato, Uyttendaele, & Membré, 2017). Thus, it is necessary to analyze the whole flowchart of chicken production, from farm to fork, considering all conditions that may lead to contamination and microbial growth in order to reduce the risk of foodborne diseases for consumers. This approach is necessary for the development of quantitative microbial risk assessment (QMRA), assuring a high level of protection of human health (CAC/GL, 2007).

Currently, one of the biggest challenges to carrying out a QMRA is the collection of real data on the whole flowchart of chicken production, because trade is branched and involves many companies located in different regions. Thus, the main objectives of the present study were to analyze time and temperature data in the chicken meat chain, fit data to distributions, and then predict foodborne pathogen growth under a real time and temperature scenario of the Brazilian chicken chain, from slaughter to consumption.

2 Materials and Methods

2.1. Data collection

2.1.1. Time and temperature data

A generic flowchart diagram from slaughter to the consumption of chicken meat was drawn based on the *Codex Alimentarius* document “Guidelines for the control of *Campylobacter* and *Salmonella* in chicken meat CAC/GL 78-2011”. The diagram was validated by 10 experts, who were food safety consultants; food researchers; sanitary surveillance officers; and professionals working in chicken slaughterhouses, food services, and supermarkets. The final version of the diagram is presented in Figure 1. Time and temperature data on all steps of chicken chain production were obtained by accessing several stakeholders inside chicken companies, distribution centers, retail outlets, and food services. These professionals were accessed by personal contact or by e-mail and were invited to participate in the study. Once they agreed, a form was sent by e-mail to each participant, who provided the required information.

The database comprised time and temperature from January 2012 and May 2018. Two scenarios were identified using the collected data: 1) chilled chain for chicken meat sold at retail and consumed at home, and 2) frozen chain for chicken consumed in food services.

A form was also sent by e-mail to the chicken slaughterhouses under Federal Inspection in the State of Rio Grande do Sul, Southern Brazil. Questions about processing time, process temperature records, equipment, and room temperatures were asked. Temperature records were compiled from 15 chicken slaughterhouses under Federal Inspection in the State of Rio Grande do Sul, Southern Brazil.

The transportation temperature from the slaughterhouse to the distribution center was evaluated through a thermocouple containing four sensors (Tenmars®, Taiwan). Each sensor was placed on different chicken meat packages, and the device was turned on just before the truck left the slaughterhouse. Once the device was turn on, the temperature was recorded every 30 seconds. The thermocouple was turned off when the truck arrived at its destination, and the truck body door was opened. The same procedure was performed from the distribution center to the retail outlet. Measurements were performed from May to November 2018 on three different days.

Records from 141 Corporate Catering Food Services located throughout the five Brazilian macro-regions were compiled between May and November 2018. The restaurants belong to the largest meal production corporation in Brazil.

Temperature records and chicken meat label information from seven retail outlets located in the State of Rio Grande do Sul were analyzed between May and November 2018. Data from Hessel et al. (2019) were used to access time and temperature data inside Brazilian residences. Finally, data obtained from the National Institute of Meteorology (INMET, n.d.) was used to access the environmental temperatures of the capital cities of each Brazilian State. For this, the maximum and minimum temperatures in the period from 01 January 2017 to 29 November 2018 registered by weather stations in each of the 27 Brazilian states were compiled. The temperatures of the capital city of each state were chosen and assumed as the temperature of each state.

2.2. Fitting data into distribution

All data compiled were organized in Excel spreadsheets where each production step (module) was put in one column. Temperature data were fitted to distributions (BetaGeneral, ChiSqd, Expon, Logistic, LogLogistic, LogNorm, Normal, Pareto, Pert, Student, Triangular, Uniform, and Weibull) using the software @Risk (Palisade corporation, version 6.3.1). As a measured of goodness of fit, the different distributions provided were ranked according to the root mean squared error for each module. The distribution choices were selected according to the study of Roccato, Uyttendaele, & Membré (2017) and Alfama et al. (2019).

Time data were not fitted to distributions, since the Pert distribution allows the completion time based on the best estimates of minimum, maximum, and the most likely values for an event. Therefore, this distribution is usually chosen to describe this parameter in terms of distribution probability (Gomes Alfama et al., 2019; Jarvis et al., 2016; Anna Roccato et al., 2017; Sant'Ana, Barbosa, Destro, Landgraf, & Franco, 2012; Smadi & Sargeant, 2013).

2.3. Predictive growth of foodborne pathogens

The effect of real time and temperature scenarios created with data gathered from the chicken production chain on the growth of *Clostridium perfringens, Escherichia coli, Listeria monocytogenes,* and *Salmonella* spp. on chicken meat was predicted, assuming that the growth of microorganisms started after the chilling step at slaughterhouses until the moment before food preparation inside homes or food services.

The behavior of all pathogens was predicted using ComBase Predictor software. Static growth models were performed with an initial level of 1 log CFU/g and the pH and water activity (aw) values of 6.0 and 0.997, respectively (da Silva, de Arruda, & Gonçalves, 2017; Gordana Kralik, Zlata Kralik, 2017; Hertanto, Nurmalasari, Nuhriawangsa, Cahyadi, & Kartikasari, 2018). Minimum and maximum temperatures were chosen according to the limits preconized by ComBase Predictor to each microorganism, although the real scenario is an extrapolation of these. Thus, the predictive primary models were built at temperatures of 7, 10, 15, 21, 36.5, and 40°C for *C. perfringens, E. coli,* and *Salmonella* spp., while for *L. monocytogenes* the temperatures used were 1, 5, 10, 15, 21, 36.5, and 40°C. The maximum growth rate (μ) (log conc./h) at each temperature was used to obtain the secondary models (square root model). The predictive secondary models were built using the square

root equation described by Ratkowsky et al. (1982) to describe μ (growth rate) as a function of the temperature.

The predicted growth of foodborne pathogens was calculated using @Risk. At this step it the relationship between the predictive model provided by ComBase and the fitted temperature distribution was employed. The growth of pathogens at each module of the chicken meat production chain was calculated by multiplying the predicted growth obtained by the Pert fitted time of distribution. The predictive model of Oscar (2007) and Juneja et al. (2007) for *Salmonella* spp. on chicken were also tested under the real scenarios for comparison.

3. Results and Discussion

3.1 Time and temperature data

Table 1 shows time and temperature data and statistical parameters obtained by distribution fitting of chicken meat modules in two identified scenarios: 1) chilled chain for chicken meat sold at retail and consumed at home and 2) frozen chain for chicken consumed in food services. From slaughter to consumption, 60,166 data points for time and temperature were compiled from the 22 modules of the chicken meat production chain.

Considering all slaughter industry modules, 7,928 data points were collected. During the slaughter process, chickens are subjected to the steps of bleeding, scalding, defeathering, head removal, evisceration, spray washing, chilling, cutting, and packing (Figure 1). Time and temperature data on the initial modules in slaughterhouses are not usually registered by companies, so the data presented in Table 1 came from interviews conducted with technical employees of the 15 companies. It was noted that information about the time and temperature of bleeding, defeathering, head removal, evisceration, and spray washing were similar among all slaughterhouses; a possible explanation for is that variations in these parameters may impact on meat quality and increase product loss. For example, if the scalding time is too short, plucking could be more difficult, whereas if the temperature is too high, shrunken or hardened meat may result.

Even though modules from bleeding to packing demonstrated temperatures able to promote bacterial growth, the time of each module was very short; for this reason, we assumed that bacterial growth did not occur. Pathogen growth in these modules was modeled and corroborated this assumption.

The objective of the chiller is to reduce the carcass temperature to below 7 ºC (MAPA, 1998). This step includes immersion of chicken carcasses in cold water or exposure to cold air, either by passing carcasses through an air blast system or holding them in a chilling room. Normally, carcasses are immersed in stainless steel tanks for 0.60 ± 0.27 h at a temperature of 2.00 ± 1.14 ºC. The data showed that slaughterhouses were in compliance with Brazilian national legislation (MAPA, 1998); however, the maximum temperature observed in this study was 7.9 ºC (Table 2), which is a non-conformed temperature.

After chilling, the carcasses are butchered, packaged, and stored. In Brazil chilled chicken meat should be butchered in rooms at temperatures below 12 ºC (MAPA, 1998). It was observed that this module was controlled in the slaughter process (average temperature of 10.52 ± 0.54 ºC, and the maximum value found was 12.4 ºC). The time spent at this module depends on the cut and ranged from 14 seconds to 40.2 min, which do not allow for bacterial growth.

After the chicken meat is packaged, the carcasses are refrigerated or frozen (Figure 1). The packing module showed a wide variation in temperature, ranging from −6 to 22.8 ºC, and the average was 2.7 ºC. Similarly, the time spent in this process varied from 10 seconds to 16 h, and the average was 1.29 h.

The data show that frozen storage at the slaughterhouse occurs at a maximum of −14.50 ºC, which is in accordance with Brazilian national legislation. The recommendation is to maintain frozen chicken at a temperature not exceeding −12 ± 2 °C (MAPA, 1998). Chilled chicken carcasses showed average temperatures of 2.51 ± 1.65 ºC, in agreement with the national legislation, which tolerates a maximum temperature of 4 ºC (MAPA, 1998). However, the maximum temperature observed in this study was 22.8 ºC (Table 2), which is an inadequate temperature, and the maximum duration of this process was 72 h. Thus, this scenario may represent a food safety risk, because it allows the growth of mesophilic microorganisms.

The next step in the frozen chicken carcass distribution chain is transport from industries to distribution centers and from these locations to restaurants or supermarkets, where the carcasses are stored. Our results demonstrate that these modules occurred at sub-zero temperatures (−14.50 °C or below; data not shown), not allowing microbial multiplication.

After that, at food services, frozen carcasses need to be thawed before cooking and consumption. This is accomplished by increasing the temperature of the

chicken. According to Brazilian legislation, chicken must be thawed with cooling equipment at temperatures below 5 ºC or by direct heating followed by immediate consumption (BRASIL, 2004). The results showed that food services thaw frozen chicken meat at average temperatures of 5.04 ± 8.04 ºC. However, some inadequate temperatures were observed, e.g., 35.00 ºC, and the maximum duration of this process was 24 h. Thus, these data may be related to the defrosting procedure occurring at room temperature, which is inadequate, since it allows microbial growth.

Cooking or other thermal processing methods are the main steps responsible for the inactivation of vegetative foodborne pathogens eventually present on chicken meat, while distribution is the step where ready-to-eat chicken meat is held at hot or refrigerated temperatures before consumption. Table 1 shows adequate average temperatures for cooking and for hot distribution after cooking in food services (higher than 70 ºC and 60 ºC, respectively) (BRASIL, 2004). At these temperatures, vegetative pathogens are inactivated, and if there was no cross-contamination good-quality chicken meat is safe for consumption. The duration of the distribution step was in accordance with Brazilian legislation (maximum = 6 h) (BRASIL, 2004).

The label on chilled chicken meat advises that it be maintained below 4 ºC and consumed within 11 to 14 days. Thus, considering these parameters recommended by slaughterhouses, the temperatures observed at the distribution center, transport to retail outlets, and storage at retail outlets are in accordance with those recommended by the industry.

Storage in retail outlets occurred at mean temperatures of 2.05 ± 3.12 ºC, while mean distribution temperatures were 5.83 ± 9.58 ºC, with a maximum of 25 ºC. The higher temperature observed during distribution (10.40 ºC) may be related to the continuously opened door of chilling equipment where chicken meat is stored for sale. In retail centers, the average temperature was 1.3 ºC higher than that recommended by the slaughter industry. Furthermore, maximum temperatures higher than 4 ºC were observed at distribution centers, during transport to retail stores, and in storage at retail stores (10.8, 24.7, and 10.4 °C, respectively).

Transport from retail stores to homes occurred at environmental temperatures of 25.67 ± 3.27 ºC, and the duration of this module varied from 4.8 min to 2 h. Under these conditions, foodborne pathogens can easily grow; to prevent this, the time must be short (Oscar, 2007; A. Roccato et al., 2015; Seo et al., 2016). The temperature and duration of storage and food exposure on tables at Brazilian homes

were obtained from previous studies (Hessel et al., 2019; Silva, D. L. D., Celidonio, & Oliveira, 2008). These studies demonstrated adequate storage time and temperatures of 3 ºC and 24 h, respectively; however, food exposure on tables represented some food safety risk, because the temperature varied from 20.86 to 29.81 ºC, and the time ranged from 0 to 2 h.

In general, the average of temperatures of the chicken meat cold chain was considered adequate from slaughter to the distribution center, although some breaches in temperature maintenance were observed. Compliance in the cold chain was mostly observed in the first modules of the chain, namely the slaughter industry (chiller, cutting, packing, cooling, and frozen storage) and at distribution centers. Similar results were reported by several authors (Baldera Zubeldia, Nieto Jiménez, Valenzuela Claros, Mariscal Andrés, & Martin-Olmedo, 2016; Daelman et al., 2013; Morelli et al., 2012; Ndraha et al., 2018) (Table 1). In retail, the average storage temperatures were 2.05 ± 3.12 ºC, while exposure of foods in supermarkets demonstrated average temperatures ranging from −20.00 to 25.00 ºC. The average temperatures were 5.83 ± 9.58, which may support some bacterial growth. Moreover, storage in supermarkets was above the Brazilian limit of temperature (<5 ºC), and for these reasons, it was considered inadequate.

In Brazil, one of the most common causes of foodborne outbreaks is failure in time and temperature control of ready-to-eat food at food services (BRASIL, 2019; Costalunga & Tondo, 2002; da Cunha et al., 2016). Perishable foods are considered of high value and high risk to consumers, because pathogen growth can occur, and the food's shelf-life can be reduced if the temperature and time of storage are not controlled (Aung & Chang, 2014; Gonçalves-Tenório et al., 2018; Anna Roccato et al., 2017). Thus, it requires the application of particular monitoring and surveillance time and temperature protocols. However, studies reported fails in this control, mostly due to inadequate equipment, the physical structure of cooling chambers, and daily work procedures, where the control of cold chain temperature is not prioritized (Baldera Zubeldia, Nieto Jiménez, Valenzuela Claros, Mariscal Andrés, & Martin-Olmedo, 2016; Garayoa, Díez-Leturia, Bes-Rastrollo, García-Jalón, & Vitas, 2014; Olmedo, Stangarlin-Fiori, Opolski Medeiros, Tondo, & Ferreira, 2018; Penedo, Jesus, Silva, Monteiro, & Ribeiro, 2015). So, food producers and retailers must systematize data collection and measure the environmental temperature in order to identify and evaluate precisely weak points of the cold chain, and food operators need to be

trained in order to overcome safety and quality food problems (Baldera Zubeldia et al., 2016; Heap, 2006; Maldonado-Simán, Martínez-Hernández, Zaragoza-Ramírez, & Rodríguez-de Lara, 2019; World Health Organization, 2002).

Temperature data were fit to Beta General and Logistic distributions (Table 1). Data provided as probability distributions make it possible to observe the variability of parameters influencing microbial counts and their probability of occurrence (Membré & Guillou, 2016; Valdramidis, 2016). This information is important to develop precise QMRA, since time and temperature data influence microbial growth. Setting these parameters as single-point 'worst-cases' may overestimate the likelihood of exposure to unacceptable numbers of pathogenic microorganisms. In addition, because most studies of storage at processing plants and distribution to the retailer are performed by the broiler industry or retailers, the complete time and temperature record of foodstuff is not fully known (Adams & Moss, 2008; Maldonado-Simán et al., 2019; A. Roccato et al., 2015; World Health Organization, 2002).

3.2. Predictive growth of foodborne pathogens

There are several factors implicated in increasing pathogen counts across the chicken meat chain, especially time and temperature conditions. So, this study predicted the behavior of foodborne pathogens from the slaughter industry to consumption of chicken meat at home or at food services in Brazil. The change in numbers of foodborne pathogens was estimated at each module from the chiller, to inside slaughterhouses, to storage in homes or until thawing at food services. The prediction of growth was carried out until these steps because cooking was assumed as adequate and able to eliminate vegetative pathogens.

In Scenario 1, according to the COMBASE model, at all steps, including slaughter, transport from the slaughterhouse to the distribution center, and storage in the distribution center, there was no growth of *C. perfringens, E. coli, Salmonella spp.,* or *L. monocytogenes* (log CFU/g <0.009) (Table 2). This result corroborates the observed maintenance of the cold chain at the first modules of the food chain and previous studies (Kusumaningrum, Van Asselt, Beumer, & Zwietering, 2016; Anna Roccato et al., 2017; World Health Organization, 2002).

During retail storage, the average temperature was 2.05 ± 3.12 ºC, and the time ranged from 0 to 120 h (Table 1). At this stage, a slight increase was observed in *L. monocytogenes* (Table 2), which can be explained by the psychrotrophic

behavior of this microorganism. Although higher temperatures were observed at slaughterhouses and during transport from slaughterhouses to distribution centers, no growth was predicted due to the short time. This fact illustrates the necessity of controlling the time and temperature binomial. According to COMBASE, all pathogens were able to grow during distribution at retail outlets, during transport from retail stores to home, and during home storage. At these modules, pathogen loads varied from 0.01 log CFU/g to 1.45 log CFU/g, and in retail distribution, the highest growth was observed for all pathogens analyzed. This result is related to breaches in the chilled chain in retail distribution, where the average temperature was 5.83 ± 9.58 ºC. Indeed, temperature abuse has been reported to occur during transportation, retail storage, and retail display of food products (Ndraha et al., 2018). At the end of the chilled chicken meat chain, *C. perfringens* achieved the highest counts, followed by *E. coli*, *Salmonella* spp., and *L. monocytogenes* (2.30, 1.99, 1.87, and 0.11 log CFU/g, respectively).

In the Scenario 2, which considered the frozen chicken meat chain, bacterial growth was observed only at thawing in food services. This result was already expected, since, normally, foodborne pathogens start to grow at 4.4 ºC, and temperatures reported for thawing were 5.04 ± 8.04 ºC (Forsythe, 2013). *E. coli* showed the highest concentration, followed by *Salmonella* spp., *C. perfringens,* and *L. monocytogenes* (1.34, 1.19, 1.08, and 0.07 log CFU/g, respectively). These results emphasize the importance of controlling the thawing procedure, which, if not controlled can allow microbial multiplication and also has been reported as high risk practice for cross-contamination (Katiyo, de Kock, Coorey, & Buys, 2019).

Inadequate handling practices of chicken meat by consumers play an important role in foodborne outbreaks, and some recommendations to avoid it are 1. not washing raw chicken before cooking in order to avoid the spread of bacteria on the kitchen surfaces, favoring cross-contamination; 2. Only thawing raw chicken meat at temperatures <5 ºC, or using thermal processing for thawing (e.g. use of a microwave or stove) if the food will be eat immediately (Carrasco, Morales-Rueda, & García-Gimeno, 2012; SEVS, 2009). Thawing chicken meat in water or running water should be avoided in order to avoid cross-contamination.

Differences among pathogen loads were observed in some modules analyzed in the chicken meat chain (Table 2). As expected, pathogen loads were higher in the chilled chain than in the frozen chain; this may be explained by the ability of some

pathogens to grow at refrigerated temperatures but not at frozen temperatures. Further, chilled food (-1 °C to 8 °C) has been shown to be more sensitive to temperature variation than frozen food (Aung & Chang, 2014; Kuo & Chen, 2010).

A comparison of data reported by Silva et al. (2008) with those observed by us revealed that chicken meat storage temperatures inside homes were higher than those observed during storage in food services. The high temperatures observed inside domestic refrigerators indicate that substantial improvements to consumer practices are required to improve perishable food preservation and reduce food safety risks (Mercier et al., 2017). The good hygienic practices and sanitary surveillance programs followed by food services generally result in better controls of time and temperature compared with those inside private homes. At home, consumers prepare foods according to their own culture and knowledge, and sanitary surveillance services are not allowed to impose sanitary laws. Corroborating this fact, during the last decade, most foodborne outbreaks registered in Brazil occurred inside private homes (BRASIL, 2019).

The growth potential (δ) of a microorganism on food is defined as the measure of the difference between the final population of a given organism at the final shelf-life of a determined food and its initial population. When δ values are negative or lower than 0.50 log CFU/g, it is concluded that microorganisms are not able to grow on this food (Sant'Ana et al., 2012). Considering the real scenarios observed in the entire chilled and frozen chains, *L. monocytogenes* was the only microorganism that presented a final level of growth under 0.50 log CFU/g (Table 2), even though this pathogen was the first to start growing in retail storage according to the COMBASE model results. The other pathogens demonstrated final growth levels of *E. coli* and *Salmonella* spp. during exposure in retail (1.34 and 1.31 log CFU/g, respectively) that were higher than 0.5 log CFU/g.

Salmonella is one of the most common foodborne pathogens worldwide, including in Brazil (BRASIL, 2019; EFSA, 2018; Nguyen et al., 2015; WHO, 2018). Despite regulatory programs intended to reduce the prevalence of this pathogen in chicken meat, this kind of food remains an important source of salmonellosis. Predictive modeling in food microbiology uses mathematical equations to describe biological processes and, once quantitative models are available, these models can be used to quantitatively describe and compare observations, such as kinetic data (Zwietering & den Besten, 2011). Thus, in our research, we also compared the

behavior of *Salmonella* spp. in the chicken chain with predictive growth models by Oscar (2007) and Juneja et al. (2007), available in the research literature (BRASIL, 2019; CDC, 2019).

The COMBASE model showed *Salmonella* spp. growth during retail distribution, transport from retail to home, and storage at home and in food service modules (Table 2), while the Oscar (2007) model demonstrated that *Salmonella* spp. was able to grow in more modules, i.e., storage at distribution centers and storage in retail stores. While COMBASE demonstrated an increase in *Salmonella* of 1.87 log CFU/g, the Oscar model demonstrated 0.71 log CFU/g (Table 2). However, no *Salmonella* growth was demonstrated by the use of the Juneja model (2007). Since considerable differences were found in the predicted growth of pathogens depending on the model used, care should be taken when choosing the model, in order to use the one that is most appropriate for each reality.

4. Limitations and perspectives

One of the major limitations of this study was to access different stakeholders of chicken meat chain in order to access data of time and temperatures. Beyond that, even access these data, they represent only a sample of the reality of the very big chicken chain production inside a continental country as Brazil. Other limitation of our study was that time of several modules were not registered by companies or other stakeholders, forcing us to obtain this information from expert opinions. Even considering the high experience of these professionals, the data obtained from them also could not represent the whole reality of time of some modules of chicken meat chain. Finally, the perspective of the present study is to use the data presented here in quantitative microbial risk assessments concerning the consumption of chicken meat.

5. Conclusion

Despite some breaches, the time and temperature data presented in this study were in compliance with the recommended parameters established by Brazilian legislation, especially in the first modules of the chicken production chain. Beta General and Logistic distributions were the most appropriate fit to temperature data, and the Pert distribution was chosen for time data.

Pathogen growth was not observed under slaughter industry and transport conditions, while slight growth was observed from storage in retail stores to home storage, depending on the model used to predict growth. All pathogens achieved a higher microbial load at the final level in homes than in food services. Considering to whole chilled chain, *L. monocytogenes* reached the lowest counts when compared to the growth of the other pathogens analyzed, although this microorganism was the first to start growing in retail storage. *C. perfringens* reached the highest counts in the chilled chain, while *E. coli* was the microorganism that multiplied better in the frozen chain. *Salmonella* spp. growth was expressly different when different models were used. Thus, care should be taken when choosing a predictive microbial model, in order to use the most suitable one for each reality. To the best of our knowledge, this study is the largest survey about chicken meat storage time and temperature performed in Brazil. On this basis, the data provided here can be useful to predict microbial growth, survival, or both, in stochastic risk assessments or as a basis for probability distributions of time and temperature data in stochastic modeling.

6. Authors' Contributions

In this research, all authors contributed effectively. Claudia Titze Hessel conducted the data collection, organization, analysis, interpretation and wrote the manuscript. Cris Rocha Pinto Magalhães, Mateus Silva de Lima and Leonardo Werlang Isolan contributed in collection data. João Pedro Pessoa, Susana de Oliveira Elias and Elis Regina Gomes Alfama contributed in statistically analysis. data interpretation, results discussion and in the writing process. Eduardo Cesar Tondo supervised the project and revised the manuscript.

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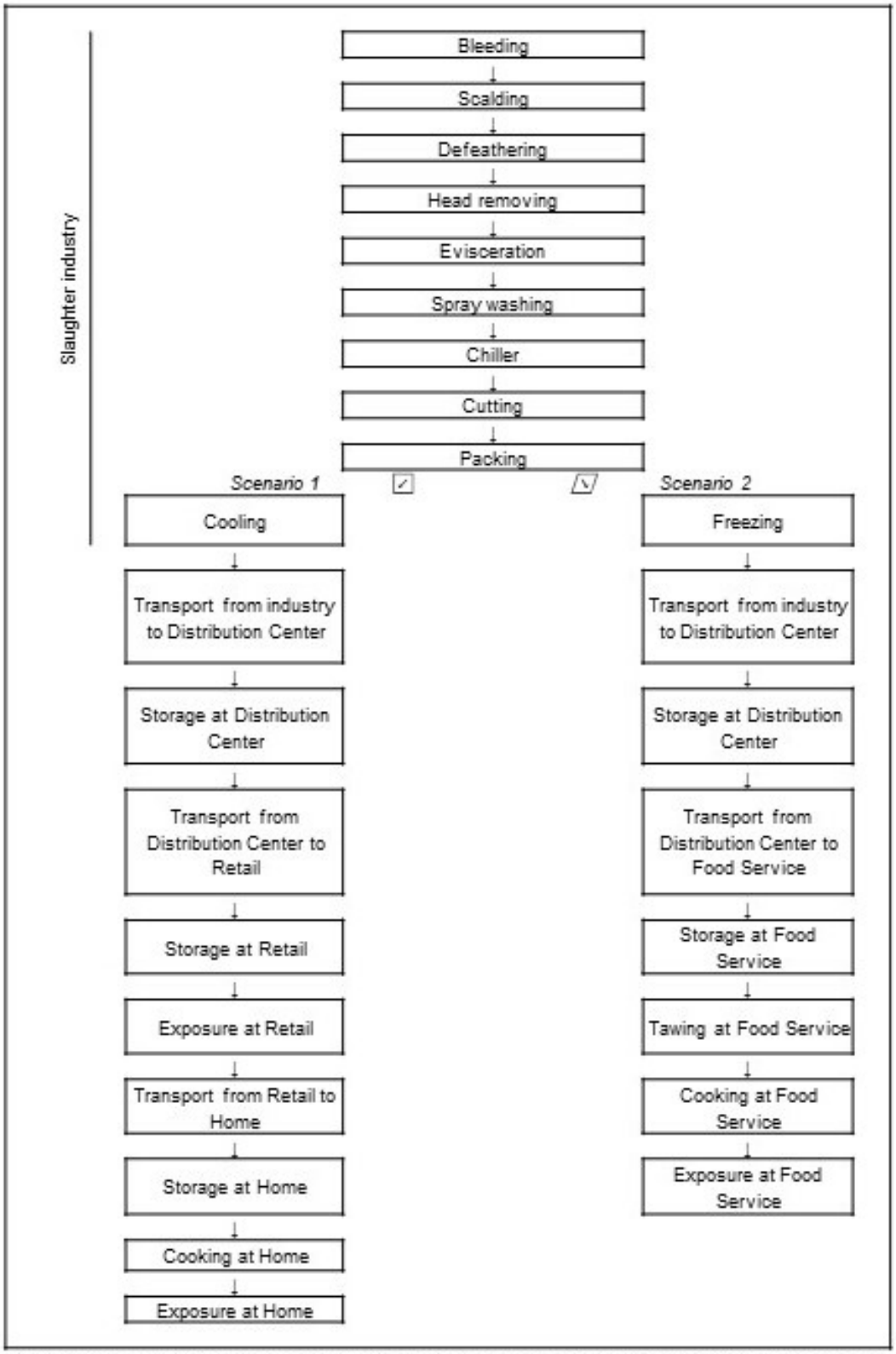

Figure 1: Flowchart of chicken meat modules on two scenarios identified: 1) chilled chain for chicken meat sold at retail and consumed at home; 2) frozen chain for chicken consumed in food services.

Table 1: Time and temperature data and statistical parameters obtained by distribution fitting of chicken meat modules.

	Local	Module	n	Temperature				Distribution according to @Risk	Time				
				Minimum (ºC)	Maximum (ºC)	Median (ºC)	Average±SD (ºC)		n	Minimum (h)	Maximum (h)	Median (h)	Average±SD (h)
Scenario 1: chilled chain	Slaughter industry	Bleeding	EO	20.86	30.00	24.02	NA	NA	7	0.002	0.08	0.05	0.05 ± 0.02
		Scalding	EO	50.00	60.00	55.00	NA	NA	7	0.002	0.03	0.02	0.02 ± 0.01
		Defeathering	EO	30.00	58.00	45.00	NA	NA	7	0.004	0.50	0.02	0.09 ± 0.18
		Head removing	EO	30.00	58.00	45.00	NA	NA	EO	0.004	0.50	0.02	NA
		Evisceration	EO	35.00	50.00	45.00	NA	NA	7	0.13	0.17	0.17	0.17 ± 0.17
		Spray washing	EO	0.00	16.00	10.00	NA	NA	EO	0.008	0.016	0.012	NA
		Chiller	1331	0.00	7.90	1.80	2.00 ± 1.14	BetaGeneral	12	0.33	1.17	0.50	0.60 ± 0.27
		Cutting	1331	1.20	12.40	10.50	10.52 ± 0.54	BetaGeneral	12	0.0039	0.67	0.09	0.17 ± 0.21
		Packing	1331	-6.00	22.80	2.80	2.70 ± 1.31	Weibull	12	0.0028	16	0.01	1.29 ± 3.68
		Cooling storage	2604	-6.00	22.80	2.60	2.51 ± 1.65	Logistic	EO	0.00	72.00	12.00	NA
		Transport from industry to Distribution Center	1648	-3.40	12.40	3.30	2.71 ± 1.97	ChiSquare	58	0.00	8.50	0.83	2.00 ± 2.35
	Distribution Center	Storage	52	-8.70	10.80	2.60	1.64 ± 3.46	Logistic	EO	1.00	48.00	12.00	NA
		Transport from Distribution Center to Retail	40205	-14.30	24.70	0.30	1.00 ± 5.25	Normal	58	0.08	8.50	0.83	2.00 ± 2.35
	Retail	Storage	888	-9.00	10.40	1.70	2.05 ± 3.12	Normal	EO	0.00	120.00	48.00	NA
		Exposure	1701	-20.00	25.00	4.15	5.83 ± 9.58	Lognorm	EO	0.00	120.00	48.00	NA
	Home	Storage	996	1.00	6.00	3.00	NA	Pert	988	0.00	48.00	24.00	NA
		Exposure	996	20.86	29.81	24.02	25.67 ± 3.27	BetaGeneral	988	0.00	2.00	0.60	NA
Scenario 2: frozen chain	Slaughter industry*	Freeze Storage	1331	-35.10	-14.50	-24.10	-23.56 ± 2.89	Weibull	EO	0.00	48.00	24.00	NA
		Tawing	337	-18.50	35.00	4.60	5.04 ± 8.04	Logistic	EO	0.00	24	4.00	NA
		Cooking	221	8.10	108.10	86.60	85.79 ± 8.30	Logistic	EO	0.08	2.00	0.50	NA
	Food Service	Exposure	1054	50.00	150.10	89.60	89.38 ± 12.27	ChiSquare	EO	0.00	6.00	2.00	NA

*Slaughter modules until packing are represented at Scenario 1. NA = not avaiable. EO = expert opinion.

Table 2: Predictive growth of foodborne pathogens at modules of chicken meat chain.

Scenario 1: Chilled chain*								
Pathogen	**Model**	**Storage at Distribution Center (log CFU/g)**	**Transport from Distribution Center to Retail (log CFU/g)**	**Storage at Retail (log CFU/g)**	**Exposure at Retail (log CFU/g)**	**Transport from Retail to Home (log CFU/g)**	**Home storage (log CFU/g)**	**Final level (log CFU/g)**
Clostridium perfringens	COMBASE	NG	NG	NG	1.21	0.64	0.54	2.30
Escherichia coli	COMBASE	NG	NG	NG	1.45	0.47	0.05	1.99
Listeria monocytogenes	COMBASE	NG	NG	0.01	0.08	0.02	0.01	0.11
Salmonella spp.	COMBASE	NG	NG	NG	1.31	0.53	0.02	1.87
Salmonella spp.	Oscar (2008)	0.04	NG	0.14	0.32	0.10	0.08	0.71
Salmonella spp.	Juneja et al. (2007)	NG	NG	NG	NG	NG	NG	NG

Scenario 2: Frozen chain*						
Pathogen	**Model**	**Storage at Distribution Center (log CFU/g)**	**Transport from Distribution Center to Food Service (log CFU/g)**	**Storage at Food Service (log CFU/g)**	**Tawing at Food Service (log CFU/g)**	**Final level (log CFU/g)**
Clostridium perfringens	COMBASE	NG	NG	NG	1.08	1.08
Escherichia coli	COMBASE	NG	NG	NG	1.34	1.34
Listeria monocytogenes	COMBASE	NG	NG	NG	0.07	0.07
Salmonella spp.	COMBASE	NG	NG	NG	1.19	1.19
Salmonella spp.	Oscar (2008)	0.04	NG	NG	0.46	0.49
Salmonella spp.	Juneja et al. (2007)	NG	NG	NG	NG	NG

* It was estimated the change in numbers of foodborne pathogens at each module from the time of chiller, in slaughter industry, until the time before preparation in homes or food services. Growth is expressed as log CFU/g; NG = no growth observed (log CFU/g < 0.009).

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Food safety behavior and handling practices during purchase, preparation, storage and consumption of chicken meat and eggs

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Abstract

The aim of this study was to assess the risk of occurrence of a foodborne outbreak and point practices determinant to achieve high Good Hygienic Practice level during handling practice from purchase to consumption of chicken meat and eggs. The risk

behavior of respondents and the risk of the occurrence of a foodborne outbreak were measured using Weighted Harmonic Outbreak Prevention Index (WHOPI). WHOPI were not correlated to socioeconomic data and perception of risks. Different profiles of handling practices were identified inside each WHOPI level. Chicken meat defrost, time and temperature of egg cooking and the point of yolk were identified as the most important procedures responsible for the WHOPI level upgrades. The consumption of chicken meat and eggs were characterized as discrete distributions. The average consumption of chicken meat was 113.48 grams/per day and eggs daily intake distribution was 0.92 units/day. Our results can be applied for future microbiological food safety risk assessments related to the consumption of chicken meat and eggs.

Keywords: Food safety; Food preparation; Good hygienic practices; Probability distribution; Food safety assessment.

1. Introduction

Foodborne outbreaks are continuing causing serious economic losses and sickness and poultry products, such as chicken meat and eggs, are among the most often implicated in foodborne outbreaks around the world. However, the consumption of these foods are an important source of protein at global level and its demand are expected to continue increasing in the incoming years (ABPA, 2017; CDC, 2014, 2018; Chai, Cole, Nisler, & Mahon, 2017; Crowe et al., 2017; Dallman et al., 2016; EFSA, 2018; MS, 2019; Nguyen et al., 2015; World Health Organization, 2015).

Consumer handling practices play an important role in foodborne diseases in the food supply chain. Improper practices at domestic kitchen involving time and temperature aspects and cross-contamination between food handlers, equipment and utensils are the main responsible of foodborne outbreaks (Beumer et al., 1998; da Cunha, Stedefeldt, & de Rosso, 2014; Evans et al., 1998; FDA, 2009; Gonçalves-Tenório, Silva, Rodrigues, Cadavez, & Gonzales-Barron, 2018; Kusumaningrum, Van Asselt, Beumer, & Zwietering, 2016; G. C. Lima, Loiko, Casarin, & Tondo, 2013; Murray et al., 2017; Ndraha, Hsiao, Vlajic, Yang, & Lin, 2018; Omari, Frempong, & Arthur, 2018; Todd, Greig, Bartleson, & Michaels, 2009)). Thus, this data highlight that food handlers are facing difficulties in controlling hazards.

Access the main difficulties to promote food safety at consumer level is essential to promote public health. Regulatory bodies and food industry professionals

can use consumer behavior information when creating targeted control strategies along the farm-to-plate chain. In addition, this information can be applied in microbiological food safety risk assessments and drive risk communication strategies for the different group profiles (Carrasco, Morales-Rueda, & García-Gimeno, 2012; CDC, 2014; Delbeke et al., 2015; Hoelzer, Pouillot, Egan, & Dennis, 2012; World Health Organisation, 2002).

Several studies were conducted across the world to identify safety risks associated with practices and knowledge of consumers while handling chicken meat and eggs (Al-Sakkaf, 2015; Bearth, Cousin, & Siegrist, 2014; Katiyo, de Kock, Coorey, & Buys, 2019; Murray et al., 2017; Trafialek et al., 2018) However, knowledge about the food safety behavior and handling practices during purchase, preparation, storage and consumption of chicken meat and eggs in Brazil is still limited and there are not accurate data about consumer phase in the foodchain of this products. Thus, the objective of this research is to assess the risk of occurrence of a foodborne outbreak and point practices determinant to achieve high GHP level during handling practice from purchase to consumption of chicken meat and eggs.

2. Materials and Methods

2.1 Questionnaire

The questionnaire was constructed regarding handling practices of chicken meat and eggs during purchase, storage, preparation practices, and consumption. Questions about Good Hygienic Practice (GHP) during food preparation and perception of risks were also included. A draft of the survey was developed using research articles and relevant questions considering the topic were included (da Cunha, Braga, Passos, Stedefeldt, & de Rosso, 2015; Elias, Tomasco, Alvarenga, Sant'Ana, & Tondo, 2015; Jacxsens et al., 2015; Kennedy et al., 2005). After the draft revision, the questionnaire suitability and applicability were verified by several food professionals and a pilot-test was performed with 10 persons conveniently chosen from different socioeconomic profiles of the Southern Brazil (gender, age, degree of instruction and family income). People were instructed to check the understanding and the wording of sentences. After the adjustments, the final version consists in 61 questions, which were approved by the Ethics Committee from the Federal University of Rio Grande do Sul. Once approved, the questionnaire was

announced by social media and *e-mail* lists and sent by GoogleForms® link to different common consumers along the entire Brazil during March and April 2018. People who consent to participate in the survey, received the questionnaire, fulfilled it and returned on-line (see Supplementary material).

The questionnaire was composed by four sections: 1) Socioeconomic data, 2) Data regarding chicken meat, 3) Data regarding eggs and 4) Data about GHP and perception of risks.

The socioeconomic data section included questions about gender, age, family income, educational level and Brazilian macro-region of residence. Information was used to verify the sample heterogeneity of the population interviewed.

The second and third sections concerned about the behavior habits of chicken meat and eggs, respectively. These two sections were divided in subsections: purchase, preparation consume and reuse. It was also asked about the practice of purchasing frozen chicken meat, in order to analyze the procedure of defrosting. The same question was not done to eggs because eggs are not sale frozen in Brazil (See Supplementary material). Initially, the participants were asked if they have the habit, i.e. “Do you eat chicken meat?”. In case of affirmative response, the subsequent questions were regarding time, temperature and Good Hygiene Practices (GHP) (Fourth section). In case of negative response, the participant was drove to the next section, i.e. “Do you buy chicken meat?”.

2.2 Socioeconomic data

Table 4 shows the information on socioeconomic data of respondents and consumption practices of chicken meat and eggs. The majority of respondents were female (82.5%), belonging to the age group 25–34 years (38%), were graduated (56.2%) and declared family income from 2 to 3 minimum wages (26.87% and 26.22%, respectively). Macro-region of most respondents was Southern Brazil (62.37%), followed by Northeast region (23.17%), Southeast (7.56%), Midwest (3.27%) and North (2.46%).

WHOPI for handling chicken and eggs were not correlated to socioeconomic questions (gender, age, family income, educational level and macro region of residence, $p > 0.005$), even that most of the respondents were female and had high-level of education.

Socioeconomic profile, consumers' practices and food safety behavior are not linearly corelated. Some authors found that gender, age, level education and region of residence influence in food safety aware and GHP level inside domestic kitchens (Agudo et al., 2002; Liu & Niyongira, 2017; Odeyemi et al., 2019; Planzer et al., 2009; Yngve et al., 2005; Zeeshan et al., 2017). On the other hand, other authors found the opposite (Katiyo et al., 2019; Omari et al., 2018); i.e. Katiyo et al. (2019) reports that in South Africa women reported following more safe practices on handling chicken than men even their knowledge levels were similar. This inconsistence might be related to the complexity of human behavior, which involves the risk perception, the habitus and optimistic bias of food handler and the absence of translation of knowledge into attitudes/practices (Al-Sakkaf, 2015; Bearth et al., 2014; de Freitas, da Cunha, & Stedefeldt, 2019; Katiyo et al., 2019; Zanin, da Cunha, de Rosso, Capriles, & Stedefeldt, 2017).

2.3 Data analysis

The questionnaire responses were analyzed using Microsoft Excel® 2013. There were no missing data, since the questionnaire was answered online. Only five responses were excluded due to incoherent information.

In order to measure the risk behavior of respondents and the risk of occurrence of a foodborne outbreak the Weighted Harmonic Outbreak Prevention Index (WHOPI) (Elias et al., 2015) was adopted. This tool was chosen because with WHOPI it is possible to measure practices and behavior attributing weights for features according to their contribution to increase the risk.

WHOPI is based on the weighted harmonic mean with some modifications. Questions about socioeconomic data were not used in WHOPI, which was composed by 26 questions related to behavior and handling practices. Questions about time, temperature, binomial time-temperature and GHP, were classified as “conforming” or “non-conforming” (Tables 1, 2, 3). In order to feed WHOPI, the value 4 was attributed to answers “conforming” and value 0 was attributed when the response was “non-conforming”. For combined responses to the questions regarding the binomial time-temperature, conform was attributed only when both conditions were conforming (time and temperature). In addition, each feature also receives weights according its contribution to outbreak prevention questions. The WHOPI calculation resulting

values were classified in three levels of GHP and preventive actions against foodborne outbreaks (Table 3). The WHOPI was obtained by Equation 1 (Eq.1).

$$WHOPI = \frac{\sum_{i=1}^{N} w_i}{\sum_{i=1}^{N} \frac{w_i}{x_i + 1}} - 1$$

where:

xi response of i question of the questionnaire;

wi=weight of i question;

questions regarding features time, temperature, binomial time-temperature ⇒ wi=4;

questions regarding feature good practices ⇒ wi=1;

N = number of question.

The data was also statistically analyzed using the Statistical Package for Social Sciences (SPSS version 21.0, Chicago, IL), adopting the significance level of 5%. Descriptive statistic was performed. Tukey-test or Pearson's chi-square test were used to rank correlation and relationship between the variables.

3. Results and Discussion

In the present study, 1217 records were collected and analyzed in order to assess the handling practices, behaviors and measure the risk of occurrence of a foodborne outbreak due to chicken meat and eggs. The results were presented initially by the socioeconomic data, following by behavior and practices on handling chicken and eggs based on WHOPI. Then behavior and practices on handling only chicken meat were analyzed and presented separated from eggs. Finally, the consumption data of chicken meat and eggs and its application in Risk Assessments were discussed.

3.1 Behavior and practices on handling chicken and eggs

In this study the WHOPI was used in order to assess the risk behavior of respondents and the risk of an outbreak occurring through their practices while handling poultry products and cooking at home. There is a lack of methods which enable estimation of the risk, then elaborate a method based on mathematical formula including the most important hazard factors can be helpful (Trafialek & Kolanowski, 2014). Several authors also developed methods to calculate risk based

on ranking questionnaire answers and building formula (Gizaw, Gebrehiwot, Teka, & Molla, 2014; Katiyo et al., 2019; Trafialek et al., 2018).

Table 5 shows the WHOPI values and classification according to the conformity level for chicken meat and eggs and by the four controls considered for the WHOPI score – time, temperature, binomial time-temperature and GHP.

The average value of WHOPI for handling chicken and eggs was 0.442 ± 0.175, belonging to the medium group. In general, the best controls practiced were related to time, following by binomial time-temperature, GHP and temperature (Table 5). These results are important because the ince the violation of time and temperature and poor GHP are some of the most important factors resulting in foodborne illnesses (Roccato et al., 2015). In addition, information regard GHP adopted inside domestic kitchen are useful for food safety programs education focused at consumer level aiming to raise consumer awareness of the risks of cross-contamination in homes and their role in its prevention. Important practices to avoid cross-contamination while cooking is washing hands, the use of different utensils for raw and ready to eat foods and properly wash and disinfect surfaces between the preparation of raw and cooked foods. Our results showed that 90.70% of respondents declared to always washing hands before, during and after cooking and 9% of them only sometimes. This result was similar between all groups regardless the WHOPI level (Chi-square test, $p > 0.05$ for all of them) (Table 6). Regarding the use of different utensils to prepare different foods or wash them during preparation, 76.50% of respondents declared to did it, while 23.40% used the same utensil for different preparations without washing them. Proper washing of cooking utensils between preparation of raw and cooked foods or ready-to-eat foods is pointed as the result to eliminate the cross-contamination route inside kitchen (Kusumaningrum et al., 2016).

In the question about the main criteria chosen before consumption of foods stored in the refrigerators, 77.61% of respondents checked more than one factor presented (smell, taste, appearance, storage time and expiration date). The observation of storage time and expiration data were considered an adequate criteria in order to prevent foodborne diseases, while smelling, tasting and observe the appearance of food were not, once pathogens can be in foods and not modify food sensorial characteristics. Only 13.22% of respondents have chosen the adequate

criteria, being that this was most misbehaved GHP practice declared by all respondents.

Regarding the reading of food labels, 47.09% of respondents declared to read information most of time, while 38.80% declared to do it always, which was considered adequate. Half of respondents declared to be very interested in food topics (51%), and also declared to access various sources of information about the topics (48.20%). The practice of reading and understanding food labels are important to improve food safety (FDA, 2011; WHO, 2017).

Finally, perception questions were not correlated to high WHOPI levels since the same trend of response was observed for the three levels group (Chi-square test, $p > 0.05$ for all of them). Regarding risk perception related to chicken meat, 63.60% of respondents believed that its consumption posed risk to consumer health. Corroborating this result, more than ten combinations of hazards were identified as, and, for this reason, the Table 6 shows only those with percentage higher than 5.0 %. Microorganisms (bacteria, fungi, parasites) were the main hazards identified by consumers as related to chicken meat (15.40%) and the top factors together were microorganisms, antibiotics and hormones (13.20%). Specifically for eggs, 66.20% of respondents believed that the consumption of eggs may affect consumer health. Among the hazards described, also a great quantity of hazards combinations was associated to the eggs consumption, and, between those, microorganisms were the principal (36.90%), followed by microorganisms and toxins (12.10%). No one respondent considered pesticides as a hazard in eggs (Table 6). The fact of respondents linked as a hazard of egg consumption the presence of *Salmonella* is positive, since this pathogen is highly associated of foodborne outbreaks due to the egg consumption (CDC, 2018; WHO, 2015).

Even though all those hazards identified demonstrated an expressive percentage of respondents with perception of risks, this seems to be not enough to change attitudes or practices inside kitchen, because the WHOPI level related to GHP was low and there was no correlation between the perception of risks and GHP. This pattern was already demonstrated by da Cunha et al. (2015) which correlated the difference between consumer own risk perceptions and tendency of an optimistic bias in food handlers - a positive outlook regarding future events, in which individuals find themselves less likely than others to experience negative events. Thus, food handlers perceived themselves as less likely than their peers to cause a foodborne

disease demonstrating the tendency of an optimistic bias. In addition, Zanin et al. (2017) showed that in the last decade, several studies were conducted in order to assess knowledge, attitudes and practices of food handlers in different sectors, so as to understand their behaviors and relate them to the causes of foodborne diseases and concluded that there is no translation of knowledge into attitudes/practices or attitudes into practices after food handlers training.

Based on WHOPI classification it was possible to characterize consumers in three behavior profiles, namely: low, medium and high (Figure 1). 11.1 % (n = 136) of respondents showed high level of GHP, 53.2 % (n = 652) medium and 35.7% (n = 438) low. Similar profile was observed in South Africa by Katiyo et al. (2019), where most consumers of chicken meat present moderate or poor practices (62%) and had moderate or poor knowledge levels (72%). Poultry products consumers are generally aware of the contamination of raw poultry meat with pathogenic bacteria. However, awareness did not necessarily imply absolute safety during preparation (Bearth et al., 2014).

Persons belonged to low WHOPI level showed deficiency in practices related to controls of time and temperature and GHP. Only 45% of person that belonged to low WHOPI level uses to control time during the transport of chilled chicken meat after purchase until home, doing it in less than 30 minutes. Even considering that this period generally do not promote significant microbial multiplication, the low concern about control of time is worrisome. In addition, persons with low WHOPI level exposes chicken and eggs prepared on table for longer than 30 minutes (77.70% and 22%, respectively). On practices related to time-temperature control, 77% of low WHOPI level respondents defrost chicken meat under water or longer than 31 minutes at room temperature, thus only 33% declared to do it properly (under refrigeration, cooking directly or in the microwave). After prepared until consumption of chicken, 60.60% of low WHOPI person maintain it at room temperature for longer than 31 minutes. On temperature control issues these persons showed improper practices on how prepare eggs and at the point of egg consumption. Only 0.9% declared to prepare eggs with cooked yolk and 45.70% consume egg with completely cooked yolk. Finally, only 8% of low WHOPI people used to consider storage time and expiration date before consuming a stored food in the refrigerator, 8% read information of shelf-life and storage conditions in food labels always before consumption and 52.30% and 57.80% did not read expiration date and observed the

Ministry of Agriculture (SIF) seal proving the correct inspection of chicken and eggs, respectively.

Looking into the medium WHOPI level, the majority of persons belonging to this group achieved adequate practices related the control of time, temperature, binomial time-temperature and GHP. Specifically to the time control, 66% of medium WHOPI level persons stored chicken meat under refrigeration in less than 30 minutes after purchase, 99.50% store it under refrigeration until 48 hours before cooking and after cooked 64.90% placed the chicken meat on table for 30 minutes maximum. In addition, most of medium WHOPI level placed chicken meat and eggs already cooked under safe binomial time-temperature conditions before consumption (55.30% and 93.70%, respectively). For defrost procedure 46% of medium WHOPI person did it properly. In relation to temperature control, most of persons belonging to medium WHOPI level declared to consume egg yolk cooked (55.20%). Finally, 13.70% of medium WHOPI level persons used to consider storage time and expiration date before consuming stored foods in the refrigerator, 58.80% read expiration date and the Ministry of Agriculture (SIF) seal on the packaging of chicken, 55.50% read the same information for eggs and 88.30% stored chicken meat capped before be reused.

High WHOPI level persons declared to have considerably better attitudes in GHP practices than other groups and achieved correct handling practices in up to 90% of most of questions. 61% of high WHOPI persons spent less than 30 minutes from the place of chicken meat purchase to refrigeration and all of them storage the chicken before cooking for less than 48 hours under adequate temperatures. For eggs, 91.20% of high WHOPI persons storage properly eggs before preparation, doing it until 30 days after purchased. 93.70% and 100% of high WHOPI persons declared to do it until 30 minutes, respectively. High WHOPI level profiles also shows high achievement. 90% and 100% high WHOPI level person did it correct. 80.70% of persons with high WHOPI level defrost chicken meat correctly. 99.20% of person belonged to this group ate eggs with cooked yolk and 32% cooked egg until obtain cooked yolk. Among GHP practices despite high WHOPI level person obtain high percentages in questions from this group, there were no statistical differences between WHOPI scores from the medium WHOPI level group (Tukey-T test, $p = 0.75$). For the main criteria considered before consumption of foods storage in refrigerator 27.90% of high WHOPI level chose the right criteria and most of persons

read expiration date and the Ministry of Agriculture (SIF) seal on the packaging of chicken and eggs (60.20% and 64.40%, respectively).

The results obtained by the present study accessed the in-house practices in relation to the handling of chicken meat and eggs from purchase to consumption. This information about food safety behavior sometimes are unknown by risk assessors and food industries and are important for food business management and public policy stakeholders, since most foodborne outbreaks occurs due to inadequate food handling (de Freitas et al., 2019; M. S. de Lima, Isolan, Hessel, Pessoa, & Tondo, 2018; Katiyo et al., 2019; G. C. Lima et al., 2013; Murray et al., 2017; Planzer et al., 2009; Todd et al., 2009; Tran-Thi et al., 2017).

In this issue, this research also tested the impact of changes in GHP practices on the upgrade of WHOPI level based on the food handler behavior characterization performed. To simulate changes in behavior, for each question and each control non-conforming practices were changed to conform practices. The results obtained were analyzed as their impact by percentage of persons upgraded in WHOPI level. By this evaluation it was possible to identify the practices that most contribute to the upgrade in the WHOPI level.

For chicken meat, only changing the defrost procedure from a non-conforming to a conforming way the upgrade from medium to high WHOPI level was of 17.35% and also upgraded most of respondents to the high level of GHP (54.32%). In addition, just by comply one control (independently of which) there was no respondent at low WHOPI level anymore. Finally, the ranking of controls which when adopted most contribute to upgrade in WHOPI level for chicken meat were the binomial time-temperature > time > GHP.

For eggs, it was important to upgrade WHOPI level change the way of cooking eggs and point of yolk consumption. Changing those practices lonely would upgrade respondents from medium to high WHOPI level in 39.80% and 15.40%, respectively, while its combination would raise 87.60% of respondents to the high WHOPI group. The controls that most contributed to the upgrade in WHOPI levels for eggs by ranking were temperature > GHP > binomial time-temperature > time. However, for eggs, comply all practices from a specific control were not enough to eliminates the low WHOPI level.

Thus, it was observed that for chicken meat and eggs combined it was more determinant to upgrade in WHOPI level, the following factors, by order, temperature,

binomial time-temperature, time and GHP. Comply solely one of those controls did not eliminates low WHOPI level group. Besides, comply two controls distribute respondents between medium and high WHOPI level and by complying three raises all food handlers to high WHOPI level.

It was observed that upgrades in WHOPI level reflects in produce food safer and with lower risks to foodborne outbreaks. Thus, focusing on the main non-conforming practiced observed in this research and which one upgrade easily the WHOPI level, is recommended that education program followed this order to optimize results in food safety compliance behavior: temperature, binomial time-temperature, time and GHP.

3.2 Behavior and practices on handling chicken meat

The habits practiced from purchase to consuming the chicken meat are presented in Table 7. In general, 81.20% of respondents bought chilled chicken meat and, among them, less than 2% bought chilled chicken at street fairs or directly from producers. The most common place chosen to buy chicken was the supermarkets or butcheries (98.60%). At the time of purchasing, 45.50% check the information about the product. After buying chilled chicken meat, the main behavior observed was transport meat during less than 30 minutes until home where meat was storage under refrigeration (72.30%). The order frequent behavior was transport chicken meat in less than 1 hour (25.50%) until home storage. No predominant behavior was identified at home, concerning the time between storage to cooking. A broad spectrum of behavior was observed, varying to 1 hour to more than 48 hours of refrigerated storage. The majority of respondents prepared roasted or fried chicken meat (90.40%) with some kind of sauce (83%). Actually, in this study, the chicken meat was considered as always cooked, since in Brazil people do not consume raw chicken. Once prepared, chicken meat was exposed to room temperature before serving by less than 30 minutes up to 1 hour (47.90% and 42.30%, respectively). When served, the chicken meat stayed from less than 30 minutes up to 1 hour at room temperature (42.10% and 38.80%, respectively). Most respondents (92.60%) stated reusing chicken meat after refrigerated storage (91.10%) in a capped container (97%), for less than 24 hours (68.4%). The consumption of reused chicken meat was mostly, at once (66.10%). Furthermore, 80.90% of the respondents bought frozen chicken meat. In relation to the defrost procedure, nearly half of the

respondents used microwaves or refrigerators to defrost chicken meat (49.20%), besides 32.10% did it at room temperature and 13.60% defrost raw chicken water. In addition, most respondents defrost chicken for 2 to 6 hours (31.50%), following by less than 1 hour (28.70%) and from 6.1 and 12 hours (16.80%). When asked where they consumed chicken meat, 46.10% of subjects declared to eat only at home and 44% in and out of their homes (Table 4).

The general, WHOPI for chicken meat handling scored 0.62 ± 0.18, being classified as medium risk level (Table 5). When looking deeply inside each control, it can be observed that in time, temperature and binomial time-temperature scores there were a statistically significant difference among all three group levels (Tukey-T test, $p < 0.000$ for all of them). Because all chicken meat was considered as cooked, feature temperature scored 1, the highest WHOPI level. The lowest value was for control related to GHP, demonstrating that people declared failures in common hygienic practices inside homes.

Based on WHOPI classification it was possible to observe that three GHP practices were similar among the three WHOPI levels ($p > 0.05$): the practice “where to buy chicken meat” and “cap the container where the chicken meat to be reused is” and “perception of risks” of chicken meat. Regarding the time to refrigerate chicken meat after purchase, 96.50% of respondents belonging to high level WHOPI did it correctly, while 85.80% of medium level, and only 50.40% from low level did the same procedure. On the other hand, time during transport between the place of purchase and the place where it would be refrigerated was the behavior that contributed the most to achieve a medium level in this control. Binomial time-temperature practices of where the chicken meat was placed until time to serve and how long it took to be served after the chicken meat was cooked demonstrated the highest difference between WHOPI levels. While only 39.40% of low level did it properly, 55.30% of medium level and 90% of high level also did it properly, being the general average (among three WHOPI levels) 53.4%. In addition, the time that the chicken meat was served on the table was also correlated to high WHOPI level, being only 22.30% correct for low level, 64.90% for medium level and 93.70% for high level.

For binomial time-temperature the questions that contributed the most for a higher WHOPI index were: “time-temperature to defrost chilled chicken meat” and the “time to prepare chicken meat”. When defrosting chilled chicken meat only, 23% of

low WHOPI level respondents did it properly, while 46% of medium level and 80.70% of high level did it properly. In addition, 39.40% of low WHOPI level, 55.30% of medium WHOPI level and 90% of high WHOPI level cares about the time to prepare chicken meat. Thus, the practices adopted by respondents for defrost chicken meat and handling cooked chicken meat highlighted the risk of cross-contamination and pathogen multiplication in low WHOPI level.

Regarding defrost procedure, it is recommended that a frozen broiler chicken should be thawed inside a refrigerator. The purpose is to maintain the surface of the broiler at a low enough temperature to prevent the growth of bacteria (FAO/WHO, 2002). However, because this procedure needs more time for defrosting and space inside refrigerators, people generally conduct defrost at room temperature, creating conditions to microbial multiplication. Moreover, because the majority of the bacteria are on the surface of chicken carcasses or inside feather folicules, and these are the places that defrost first, the probability of bacterial multiplication is high when not conducted inside refrigeration. Other important risk associated to defrost are the drops from the carcasses that can be sources of cross-contamination, spreading pathogens inside kitchens (Carrasco et al., 2012; Elias et al., 2015; Harrison, Griffith, & Tennant, 2001; Katiyo et al., 2019).

3.3 Behavior and practices on handling eggs

The habits practiced for purchase, transport, preparation and consumption of eggs are presented in Table 8. The majority of respondents bought eggs (95.30%) from greengrocers or local markets (67.80%), while 5.20% acquired eggs directly from producers and 24% from various places mentioned above. In addition, when buying eggs, 51.50% of respondents observed the egg origin, expiration date and the label indicating sanitary fiscalization by the Ministry of Agriculture on the packaging. At home, 90.20% of people declared that eggs were stored in refrigerators and 9.40% at room temperature, remaining, mostly, between 8 and 15 days (41.80%) and between 1 and 7 days (36.70%), until preparation. Regarding the preparation, 97.10% of respondents prepared eggs by cooking or frying, until obtain completely cooked yolks (76.50%), while the remaining prepared raw or underdone yolks or omelets (10.70%). The omelets were considered as preparations containing yolk not completely cooked eggs and, because of that, they were jointed with underdone yolks. The main behavior observed was the preparation of eggs less than 30 minutes

before serving (92.40%), exposing them at room temperature (86.40%), being consumed in less or until 30 minutes (88.90%). Only 25.0% of the respondents reported to reuse eggs. In case of reusing, they were mostly storage under refrigeration (93.30%) in a capped container (89.90%) and consumed at once (87.54%), less or until 1 day (87.50%) after storage. Furthermore, 75.70% of respondents consumed eggs only at home, being that 56.30% stated to eat cooked yolk, 33.60% raw or underdone yolk and 10.10% at both cooking stages (Table 4).

Good hygienic practices (GHP) related to eggs were classified in a medium WHOPI level (0.62 ± 0.15). Interestingly, two features were at high level (time and binomial time-temperature) and two in a low level (temperature and GHP) (Table 5). The two first are in high level because more than 90% of respondents declared that did not reuse eggs, and when they did it, the eggs were stored under refrigeration in a capped container and consumed until 5 days. Further, the lowest WHOPI value (0.18 ± 0.29) was identified for the feature temperature, because 68.0% of respondents declared to cook and consume eggs with raw or soft yolks. Even all groups showing this risk behavior, this trend was significantly more associated to low WHOPI levels, where 98.90% respondents declared to cook eggs only at raw yolk point.

Morris 1990 conducted a risk assessment of *Salmonella* Enteritidis in eggs, and identified that poor refrigeration, improper storage of pooled eggs, use of raw eggs, time and temperature abuse of eggs were the most important risk factors for the occurrence of salmonellosis. In our study, the conditions of time, temperature and binomial time-temperature handling eggs from purchase to consumption, according respondents, were mostly in accordance to GHP, which will be discussed further in this manuscript.

Differently than chicken meat, the source of eggs varied significantly. Among places were eggs were bought greengrocer/local market, directly from producer and door-to-door grocery selling were declared. Attention should be taken especially from those acquired directly from small producers and door-to-door grocery selling, where, many times, there is no GHP implementation or regular sanitary inspections. The source of eggs may influence the microbial contamination of the products. According to Gast & Beard (1992), fresh laid eggs naturally may contain no more than a few hundred *Salmonella* cells. Thus, is important its control at primary production, due to procedures to prevent the growth of these populations along transport, processing

and storage (Richard K. Gast, Guraya, Guard, & Holt, 2010). In addition, once *Salmonella* Enteritidis is introduced into the albumen, it can reach the yolk and rapidly multiply during unrefrigerated storage, being those contaminated eggs a serious threat to consumers (Humphrey, 1994).

Most of respondents declared to prepare and consume eggs with completely cooked yolk, which is in accordance with the sanitary regulation 78/09 and previous studies (Elias et al., 2015; Rio Grande do Sul, 2009).

3.4 Consumption data of chicken meat and eggs and its application in Risk Assessments

In the survey, besides access food safety behavior on handling chicken meat and eggs, the characteristics of respondents according to them consume habits practiced also were included. This data sets can be used in microbiological or chemical exposure assessments linked to chicken meat and eggs by feeding data for the exposure assessment. The consumer intake by portion size, frequency of consumption and consumption for chicken meat and eggs are in Table 4.

The profile of respondents revealed a huge consume of chicken meat (96.79%) in a frequency of 2 to 3 times a week (45.67%), eating in one meal a day (74.40%) at least 90 - 100 grams per meal (46.98%). The profile for eggs showed that 97.54% of people interviewed declared to consume eggs 2 to 3 times a week (37.86%). The amount of consumption per meal was one and two units and their percentages were very similar, 47.31% and 46.12%, respectively (Table 4).

To calculate the daily consumption and discrete distribution function the percentage of the respondents corresponding to a certain frequency and portion size consumed data were extracted from the data set with SPSS. For chicken, the results were already expressed in grams and for eggs each unit of eggs were multiplied by 55 grams.

The distributions of the daily consumption data are on Table 9. It is possible to observe that for chicken meat and eggs the median are similar from the mean. However, skewness and kurtosis of chicken daily consumption distribution clearly indicate that data are not normal distributed (skewness = 0.20 and kurtosis = -0.921), being the distribution with large tail to the right (most values are above average) and platykurtic (flatter than the normal distribution). The large tail to the right and platykurtic shape in the distribution indicate that most consumers eat

medium portions and a few consumers eat large portions (comparison of median, 95th percentile, and maximum). Therefore, the distribution of consumption of chicken meat observed in one day ranged from a minimum of consumption of once a week in one meal in the portion of less than 60 g (0.01%, n =23) to a consume of more than 6 days a week in at least 4 meals eating a portion of 100 grams at least (no cases). The highest amounts observed in this study were the following: eating 6-7 times a week, 4-5 meals a portion of 60 - 80 grams. Thus, considering all situations obtained in this study, the consumption of chicken meat in a day range from 60 grams/day to 480 grams/day, being the median consumption 95 grams/per day, which correspond to a regular filet. This result is higher than other results obtained by other surveys conducted in Brazil, which showed chicken consumed ranging from 31.10 to 41.10 grams/day (ABPA, 2017; Brasil, 2009).

For eggs the skewness showed the large tail to the right, kurtosis with leptokurtic distribution with fatter tails and central peak higher and sharper (skewness = 3.26 and kurtosis = 15.54). The distribution shape indicates regular consumption of eggs (mean, median, 75th percentile), however the greater kurtosis indicates that extreme values occasionally occur, means that the probability of extreme events is greater than that implied by the normal curve. In the same way, eggs consume practiced ranged from one unit of egg in one meal once a week (n=142, 11.66%) to more than 2 eggs, in 4 or more meals at least 6 times a week (no cases). The maximum intake scenario observed for eggs in this study was the intake of to 2 eggs, in 4 or more meals at least 6 times a week (n = 6, 0.5%). The eggs daily intake observed in this paper was 0.92 units/day. Another survey conducted in Brazil informs that the consumer data was 190 units/year/person, which corresponds to 0.52 units/day/person (ABPA, 2017). This study considered in the calculation of intake only persons who eat chicken meat and eggs, differently from the others found who consider all population. For this reason, the grams consumed per person was higher.

4. Limitations and perspectives

The major limitation of this study was due to the internet access and the population surveyed. Females and young adult age group were more represented, and this group may not represent the risk behavior and the risk of the occurrence of a foodborne outbreak by Brazilian consumers. In addition, handling practice from

purchase to consumption of chicken meat and eggs assessed in this study were self-reported, so non-conforming practices and misconceptions may not have been truly reported. Even with these limitations, the results obtained are useful since this is the first study conducted in Brazil regard consumer behavior and practices of chicken meat and eggs from purchase to consumption at home and the risk of the occurrence of a foodborne outbreak due to it. Furthermore, the perspective of the study is to apply the data surveyed in microbiological food safety risk assessments related to the consumption of chicken meat and eggs and targeted risk communication strategies for the different group profiles. Finally, the results of this study could also support education program to improve consumer awareness on food safety practices.

5. Conclusion

The present study assessed the risk of occurrence of a foodborne outbreak and the determinant point practices to achieve high Good Hygienic Practice level during handling practice from purchase to consumption of chicken meat and eggs. This is the first time that this data is carried out in Brazil.

Most respondents showed medium level of GHP procedures. The main non-conforming practiced while handling chicken meat and eggs were related to fail on controls in time to transport chilled chicken meat after purchase to home and expose chicken and eggs prepared on table for longer than 30 minutes, defrost chicken meat, point of egg yolks consumed and the criteria considered before consuming a stored food in the refrigerator. Controls in temperature showed higher impact to upgrade in WHOPI level, followed by binomial time-temperature, time and good practices. Our findings reflect the needs of food safety programs education focused at consumer level aiming to raise consumer awareness of the risks of cross-contamination in homes and their role in its prevention. Finally, these results combined, offer useful insights into food handling behavior and practices for microbiological food safety researches.

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Table 1: Questions and conformity level of responses to the questions regarding time, temperature and good practices used in the Weighted Harmonic Outbreak Prevention Index (WHOPI).

Feature	Practice	Confirmity*	Weight
Time			
After buying chilled chicken meat, how long does it take between the place of purchase and the place where it will be refrigerated?	Less os until 30 minutes	C	4
	Between 31 minutes and 1 hour	NC	0
	Between 1,1 hour and 2 hours	NC	0
	More than 2 hours	NC	0
If it is stored under refrigeration before preparation: for how long the cooled chicken meat is stored until prepared (cooked)?	Less or until 48 hours	C	4
	More than 48 hours	NC	0
When served, how long is the chicken meat on the table?	Less or until 30 minutes	C	4
	Between 31 minutes and 1 hour	NC	0
	Between 1,1 hour to 2 hours	NC	0
	More than 2 hours	NC	0
How long are eggs stored before preparation?	Less than 30 days	C	4
	More than 30 days	NC	0
How long are the eggs being served on the table?	Less than 30 minutes	C	4
	Between 30 minutes and 1 hour	NC	0
	Between 1,1 and 2 hours	NC	0
	More than 2 hours	NC	0
How long do the reused eggs continue to be consumed?	Less or until 5 days	C	4
	More than 5 days	NC	0
Temperature			
How do you cook chicken meat?	Raw or underdone	NC	0
	Cooked	C	4
How do you cook eggs?	Cooked or fried with cooked yolk	NC	0
	Cooked, fried with underdone yolk or omelette	C	4
	Both methods	NC	0
When you consume eggs, at which point do you consume the yolk?	Raw or underdone	NC	0
	Cooked	C	4
	Both points	NC	0
Good Practices			
Where do you buy chilled chicken?	Supermarket	C	4
	Butchery	C	4
	Street fair	NC	0
	Directly from producer	NC	0
Is the container where the chicken meat to be reused capped?	Yes	C	4
	No	NC	0
Where do you buy eggs?	Supermarket	C	4
	Greengrocer/ local Market	C	4
	Street fair	NC	0
	Directly from producer	NC	0
	Door-to-door grocery selling	NC	0

Is the container where the eggs to be reused capped?	Yes	C	4
	No	NC	0
Do you wash your hands before, during and after preparing food?	Yes	C	4
	No	NC	0
In relation to utensils used during the preparation of food, do you:	Use different utensils or wash between preparations	C	4
	Use the same utensils for different preparations without washing them	NC	0
What is your main criteria to consume a stored food in the refrigerator:	Smell	NC	0
	Taste	NC	0
	Appearance	NC	0
	Storage time	C	4
	Expiration date	C	4
	2 or more factors together	NC	0
When buying chicken meat do you look at the packaging for the origin of the product, the expiration date and if there is a seal from the Ministry of Agriculture (SIF)?	Yes	C	4
	No	NC	0
When you buy eggs, do you observe the product origin, expiration date and the Ministry of Agriculture (SIF) seal on the packaging?	Yes	C	4
	No	NC	0
		NC	0
How often do you read information of shelf-life and storage conditions in food labels:	Always	C	4
	Most of time	NC	0
	Rarely	NC	0
	Never	NC	0
In your opinion, can the consumption of chicken meat pose any risk to consumer health?	Yes	C	4
	No	NC	0
In your opinion, can the consumption of eggs pose any risk to consumer health?	Yes	C	4
	No	NC	0

*C= conforming; NC= non-conforming

Table 2: Questions and conformity level of responses to the questions regarding the time-temperature binomial used in the Weighted Harmonic Outbreak Prevention Index (WHOPI) questions.

			Feature Time					
			How long before serving is the chicken meat cooked?					
			Less than 30 minutes	Between 31 minutes and 1 hour	Between 1,1 and 2 hours	More than 2 hours		
	Until the time to serve, where is the ready chicken?	Immediate consumption	C					
		Refrigerator	C	C	C	C		
		Room temperature	C	NC	NC	NC		
		2 or more methods[a]	C	NC	NC	NC		
			How long will the reused chicken meat be stored before it is consumed?					
			Less than 12 hours	Between 12,1 and 24 hours	Between 24,1 and 48 hours	More than 48 hours		
Feature Temperature	Where is the chicken meat to be reused stored?	Refrigerator	C	C	C	C		
		Room temperature	C	NC	NC	NC		
		2 or more methods[a]	C	NC	NC	NC		
			How long does it take to defrost the chicken meat?					
			Less than 1 hour	More than 1 hour	Between 2 and 6 hours	Between 6,1 and 12 hours	Between 12,1 and 24 hours	More than 24 hours
	How do you defrost the chicken meat?	At room temperature	NC	NC	NC	NC	NC	NC
		Microwave or refrigerator	C	C	C			
		Immersed in water	NC	NC	NC	NC	NC	NC
		Cooking directly	C					
		2 or more methods[b]	NC	NC	NC	NC	NC	NC
			How long before serving the eggs are cooked?					
			Less than	Between 31	Between 1,1	More than 2		

		30 minutes	minutes and 1 hour	and 2 hours	hours
Until the time to serve, where is the ready egg?	Immediate consumption	C			
	Refrigerator	C	C	C	C
	Room temperature	C	NC	NC	NC
	2 or more methods[a]	C	NC	NC	NC
		How long will the reused eggs be stored before it is consumed?			
		Less or until 1 day	Between 2 and 5 days	Between 6 and 7 days	More than 7 days
Where is the eggs to be reused stored?	Refrigerator	C	C	NC	NC
	Room temperature	NC	NC	NC	NC
	2 or more methods[a]	NC	NC	NC	NC

[a]: The methods are immediate consumption, refrigerator and room temperature. The response of incorrect time-temperature is considered incorrect.
[b]: Methods are at room temperature, microwave or refrigerator, immersed in water, cooking directly. The response of incorrect time-temperature is considered incorrect.
C= conforming; NC= non-conforming.

Table 3: Classification according to the conformity level of the Weighted Harmonic Outbreak Prevention Index (WHOPI).

WHOPI	Conformity level
0–0.35	Low
0.36–0.7	Medium
0.71–1	High

Table 4: Socioeconomic characteristics of respondents and consumption practices of chicken meat and eggs.

Feature	Practice	Answer	Frequency	
			n	%
Socioeconomic	Gender	Male	212	17.42
		Female	1005	82.58
	Age group	Less than 18 years old	5	0.41
		18 to 24 years old	202	16.6
		25 to 34 years old	574	47.16
		35 to 44 years old	221	18.16
		45 to 54 years old	88	7.23
		55 to 64 years old	102	8.38
		65 years old or more	25	2.05
	Family income	Less than 1 minimum wage	77	6,33
		1 minimum wage	222	18.24
		2 minimum wages	327	26.87
		3 minimum wages	319	26.22
		4 minimum wages	231	18.98
		More than 4 minimum wages	41	3.37
	Education[a]	Elementary education	8	0.66
		High school	68	5.59
		Under graduation	457	37.55
		Graduation	684	56.2
	Macro region of residence[b]	South	759	62.37
		Southeast	92	7.56
		Midwest	40	3.27
		Northeast	282	23.17
		North	30	2.46
Chicken meat	Do you consume chicken meat?	Yes	1178	96.79
		No	39	3.20
	Weakly, how often do you eat chicken meat?	Once a week	204	17.32
		2 to 3 times a week	538	45.67
		3 to 5 times a week	355	30.12
		6 to 7 times a week	81	6.88
	How many meals do you eat chicken meat in a day?	1 meal	875	74.40
		2 meals	290	24.66
		3 meals	9	0.76
		4 or more meals	2	0.17
	How much do you eat per meal?	Less than 60 grams	52	4.34
		60 - 80 grams	380	32.28

		90 - 100 grams	553	46.98
		More than 100 grams	193	16.4
	Where do you eat chicken meat?	Only at home	544	46.10
		Only out of home	115	9.90
		Both places	519	49.00
	Do you eat eggs?	Yes	1187	97.54
		No	30	2.47
	Weakly, how often do you eat eggs?	Once a week	227	19.06
		2 to 3 times a week	449	37.86
		3 to 5 times a week	267	22.51
		6 to 7 times a week	244	20.57
	How many meals do you eat eggs?	1 meal	926	78.41
		2 meals	218	18.46
		3 meals	26	2.2
Eggs		4 or more meals	11	0.93
	What is the approximate amount per meal?	1 egg	561	47.31
		2 eggs	547	46.12
		More than 2 eggs	78	6.58
	When you consume eggs, at which point do you consume the yolk?	Raw or underdone	399	33.60
		Cooked	669	56.30
		Both points	119	10.10
	Where do you eat eggs?	Only at home	899	75.70
		Only out of home	16	1.30
		Both places	272	22.90

[a] Refers to the partial or complete education level.
[b] Refers to five Brazilian Macroregions.

Table 5: Weighted Harmonic Outbreak Prevention Index (WHOPI) value and classification according to the conformity level of Good Hygienic Practices for chicken meat and eggs.

		Feature				
		Time	Temperature	Binomial Time-temperature	Good practices	General
		Mean ± st.dv* Classificationᵘ	Mean ± st.dv Classification	Mean ± st.dv Classification	Mean ± st.dv Classification	Mean ± st.dv Classification
Poultry product	Chicken meat	0.52 ± 0.40^{aA} Medium	1^{bA} High	0.39 ± 0.38^{cA} Medium	0.32 ± 0.19^{dA} Low	0.62 ± 0.18^{eA} Medium
	Eggs	0.90 ± 0.26^{aB} High	0.18 ± 0.29^{bB} Low	0.90 ± 0.27^{aB} High	0.27 ± 0.19^{cB} Low	0.62 ± 0.15^{dB} Medium
	General	0.63 ± 0.34^{aC} Medium	0.29 ± 0.28^{bC} Low	0.44 ± 0.33^{cC} Medium	0.32 ± 0.19^{bA} Low	0.44 ± 0.17^{cC} Medium

* = Lower letter shows significant differences among features, capital letter shows significant differences among poultry products.

ᵘ = Classification according to the conformity level of the Weighted Harmonic Outbreak Prevention Index (WHOPI) questions.

Table 6: Good Hygienic Practices and perception of risk of respondents.

Feature	Practice	Answer	Frequency N	Frequency %
Good Practices	Do you wash your hands before, during and after preparing food?	Yes	1072	90.70
		No	2	0.20
		Sometimes	107	9.00
	In relation to utensils used during the preparation of food, do you:	Use different utensils or wash between preparations	901	76.50
		Use the same utensils for different preparations without washing it	277	23.40
	What is your main criteria to consume a stored food in the refrigerator:	Smell	83	6.80
		Taste	8	0.60
		Appearance	32	2.60
		Storage time	99	8.10
		Expiration date	52	4.30
		Smell and taste	99	8.10
		Taste, smell and appearence	52	4.30
		Smell, appearence, storage time and expiration date	120	9.90
		Smell, appearence and expiration date	96	7.80
		Smell, taste, storage time and appearance	110	9.00
		Other factors together	466	38.30

	How often do you read information of shelf-life and storage conditions in food labels:	Always	473	38.80
		Most of time	583	47.90
		Rarely	153	12.50
		Never	8	0.60
	How do you assess your degree of interest in food:	Very desinterested	11	0.90
		Desinterested	29	2.40
		Neutral	197	16.20
		Interested	360	29.60
		Very interested	620	51.00
	What source of food information do you use?	Television	22	1.80
		Internet	527	43.30
		Journal, books, magazines	48	3.90
		Research articles, professional consulting, courses	28	2.30
		Family or friends	5	0.40
		2 or more factors together	587	48.20
	In your opinion, can the consumption of chicken meat pose any risk to consumer health?	Yes	774	63.60
		No	443	36.40
Perception of risks	Among the hazards described, which of these hazards may be associated with the consumption of chicken meat?	Microorganisms (bacteria, fungi, parasites)	120	15.40
		Toxins	3	0.380
		Pesticides	1	0.10
		Hormons	56	7.20
		Antibiotics	2	0.30
		Microrganisms and hormons	93	11.90
		Microrganisms,antibiotics and hormons	103	13.20
		Microrganisms, toxins, pesticides, hormons, antibiotics	72	9.30
		Other factors together	327	42.10
	In your opinion, can the consumption of eggs pose any risk to consumer health?	Yes	807	66.20
		No	410	33.70
	Among the hazards described, which of these hazards may be associated with the consumption of eggs?	Microorganisms	300	36.90
		Toxins	11	1.40
		Pesticides	0	0.0
		Hormons	24	3.00
		Antibiotics	2	0.20
		Microrganisms and hormons	51	6.30
		Microrganisms,antibiotics and hormons	56	6.90
		Microrganisms and toxins	98	12.10
		Microrganisms, toxins, hormons	43	5.30
		Microrganisms, toxins, hormons and antibiotics	46	5.70

	Microrganisms, toxins, pesticides, hormons and antibiotics	45	5.50
	Other factors together	135	16.70

Table 7: Characteristics of respondents according to purchase, transport and preparation of chicken meat.

			Frequency n	%
Purchase	Do you buy chilled chicken meat?	Yes	998	81.20
		No	219	18.80
	Where do you buy chilled chicken meat?	Supermarket or butchery	984	98.60
		Street fairs or directly from producer	6	0.60
		Both places	8	0.80
	When buying chicken meat do you look at the packaging for the origin of the product, the expiration date and if there is a seal from the Ministry of Agriculture (SIF)?	Yes	457	45.80
		No	541	54.20
Storage	After buying chilled chicken meat, how long does it take between the place of purchase and the place where it will be refrigerated?	Less or until 30 minutes	722	72.30
		Between 31 minutes and 1 hour	254	25.50
		Between 1,1 hour and 2 hours	19	1.90
		More than 2 hours	3	0.30
	How long the cooled chicken meat is stored until cooked?	Less or until 1 hour	141	14.30
		Between 1,1 and 4 hours	205	20.70
		Between 4,1 and 12 hours	191	19.30
		Between 12,1 and 24 hours	184	18.60
		Between 24,1 and 48 hours	132	13.40
		More than 48 hours	135	13.70
Preparation	Do you cook chicken meat?	Yes	1100	90.40
		No	117	9.60
	How do you cook chicken meat?	With sauce	82	7.40
		Roasted	80	7.20
		Fried	26	2.40
		2 or more methods	913	83.00
Distribution	How long before serving chicken meat is cooked?	Less than 30 minutes	527	47.90
		Between 31 minutes and 1 hour	465	42.30
		Between 1,1 and 2 hours	79	7.20
		More than 2 hours	29	2.60
	Until the time to serve,	Immediate consumption	9	0.80

	where is the ready chicken?	Refrigerator	93	8.5
		Room temperature	966	88.0
		2 or more methods	32	2.70
	When served, how long is the chicken meat on the table?	Dont serve chicken meat on the table	149	13.50
		Less or until 30 minutes	463	42.10
		Between 31 minutes and 1 hour	427	38.80
		Between 1,1 hour to 2 hours	48	4.40
		More than 2 hours	13	1.20
	Do you reuse chicken meat?	Yes	1019	92.60
		No	82	7.40
	Is the chicken meat reused at one time or depends on the number of serving?	One time	672	66.10
		Depends on the number of serving	345	33.90
Reuse	Where is the chicken meat to be reused stored?	Refrigerator	747	73.40
		Freezer	47	4.60
		Under refrigeration	164	16.10
		Room temperature	19	1.90
		2 or more methods	40	3.90
	How long will the reused chicken meat be stored before it is consumed?	Less than 12 hours	217	21.40
		Between 12,1 and 24 hours	478	47.00
		Between 24,1 and 48 hours	215	21.20
		More than 48 hours	106	10.40
	Is the container where the chicken meat to be reused capped?	Yes	988	97.00
		No	31	3.00
	Do you buy frozen chicken meat?	Yes	924	80.90
		No	218	19.10
	How long does it take to defrost the chicken meat?	Less than 1 hour	265	28.70
		More than 1 hour	109	11.80
		Between 2 and 6 hours	291	31.50
		Between 6,1 and 12 hours	156	16.80
Defrost		Between 12,1 and 24 hours	92	9.90
		More than 24 hours	11	1.20
	How do you defrost the chicken meat?	At room temperature	296	32.10
		Microwave or refrigerator	455	49.20
		Immersed in water	126	13.60
		Cooking directly	2	0.20
		2 or more methods	45	4.90

Table 8: Characteristics of respondents according to purchase, transport and preparation of eggs.

			Frequency n	%
Purchase	Do you buy eggs?	Yes	1166	95.80
		No	51	4.20
	Where do you buy eggs?	Supermarket	22	2.00
		Greengrocer/ local Market	791	67.80
		Street fair	5	0.40
		Directly from producer	61	5.20
		Door-to-door grocery selling	5	0.40
		Two or more places	282	24.00
	When you buy eggs, do you observe the product origin, expiration date and the Ministry of Agriculture (SIF) seal on the packaging?	Yes	600	51.50
		No	566	48.50
Storage	Where are the eggs stored until cook?	At room temperature	110	9.40
		In refrigerator	1051	90.20
		Both places	5	0.30
	How long are eggs stored before preparation?	Less than 1 day	20	1.60
		Between 1 and 7 days	427	36.70
		Between 8 and 15 days	487	41.80
		Between 16 and 30 days	208	17.90
		More than 30 days	24	2.10
Preparation	Do you cook eggs?	Yes	1181	97.10
		No	36	2.90
	How do you cook eggs?	Cooked or fried with cooked yolk	904	76.50
		Cooked, fried with underdone yolk or omelette	126	10.70
		Both methods	151	12.80
	How long before serving the eggs are cooked?	Less than 30 minutes	1092	92.40
		Between 31 minutes and 1 hour	69	5.80
		Between 1,1 and 2 hours	5	0.40
		More than 2 hours	15	1.30
Distribution	Until the time to serve, where is the ready egg?	Immediate consumption	25	2.10
		Refrigerator	111	9.40
		Room temperature	1020	86.40
		2 or more methods	25	2.10
	When served, how long is the egg on the table?	Less or until 30 minutes	1050	88.90
		Between 31	120	10.20

		minutesand 1 hour		
		Between 1,1 hour to 2 hours	9	0.80
		More than 2 hours	2	0.20
	Do you reuse eggs?	Yes	297	25.0
		No	891	75.00
	Is the egg reused at one time or depends on the number of serving?	One time	260	87.54
		Depends on the number of serving	37	12.45
Reuse	Where is the eggs to be reused stored?	Refrigerator	277	93.30
		Room temperature	13	4.40
		2 or more methods	7	2.40
	How long will the reused eggs be stored before it is consumed?	Less or until 1 day	260	87.50
		Between 2 and 5 days	33	11.10
		Between 6 and 7 days	2	0.70
		More than 7 days	2	0.70
	Is the container where the eggs to be reused capped?	Yes	267	89.90
		No	30	10.10

Table 9: Distribution of daily consumption of chicken meat and eggs and percentage of nonconsumers.

	Daily consumption (g/day)												Discrete distribution function*
Product	Mean	Median (50^{th} percentile)	SD	Minimum	Maximum	5%	10%	25%	75%	90%	95%	% nonconsumers	
Chicken meat	110.38	95	54.27	60.00	480.00	60.00	70.00	70.00	95.00	120.00	240.00	3.20	Discrete ({60; 70; 95; 120; 140; 190; 210; 240; 280; 285; 360; 480};{42; 294; 397; 145; 78; 150; 2; 53; 1; 5; 2; 1})
Eggs	110.70	110	88.34	0	660.00	55.00	55.00	55.00	110.00	220.00	220.00	2.47	Discrete ({1; 2; 3; 4; 6; 8; 12};{223; 213; 18; 54; 16; 1; 6})

* Discrete ({a1; ... ; ax}]; {[b1; ... ; bx}] where [a] is the portion weight expressed in grams and [b] is the corresponding number of consumers.

5.4. Artigo científico 4:

Quantitative Risk Assessment of Human Salmonellosis linked to Brazilian Chicken Meat

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Abstract:
A quantitative microbial risk assessment (QMRA) was carried out in order to calculate the risk of human salmonellosis due to consumption of chicken meat in Brazil. The model followed the framework proposed by the *Codex Alimentarius* and considered modules from the chicken slaughter to the consumption at home and at food services. It was created 15 scenarios modelling temperatures variation during processing, different *Salmonella* prevalences and concentrations*, Salmonella* reductions due to scalding, spray washing and immersion in chiller water containing sanitizers. Primary and secondary predictive microbiology models were created focusing the growth of Brazilian *Salmonella* strains on chicken meat. Consumption habits inside Brazilian homes were identified and considered in QMRA which were carried out using @RISK and Monte Carlo simulations. Results indicated that the reduction in initial prevalence and in cross-contamination are important to risk mitigation of salmonellosis at home and also at food services. Reduction in 50% of initial baseline prevalence reduced 50.05% of the salmonellosis risk, however reduction in initial concentration did not affect the risk. Reduction in *Salmonella* concentration due to processing or by antimicrobial agents applied at slaughter industry had no effect in salmonellosis risk reduction, however it contributes to the decrease of *Salmonella* contamination on carcasses The baseline model considered

an initial prevalence of 4.04% and the risk of *salmonellosis* was predicted in 8.092 cases in 1000 exposures at home and 7.95 cases in 1000 exposures at food services. Brazilian epidemiological data does not report so many salmonellosis cases, thus potential reasons for this overestimation are discussed in this study. The QMRA model also emphasizes that risk mitigation strategies needs to be implemented in different chicken meat production steps, from farm to consumption, aiming to effectively reduce the risk of salmonellosis due to consumption of chicken meat.

Keywords: QMRA; probabilistic assessment; salmonellosis; foodborne disease; public health; poultry products.

1. Introduction

Chicken meat products are consumed at global level and are an important source of protein. The demand of these products are expected to increase due to population growth and the rising of individual consumption (Chapman, Otten, Fazil, Ernst, & Smith, 2016; FAO, 2019; Nauta, Jacobs-Reitsma, & Havelaar, 2007). Despite the expressive growth in production and consumption, chicken meat still being associated with foodborne outbreaks and *Salmonella* spp. is the most common etiological agent of these events (EFSA, 2018; OzFoodNet, 2015).

Currently, Brazil states as the third major producer and leads the chicken meat exports in the world, producing over 4 million tons of exports in 2018 (ABPA, 2019; FAO, 2019). In addition, over 97% of the Brazilian population consume chicken meat (Hessel et al., 2019), highlighting the importance of controlling *Salmonella* in the Brazilian chicken meat production.

Even though government and chicken meat industries put a lot of effort to control *Salmonella*, these food products still being susceptible to *Salmonella* contamination because chicken productive chain is long and complex, and Food Safety Management Systems based on Good Hygienic Practices (GHP) and Hazard Analysis and Critical Control Points (HACCP) alone seems to be not completely effective. Consequently, in order to reduce the risk of salmonellosis due to chicken meat consumption, it is necessary to adopt new food safety approaches, which can work together with high level of GHP and HACCP controls. Quantitative risk assessment (QMRA) is a valuable tool and its development offers a scientific basis

approach for food risk management, providing outcomes of the most effective risk management options (Enger, Nelson, Clasen, Rose, & Eisenberg, 2012; Hoelzer, Pouillot, Egan, & Dennis, 2012; Membré & Guillou, 2016).

Considering the importance of chicken meat trade and its impact in public health, this work aims to perform a QMRA to calculate the risk of salmonellosis due to consumption of chicken meat contaminated with *Salmonella* spp. in Brazil. This study also aimed to identify the best mitigate strategies able to reduce the risk of salmonellosis due to chicken meat consumption.

2 Materials and Methods

The current QMRA followed the framework proposed by the *Codex Alimentarius* (CAC/GL, 2011) which is presented below, in order to provide an estimate of the risk of human salmonellosis due to consumption of chicken meat at domestic kitchens and in Brazilian food services.

2.1. Hazard Identification

Hazard identification is defined as the identification of biological, chemical and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods (CAC/GL, 2007). In the present work, *Salmonella* was identified as the biological hazard associated with the consumption of chicken meat in Brazil due to its frequent involvement with Brazilian foodborne outbreaks. From 2009 to 2018, 2,431 outbreaks were reported in the country, and *Salmonella* spp. was responsible for 11.3% of the cases (BRASIL, 2019). In addition, a specific strain of *Salmonella* spp., *Salmonella* Enteritidis (SE86) was the main cause of outbreaks from 1993 to 2012, in the State of Rio Grande do Sul, and is probably the most studied foodborne pathogen in southern Brazil (Ritter et al., 2019; E. C. Tondo & Ritter, 2012; Eduardo Cesar Tondo, Ritter, & Casarin, 2015; Wagner, Silveira, & Tondo, 2013).

2.2. Hazard Characterization

Following (CAC/GL, 2007), quantitative evaluations of the nature of the adverse health effects associated with *Salmonella* in chicken meat were performed at hazard characterization carried out in this study.

Several studies of the pathogenicity of *Salmonella* in humans and dose–response relationships were summarized in the WHO report “Risk Assessment of *Salmonella* in eggs and broiler chickens” (FAO/WHO, 2002). However, for conservativeness, the dose-response model adopted in our QMRA was the Beta-Poisson as proposed by WHO/FAO (2002). This model predicts the percentage of the population which responds to a certain level of pathogens (number of *Salmonella* ingested) (Teunis & Havelaar, 2000; Teunis, Nagelkerke, & Haas, 1999). The α and β parameters are fitting parameters used to describe the variability in the susceptibility to illness among different individuals in the population upon exposure to a dose of *Salmonella* (Vose, 1998). In our model it was used α and β parameters adopted by FAO/WHO (2002) (Table 1 and 2).

By adopting the Beta-Poisson model, the dose-response relationship assumed is that one single *Salmonella* cell is capable to infect and cause disease. This model was used to evaluate human dose responses for *Salmonella* in several QMRA published (Smadi & Sargent, 2012; Haas, Rose, & Gerba, 2014; Holcomb et al., 1999; World Health Organisation, 2002).

2.3 Exposure assessment

Following (CAC/GL, 2007), quantitative evaluations of the intake of the biological agents via the consumption of chicken meat were carried out at exposure assessment of this study. The present risk assessment model comprised modules of chicken meat from slaughter industry until consumption. For consumption at home was assumed the consumption of chilled chicken meat and 21 modules were described, while for chicken meat consumed at food services it was assumed frozen chicken meat chain and 20 modules were described (Figure 1). The flowchart diagram from slaughter to the consumption of chicken meat was based on the *Codex Alimentarius* document “Guidelines for the control of *Campylobacter* and *Salmonella* in chicken meat CAC/GL 78-2011” and validated by experts and field professionals.

Tables 1 and 2 summarizes the inputs used for the risk calculations for chicken meat consumption at homes and food services, respectively. The first and

second columns represents the local and step of chicken meat production considered in the model, respectively. The third column is a text description of the variable. The fourth column describes the symbol representing the formula. The next columns are the variable category and the unit assumed in the distribution, value, or formula, which represents the value of the cells. The last column (source) represents the source of the information used to determine the value of the variables.

2.3.1 Slaughter to consumption at home model *inputs*

The risk assessment model of this study comprised 21 different modules for chilled chicken meat from slaughter industry until consumption at homes and they are briefly described below (Figure 1 and Table 1).

Slaughter industry

Step1: Bleeding: The initial prevalence of *Salmonella* contamination in chicken meat considered in our model was based on prevalence published by De Lima et al. (2018). The study of De Lima et al. (2018) is the major report of *Salmonella* spp. prevalence in chicken meat reported in Brazil, which analyzed 77,165 chicken carcasses produced in slaughterhouses of Southern Brazil from 2006 to 2015.

Other prevalence numbers were reported in Brazil and were considered in the present study, as different modelled scenarios. For example, the review and meta-analysis of Hessel et al. (2020a) found the average prevalence of 14.04%, while the Ministry of Agriculture, Livestock and Supply (MAPA) reported 17.76% (MAPA, 2018). Borges et al. (2019) published a *Salmonella* prevalence of 49% in broiler slaughterhouses under the federal inspection system from the state of Rio Grande do Sul (Southern Brazil).

The initial concentration of *Salmonella* spp. was provided by three studies in which *Salmonella* were counted before the bleeding step inside the slaughter line. Rivera-Pérez (2014) analyzed the *Salmonella* contamination of broiler at bleeding and at different points of slaughter process and reported counts of 6.1 log CFU/carcass at bleeding. Assuming an average chicken carcass weight of 2.24 kg (data reported by MAPA), the *Salmonella* contamination was 0.0027 log CFU/g. Borges et al., (2019) used qPCR to quantify *Salmonella* in Brazilian poultry

slaughterhouses under the federal inspection and reported 1.6 log CFU/mL of *Salmonella* in cloacal swabs. Finally, Kotula (1995) reported 6.28 log CFU/g of *Salmonella* spp. on broiler chickens entering the processing plant and this study was used in the "Risk assessments of *Salmonella* in eggs and broiler chickens" published by FAO/WHO (2002). These three values were used to describe initial *Salmonella* concentration by Pert distribution.

Temperature and time of chicken meat chain were obtained from Hessel et al. (2020b). The research evaluated the Brazilian chicken meat chain from slaughter to consumption in terms of time and temperature data, according to the CAC/GL 78-2011 flowchart and fitted the data into distributions.

The increase in pathogen concentration in the modules was modeled using the predictive models generated by Pessoa et al. (2020). In the work of Pessoa at al. (2020) the growth of five *Salmonella* serovars (*S.* Enteritidis, *S*. Typhimurium, *S*. Heidelberg, *S.* Saintpaul and *S.* Infantis) were predicted on raw chicken meat. These serovars were used because they are important to the Brazilian poultry production (Brasil, 2012). The growth of *Salmonella* on chicken meat was assumed between 7ºC and 45ºC, since at 5°C no *Salmonella* growth was observed on raw chicken meat by Pessoa et al. (2020).

Step 2: Scalding: Our model considered the reduction found in scientific literature for *Salmonella* concentration at this step. Table 3 describe the referred studies and the distribution inputted in the model. At scalding, hot water is used to facilitate the removal of feathers at defeathering step (Barbut, 2015; Buhr et al., 2014; Russell, 2003), and, the temperatures of the hot water used for scalding (50 to 60 °C) can contribute to stop and reduce the bacterial counts present on skin (Rouger, Tresse, & Zagorec, 2017). Indeed, according to Hessel et al. (2020b) the temperature of scalding in Brazilian slaughterhouses was 55.00ºC (median).

Step 3: Defeathering: At this step, the feathers are mechanically removed from the scalded birds. Usually, in large-scale slaughterhouses, feathers are removed using rotating rubber fingers (Barbut, 2015). However, the rubber fingers surface have been showed as source of bacterial contamination inside slaughterhouse (Clouser, Doores, Mast, & Knabel, 1995; Nde, McEvoy, Sherwood, & Logue, 2007; Rouger et al., 2017; Veluz, Pitchiah, & Alvarado, 2012). Our model considered that

Salmonella spp. numbers increase due to chicken meat defeathering at slaughter. Table 4 summaries the considered scientific data used in the model.

Step 4: Head removing: At this step the chicken head is removed from the carcass and the head is commonly rinsed after each insertion (Barbut, 2015). No studies were found considering the microbial impact of this step, so this step was not considered in the model.

Step 5: Evisceration: During evisceration, the carcasses are open by cut and the digestive tract, giblets and inedible viscera are separated from the carcasses. This step is considered important for cross-contamination because the microbiota present at high counts in the digestive tract of chicken can contaminate other carcasses (Rouger et al., 2017). In our model, the increase in *Salmonella* counts due to chicken meat slaughter steps is showed in Table 4. For time and temperature parameters, the data of Hessel et al. (2020b) was considered and the increase in *Salmonella* level was predicted by the model of Pessoa et al. (2020).

Step 6: Spray washing: At this point, spray showers are positioned in such a way to remove organic matter (blood, viscera, fecal contamination) generally from the top to down of carcasses (Barbut, 2015). In Brazil chicken carcasses can only be washed using potable water, without the present of sanitizers. Only 0.2 to 2.0 ppm of free chlorine may be present because these levels are allowed for potable water. Cold water can remove or contribute to a decontaminating effect by rinsing the surface of carcasses, removing bacterial contamination (Demirok et al., 2013). The reduction in *Salmonella* counts due to spray washing considered in our model is described in Table 3.

Step 7: Chiller: In Brazil prior to chilling, there is the visual inspection and laboratory screening conducted by inspectors to ensure the absence of fecal contamination on carcasses and the integrity of carcasses (MAPA, 1998). Inspection is essential to ensure that only wholesome birds that are free of disease reach the market (Barbut, 2015). In Brazil, the inspection is carried out by MAPA officers.

The most common methods used to chill chicken meat include the immersion of carcasses in cold water, air chilling, spray chilling (intermittent water spraying), and

combinations of these methods (Barbut, 2015). At this step several technologies have been studied to reduce of *Salmonella* on chicken carcasses, such as application of physical and antibacterial treatments (Loretz, Stephan, & Zweifel, 2010). In Brazil, the most common chiller method is carried out by immersion the carcasses in cold water, and, after this step the carcass temperature must to be reduced to below 7 ºC (MAPA, 1998). According to Hessel et al. (2020b) the chiller step in several Brazilian companies occurs at 2.00 ± 1.14 ºC, not allowing *Salmonella* multiplication. Further, in our model the reduction on microbial load due to chiller was considered. The reduction by chiller is showed in Table 3.

Antibacterial treatments have been studied as interventions to reduce the bacterial load at slaughter poultry industry, however they are not allowed in Brazilian Slaughter houses until the time this study was done. Based on this kind of intervention is used in different countries and is referred in *Codex Alimentarius* CAC/GL 78-2011, we considered the reductions in our model. Loretz et. al (2010) conducted an extensive literature survey of antibacterial treatments applied in poultry industry and its bacterial reductions. The evaluated studies used physical, chemical or both methods to decontaminate chicken carcasses and scenarios were created to analyze the *Salmonella* reductions generated by these methods inside slaughter industry. The data of *Salmonella* reduction obtained by Lorentz et al (2010) were described by Pert distribution and inputted in the model (Table 3).

Step 8: Cutting: At this step the carcass is cut in pieces manually or by machinery. This transformation operations increase the surface area of meat in contact with working surfaces and air, allowing cross-contamination. Consequently, the level of bacteria is higher in transformed products than on primary cuts (Álvarez-Astorga, Capita, Alonso-Calleja, Moreno, & García-Fernández, 2002; Rouger et al., 2017; Veluz et al., 2012). Corroborating to this information, the meta-analysis of Hessel et al. (2020c) showed that entire carcasses had lower *Salmonella* prevalence than chicken meat cuts (19.7% vs. 23%). At this step, temperature parameter reported by Hessel et al. (2002b) was used, which showed that the average temperature of cutting rooms in Brazilian slaughter houses was 10.52 ± 0.54 ºC, what is in accordance to the Brazilian legislation. The increase in *Salmonella* level was predicted by the model of Pessoa et al. (2020).

Step 9: Cross-contamination: The cross contamination between carcasses or cuts may occur by direct contact or through contact with contaminated surfaces, being the foodstuff production, food handlers, air and equipment surfaces the main sources of contamination (Álvarez-Astorga et al., 2002; Rouger et al., 2017; Veluz et al., 2012). In our study, it was assumed that cross-contamination occurs during cutting, from hands to chicken and from surfaces to chicken meat. In the present study, the modules published by Smadi & Sargent (2012) were used, because they summarized the transfer rates due to cross-contamination studies for *Salmonella*.

Step 10: Packaging: Whole poultry, cut up parts or minced meat are commonly packaged in small retail packages or large combos for industrial use. At packaging, the chicken meat or cuts are pack in primary and secondary packaging. The packaging material is design to protect moisture loss due to evaporation, cross contamination by bacteria, dust and foreign matter, while also provides room for the processor to advertise its product (e.g., company's logo, recipes, nutritional information) (Barbut, 2015). According to Hessel et al. (2020b), in Brazil, at this step, the average temperatures were 2.51 ± 1.65 ºC, what is in agreement with the Brazilian legislation, which tolerates a maximum temperature of 4 ºC (MAPA, 1998). In this work, for time and temperature parameters, the study of Hessel et al. (2020b) was considered and the increase in *Salmonella* level was predicted by the model of Pessoa et al. (2020).

Step 11: Storage: Until transport, the packaged chicken meat must be storage in cold chambers at temperature bellow 4 ºC (MAPA, 1998). The period varied from 0 to 24 hours, according to slaughter processing and sells demand. For time and temperature parameters, the work of Hessel et al. (2020b) was considered and the increase in *Salmonella* level was predicted by the model of Pessoa et al. (2020).

Step 12: Transport from industry to Distribution Center: In Brazil, usually transport occurs under chilled temperature (<4 ºC) and takes maximum 8.50 hours (Hessel et al. (2020b). For time and temperature parameters, the work of Hessel et al. (2020b) was considered and the increase in *Salmonella* level was predicted by the model of Pessoa et al. (2020).

Distribution Center

Step 13: Storage: At distribution centers, chicken meat is storage at chilled temperatures and arranged to be distributed to food channels. For time and temperature parameters the work of Hessel et al. (2020b) was considered and the increase in *Salmonella* level was predicted by the model of Pessoa et al. (2020).

Step 14: Transport from Distribution Center to Retail: Transport occurs under chilled temperature at average of 2.71 ± 1.97 ºC and takes maximum 8.50 hours (Hessel et al., 2020b). The increase in *Salmonella* level was predicted by the model of Pessoa et al. (2020).

Retail

Step 15: Storage: The storage in retail outlets occurred at mean temperatures of 2.05 ± 3.12 ºC. Usually, chicken meat products stay no longer than 5 days at retail until be sold (Hessel et al., 2020b). The short time at storage is related to the short shelf-life of chilled chicken meat, which according to our study is between 11 to 14 days. In our work, for time and temperature parameters, the work of Hessel et al. (2020b) was considered and the increase in *Salmonella* level was predicted by the model of Pessoa et al. (2020).

Step 16: Exposition: At this step, the highest temperature observed in the study of Hessel et al. (2020b) was 10.40 ºC. The break of cold chain at this step might be related to the open doors of chilling equipment where chicken meat is stored for sale. Studies showed that *Salmonella* prevalence on poultry meat may vary with the distribution channels. In our work, for time and temperature parameters the work of Hessel et al. (2020b) was considered and the increase in *Salmonella* level was predicted by the model of Pessoa et al. (2020).

Step 17: Transport from Retail to Home: Usually in Brazil, the transport from retail stores to homes occurred at environmental temperatures and takes maximum 2 hours (Hessel et al., 2020). Hessel et al. (2020) accessed the National Institute of Meteorology of Brazil and observed the maximum and minimum official temperature registered by the official weather station from each capital state, between 01 January

2017 and 29 November 2018. The reported average of environmental temperature of 25.67 ± 3.27 ºC was considered in the present study (INMET, n.d.) (Hessel et al., 2020b). This parameter was described by Pert distribution and inputted in the model. The increase in *Salmonella* level was predicted by the model of Pessoa et al. (2020).

Home

Step 18: Storage: The temperature and duration of storage at Brazilian homes were obtained from previous studies (Hessel et al., 2019; Silva, D. L. D., Celidonio, & Oliveira, 2008). These studies demonstrated adequate storage time and temperatures of 3 ºC and 24 h, respectively. These parameters were inputted in the model as Pert distribution. The increase in *Salmonella* level was predicted by the model of Pessoa et al. (2020).

Step 19: Cooking: The thermal inactivation of *Salmonella* occurs during chicken meat cooking. Despite the consumption of raw or undercooked food is one of the main causes of salmonellosis, in Brazil, it is not usual the consumption of raw or undercooked chicken meat. (Hessel et al., 2019; Roccato et al., 2015). Thus, in our model it was assumed that all viable *Salmonella* on chicken meat were inactivated by adequate cooking, i.e. using temperatures ≥ 70^{o}C.

Step 20: Cross-contamination: In our model it was considered that cooked chicken meat could be contaminated by *Salmonella* after thermal processing due to cross-contamination inside kitchens, before the consumption. Cross contamination from contaminated kitchen surfaces, fresh ingredients and chicken meat, due to the lack of personnel hygiene possess greater significance in spreading *Salmonella* (Carrasco et al., 2012; Luber, 2009). In our QMRA, the models published by Smadi & Sargent (2012) were used to simulate the postcooking cross-contamination inside home kitchens. These authors summarized the transfer rate from cross-contamination studies for *Salmonella.* The transfer rate considered in our model was from raw chicken to hands, then from hands to cooked chicken and the transfer from raw chicken to cutting board (or plate) and from cutting board (or plate) to cooked chicken. The probability that hands were not washed after handling raw meat and the probability that the same cutting board (or utensil) was used for raw meat and for cooked meat was estimated based on the publication of Hessel et al. (2019). The

work assessed the behavior and handling practices during purchase, storage, preparation practices, and consumption of chicken meat, from purchase to consumption, inside homes in Brazil.

Step 21: Consumption: At this step we considered the amount of chicken meat consumed by Brazilian people in order to predict the probability of human exposure to *Salmonella* via consumption of chicken meat. The data regarding consumption habits of chicken meat at Brazilian homes was obtained from Hessel et al. (2019). According to this publication, 96.79% of Brazilian population eats chicken meat at least twice a week and at least 60 grams per serving. Considering the Brazilian population of 210.147.125 (IBGE, 2019), we assumed that 203.40.140.229 inhabitants are exposed to *Salmonella* due to the consumption of chicken meat. Is highlighted that these data might be super estimated, since it is based on one study and the consumption data was assumed without adjustments for food losses, i.e. cooking and plate loss. Therefore, per capita consumption statistics may overestimate actual chicken meat consumed by the Brazilian population.

2.3.2 Slaughter to consumption at food service model *inputs*

The risk assessment model comprised 20 modules assuming the consumption of frozen chicken meat at food services (Figure 1 and Table 2).

The initial steps of frozen chicken meat chain are equal to the chilled chicken chain already described in this study (from bleeding to cutting at slaughterhouse) (item 2.3.1). Then, following steps (freezing at slaughterhouses to storage at food service) differs in terms of time and temperature. Hessel et al. (2020b) reported that these steps in Brazil occurs at a maximum of −14.50 ºC, which is in accordance with Brazilian legislation. The recommendation is to maintain frozen chicken at a temperature not exceeding −12 ± 2 °C (MAPA, 1998). These temperatures do not allow *Salmonella* multiplication.

Step 17: Tawing: We assumed that frozen carcasses need to be thaw prior cooking. According to the Brazilian legislation, chicken must be thawed inside cooling equipment at temperatures below 5 ºC or by direct heating followed by immediate consumption (BRASIL, 2004). The results of Hessel et al. (2020b) showed that Brazilian food services thawed frozen chicken meat at average temperatures of 5.04

± 8.04 ºC, however, some inadequate temperatures (35.00 ºC) were observed, which might be related to the defrosting procedure at room temperature. In our study, for time and temperature parameters the work of Hessel et al. (2020b) was considered and the increase in *Salmonella* level was predicted by the model of Pessoa et al. (2020).

Step 18: Cooking: In Brazil, food services must thermally process food using temperatures higher than 70 ºC, and the cooked food must be distributed at 60 ºC or more (BRASIL, 2004). At these temperatures, viable *Salmonella* cells are inactivated, and if there was no cross-contamination, chicken meat is considered safe for consumption. The study of Hessel et al. (2020b) showed that these temperatures in Brazilian food services were adequate, thus, in our model it was assumed that chicken meat was sufficiently cooked to inactivate all *Salmonella* cells.

Step 19: Cross-contamination: For cross-contamination at food service it was used the modules of Smadi & Sargent (2012), as previously described in item 2.3.1 (Step 20: Cross-contamination at home).

Step 20: Consumption: In this module we used the same data of consumption at Brazilian home, which came from the study of Hessel et al. (2019). In this step it was estimated the number of *Salmonella* present on the chicken meat at consumption and the probability of human infection by *Salmonella* due to the consumption of chicken meat at Brazilian food service. The data might super estimated the risk, since the model do not considered adjustments for food loses.

2.4 RISK CHARACTERIZATION

The main objective of the present study was to perform a QMRA to calculate the risk of salmonellosis due to consumption of chicken meat contaminated with *Salmonella* spp. in Brazil, and also to identify different risk-mitigation strategies, thereby decreasing the influence of variation (variability and uncertainty).

Several scenarios were tested to evaluate the impact of specific risk management improvements on the final risk of infection by *Salmonella* in the overall Brazilian population. The effectiveness of each of the tested risk mitigation strategies

was measured as the percentage of reduction on the predicted probability of infection due to consumption of one meal keeping all other original parameters and probability distributions constant.

The baseline scenario represents the current knowledge regarding Brazilian chicken meat chain and *Salmonella.* The initial prevalence of 4.04% was used because this number came from the analyses of the results of the Official Pathogen Reduction Program conducted by the Ministry of Agriculture, Livestock, and Supply in Rio Grande do Sul State, Southern Brazil (de Lima, Isolan, Hessel, Pessoa, & Tondo, 2018).

Scenario 1, 2, 3 and 4 considered other *Salmonella* prevalence numbers on chicken meat in Brazil. At scenario 1 the initial *Salmonella* prevalence was 14,04%. This data was obtained by the meta-analysis of *Salmonella* prevalence in Brazil (Hessel et al. 2020a). Scenario 2 considered the *Salmonella* prevalence of 17.76%, considered the official data of MAPA. This data was obtained from the official report after the estimative of the *Salmonella* prevalence on chicken carcasses of the five Brazilian Macro-regions between 2017 and 2018. Scenario 3 considered the *Salmonella* prevalence on chicken of 49% (Borges et al., 2019). Scenario 4 considered the reduction of 50% of the initial baseline prevalence (4.04%), i.e. a prevalence around 2.0%.

Scenario 5 considered additional *Salmonella* contamination at defeathering and evisceration (Table 4). These slaughter steps are considered source of *Salmonella* due to cross contamination, either by the equipment or food handlers (Clouser et al., 1995; Cox, Berrang, & Cason, 2000; Göksoy, Kirkan, & Kök, 2004; Nde et al., 2007; Nidaullah et al., 2017; Rivera-Pérez, Barquero-Calvo, & Zamora-Sanabria, 2014).

Scenario 6 considered 50% of reduction in *Salmonella* concentration at the beginning of slaughter process (at bleeding step). Furthermore, scenario 7 consider a 50% reduction in the prevalence and concentration of *Salmonella* on chicken meat at bleeding step. These scenarios were tested since previous QMRA showed that reducing *Salmonella* concentration and/or prevalence the risk of infection might be reduced (Pouillot et al., 2012; Smadi & Sargeant, 2013; Zhu et al., 2017).

Scenario 8 considered the maintenance of chicken meat at maximum temperature of 7ºC since the industry to home storage. The maintenance of chicken meat in cold chain is identified as a key procedure to reduce microbial load, including

pathogens. In addition, the violation of cold chain, specially at the end of food chain is related to foodborne outbreaks occurrence (Aung & Chang, 2014; Carneiro, Cabello, Albuquerque-Junior, Jain, & Candido, 2015; Lundén et al., 2014).

Scenario 9 considered the reduction in *Salmonella* counts at scalding, spray washing and chilling step at slaughter (Table 3). Previous works showed that scalding, washing and chiller treatments can be effective in reducing bacterial loads and prevent multiplication (Loretz et al., 2010; Matias, Pinto, Cossi, & Nero, 2010; Northcutt, Cason, Smith, Buhr, & Fletcher, 2006; Rivera-Pérez et al., 2014).

Scenario 10, 11, 12, 13 and 14 considered the reduction of *Salmonella* concentration due to antimicrobial treatments used in chiller at slaughter industry. It was considered *Salmonella* reductions after chicken meat treatment with acetic acid, lactic acid, cetylpyridinium chloride, trisodium phosphate and combined treatment (Table 3, adapted from Loretz et al., 2010).

Finally, scenario 15 considered the 50% reduction in prevalence and concentrations of *Salmonella* on chicken meat at slaughter industry, added of a *Salmonella* reduction due to scalding, spray washing, chiller and the maintenance of the cold chain at maximum temperature of 7ºC until chicken meat was cooked. In this scenario, all factors which might contribute to reduce the risk of salmonellosis were considered in the model.

The QMRA model was built in an Excel spreadsheet (Microsoft, Redmond, WA) and simulated using @Risk software version 7.5 (Palisade Corporation). Hundred thousand iterations were performed using Latin Hypercube sampling to increase the reliability of the software to reproduce the defined distributions. Every simulation of the current model represented a randomly chosen chicken meat from the bleeding step at slaughter industry thought all steps of chain until consumption. Spearman's correlation coefficients were used for sensitivity analysis of the baseline model and scenarios to determine the effect of input variables on the probability of illness per serving and on the number of illness cases in Brazil. Variability and uncertainty were described for each parameter included in the model at Tables 1 and 2. Each parameter was designed as C, if was calculated; F, when fixed; V, when considered as variability and U, for uncertainty. These parameters are discussed below.

3. Results and Discussion

3.1. QMRA of *Salmonella* in chicken meat consumed at home

Baseline scenario of chicken meat consumed at home considered chilled chicken meat slaughtered under official inspection in Brazil and sell at retail. In this baseline scenario we assumed a *Salmonella* prevalence of 4.04% on raw chicken and an average concentration of 1.6 log CFU/g. Time and temperature parameters of the slaughter process followed information obtained in a previous study of Hessel et al. (2020) which evaluated real situations inside industries. In this baseline scenario it was assumed that inside slaughter industry, scalding, spray washing and chiller steps reduced *Salmonella*, while at cut step cross-contamination occurs from food handlers' hands to chicken and from surfaces to chicken. At home, we assumed that the thermal processing during cooking of chicken meat inactivates all viable *Salmonella*, thus the numbers of *Salmonella* on chicken meat just before consumption comes from cross-contamination occurred after cooking (Figure 1 and Table 1).

Considering this baseline scenario, the risk of infection by *Salmonella* due to the consumption of chicken meat at homes in Brazil was 0.008092 (8.092 in 1000 exposures), ranging from 0.003923 to 0.032498 (Table 5, Figure 2Aa). The number of cases of infection in the population exposed was 163,809,233.27, which represents 77.94% of the Brazilian population, considering the reported population of 210,147,125 (IBGE, 2019). Epidemiological data reports only 274 salmonelosis in Brazil between 2009 and 2018 (BRASIL, 2019) showing that, possibly, the risk calculated is super estimated and might does not reflect the Brazilian reality of salmonellosis. Although the predicted rate of salmonellosis is not in agreement with the recent epidemiological data, one should not automatically conclude that the QMRA does not provides reliable predictions. Thus, it is important to validate not only the final output but also the inputs and outputs of each unit operation and pathogen event in the model (Oscar, 2004). Some possible reasons for this overestimation are: (1) The high percentage of population exposed, linked to the huge chicken meat consumption on Brazil (Hessel et al., 2019). Indeed, the risk due to consumption of contaminated chicken meat with *Salmonella* depends on the set of circumstances

existing during the consumption of each individual meal. Thus, the calculation assumes the risk per serving where each chicken meat eaten had the same predicted risk of illness, and that the risk from each exposure was independent from other exposures (Pouillot et al., 2016), (2) the additional contamination in slaughter process at defeathering and evisceration, that might not occur at every chicken meat production, (3) the combination of different cross-contamination inputs (cross-contamination at industry and at home due to unwashed hands, and cutting boards after handling raw chicken meat), that might not occur always, (4) The model considered the worst scenario, assuming that 1 CFU of *Salmonella* would be able to cause salmonellosis, which, in reality, higher infective doses of *Salmonella* have been reported Akil et al (2019), (5) the real numbers of salmonellosis cases occurred in Brazil probably are under estimated due to subnotification.

Spearman's rank correlation was performed to identify predictive parameters that were most highly correlated with the contamination of *Salmonella* per serving (Figures 2Ab). The sensitivity of the baseline model outcomes to input values and model parameters, revealed that the mean number of infection cases was the most sensitive to *Salmonella* ingestion (Spearman's rank correlation = 0.93), cross-contamination through cutting board at industry (Spearman's rank correlation = 0.24), initial *Salmonella* prevalence (Spearman's rank correlation = 0.1), temperature at storage at retail (Spearman's rank correlation = 0.07) and rate of bacterial transfer from raw chicken to cutting board (Spearman's rank correlation = 0.05). Otherwise, hand washing at home and temperature during transport from distribution center to retail contribute to decrease the risk on *Salmonella* infection due to chicken meat consumption (Spearman's rank correlation = - 0.05 and - 0.01).

The higher risk of *Salmonella* infection related to the quantity of *Salmonella* ingested is related to the circumstances existing during the consumption of each individual meal. It means that people who consume fewer chicken meat have lower risk of contamination by *Salmonella,* what is very obvious. However, it is important to highlight that the number of infection cases do not represent the number of salmonellosis cases, but the number of people contaminated by at least 1 cell of *Salmonella*. The amount of *Salmonella* cells necessary to cause salmonellosis, i.e. the infective dose, can influence the development or not the disease, because salmonellosis occurrence will be depends on the immunity of each person contaminated by the pathogen, the severity of the *Salmonella* strain (Pouillot et al.,

2016), among other factors. For example, if a 10.000 CFU of *Salmonella* is assumed as the infective dose for salmonellosis, 100 cfu transferred to chicken meat by cross-contamination would take 96 hours at 10 ºC before causing the disease. If 1.000.000 CFU is assumed as the infective dose, the chicken meat should be exposed for 151 hours at the same temperature to cause salmonellosis. These exposure periods were not observed inside homes or food services (Hessel et al, 2019), suggesting that this kind of situation may reflect better the reality and also can explain why the calculated numbers of salmonellosis were very different from official epidemiological data about salmonellosis in Brazil.

Cutting board at industry was identify as an important source of cross-contamination during the slaughtering process. Bacteria from contaminated carcasses can adhere to surfaces and form biofilms, providing a source of cross-contamination to the next carcasses processed on that surface (Akil & Ahmad, 2019; Carrasco, Morales-Rueda, & García-Gimeno, 2012; Chaves, Han, Dawson, & Northcutt, 2011; Voidarou et al., 2007). In the study of Rivera-Pérez, Barquero-Calvo, Zamora-Sanabria (2014) carcasses at cut step showed higher incidence of *Salmonella* than to carcasses at chiller step (Rivera-Pérez et al., 2014). This finding emphasizes that *Salmonella* prevalence and concentration should be controlled inside slaughter processing, aiming to prevent or reduce cross-contamination. One key point to reduce the risk of salmonellosis is the initial prevalence of *Salmonella* of carcasses that get in the industrial facilities. When chicken flocks are infected at farm level, *Salmonella* can be carried in the gastrointestinal tract of chickens, and be readily transferred to carcasses through fecal contamination during slaughter and further spread to other carcasses by cross-contaminated (Arsenault, Letellier, Quessy, Normand, & Boulianne, 2007; Jeong, Chon, Kim, Song, & Seo, 2018). Other important preventive measure is to maintain high slaughter hygiene practices and adequately performed disinfection procedures, since *Salmonella* cross-contamination and recontamination episodes have been connected to poor sanitation practices, poor equipment design, and deficient control of ingredients (Carrasco et al., 2012).

Regard cross-contamination at home, these pathway of cross contamination and recontamination of chicken meat from contaminated kitchen surfaces, fresh ingredients, and due to the lack of personnel hygiene was deemed to possess greater significance in spreading *Salmonella* than undercooking poultry meat, since

depends greatly of the good hygiene practices adopted by food handlers (Hessel et al., 2019; Rajan, Shi, & Ricke, 2017). Hessel et al (2020c) when analyzing the behavior of chicken consumers observed lower good hygiene practices, demonstrating that people declared failures even in simple hygienic practices inside homes. Regarding the use of different utensils to prepare different foods or wash them during preparation, 23.40% of the respondents declared to use the same utensil for different preparations without washing them. Proper washing of cooking utensils between preparation of raw and cooked foods or ready-to-eat foods was pointed as important procedures to eliminate cross-contamination route inside kitchen (Kusumaningrum, Van Asselt, Beumer, & Zwietering, 2016). Poor GHP are among the most important factors resulting in foodborne illnesses and risk assessment has been demonstrated this procedure as one of the most important factors contributing to increase the risk of foodborne diseases (Hessel et al., 2020c; Smadi & Sargeant, 2013).

Temperature during transport from distribution center to retail are strongly influenced by the open-and-close door during retail supply (Hessel et al., 2020b). This procedure might be important since temperature influence directly the microbial multiplication. Thus, the maintenance of cold chain is important to keep the microbial load down, and, consequently reduce the risk.

Scenario 1 to 3 were performed considering other *Salmonella* prevalence in chicken meat reported in Brazil. Baseline scenario consider initial prevalence of 4.04%, while in scenario 1, the initial *Salmonella* prevalence was 14.04%. Considering this prevalence, the risk of infection increases 132.30% (1.87 in 100 exposures). At scenario 2 (*Salmonella* prevalence of 17.76%) the calculated risk was 3.542 in 100 servings, representing 337.71 % of increase to the baseline scenario. Finally, at scenario 3, which considered *Salmonella* prevalence of 49%, the risk of infection was 9.85 in 100 servings (1117.25% higher than baseline scenario). Table 3 shows all these results. Is possible to observe that the increase in initial *Salmonella* prevalence is closely related to the increment of risk of salmonellosis. The close relation of prevalence and risk might be related to the influence in prevalence in our model, as observed in the sensitivity analyzes described in Figure 2.

Scenario 4, which considered 50% reduction of the initial baseline *Salmonella* prevalence, demonstrated that the risk of infection per serving was 0.004042 (4.042

in 1000 consumes), representing 50.05 % of risk reduction, comparing to the baseline scenario. Other QMRA models reported different outcomes for *Salmonella* prevalence reduction. For example, Smadi & Sargent (2012) demonstrated that a 50% reduction of the initial *Salmonella* prevalence in Canadian retail reduced the risk to 0%, while in a Chinese scenario, the reduction of *Salmonella* prevalence on chicken meat from 41.8% to 8.8% resulted in a risk reduction of only 10% (Zhu et al., 2017).

The comparison of different QMRA models for *Salmonella* on chicken should be made with attention, since different models have investigated different contamination pathways and made different types of assumptions. For example, Akil et al. (2019) performed a QRMA for human salmonellosis resulting from the consumption of broiler chicken. The authors combined the outputs of all modules considered in their model, then carried out risk simulation using with 10,000 iterations, while our model used 100,000 iterations. Additionally, Jeong et al. (2018) analyzed the effects of variables of the retail-to-table pathway on the likelihood of salmonellosis due to broiler consumption at Korean slaughterhouses. The growth rate used for Jeong et al (2018) is the Juneja et al. (2007) model. The predictive model of Juneja et al. (2007) was developed using raw chicken tenderloins without indigenous microbiota, which is different to what was done in our study, because Pessoa et al. (2019) dis not inactivate chicken meat microbiota. Furthermore, careful should be taken when comparing different QMRA, since formulas, units, and input settings used could be different and influence the final risk results.

Scenario 5 simulates an increase in *Salmonella* contamination due to defeathering and evisceration. The risk in this scenario was 8.097 in 1000, similar to baseline scenario. *Salmonella* are commonly present on a portion of the poultry carcasses in the processing plants and if proper measures are not taken, this may lead to cross contamination (Berghaus et al., 2013; Wu et al., 2014). Guo et al. (2011) showed that each of processing steps may act as a source of *Salmonella* contamination or cross contamination, and it has been reported that 48% of chicken and 17% of turkey were contaminated during processing. The lack of influence in the risk could be related to the low increase in *Salmonella* load at these steps (0.8 log UFC/g for defeathering and 0.15 log UFC/g for evisceration (Table 4). These numbers came from scientific studies, but inside slaughter industry these values might be variable among industries and chicken batches. In addition, other factors

besides defeathering and evisceration have related to raise *Salmonella* contamination inside slaughter industry, such as unhygienic carcass handling, soiled slaughter equipment, spreading contaminated water from scalding and chilling steps and spreading of wastes from post evisceration (Boonprasert et al., 2014; Hamidi et al., 2014; Schambach et al., 2014; Wang et al., 2014; Folk, 2008; Henry et al., 2012).

Scenario 6 evaluated the 50% reduction of *Salmonella* concentration and the risk was not modified, when compared to baseline scenario. It shows that only reducing *Salmonella* loads at beginning of industrial process there was no significant reduction in risk of salmonellosis. In the Canadian QMRA, the reduction of concentration levels of *Salmonella* at retail by 50%, reduced the risk of salmonellosis in 40% (Smadi & Sargeant, 2013). However, at scenario 7 where both the prevalence and concentration were reduced in 50%, the risk was proportionally reduced in 50.09% (4.039 in 1000 cases). The reduction obtained by reducing both prevalence and concentration by 50%, was probably due to prevalence reduction. Despite the absence of risk reduction by concentration, reduction in microbial load is essential to reduce cross-contamination (Arsenault et al., 2007; Carrasco et al., 2012; Rajan et al., 2017). Thus, this strategy must not be ignored.

Scenario 8 represents the implementation of cold chain since industry up to homes. The risk reduction was 1.70%, comparing to baseline scenario. The low contribution of temperature in risk reduction is explained by the comply temperature of chicken meat chain in Brazil and cold chain maintenance (Hessel et al., 2020b). The cold chain is responsible for the food safety and preservation, since perishable foods, such as chilled chicken meat, in the proper temperature range slow microbiological decay processes. Failing in cold chain may stimulate the growth of pathogens and spoilage microorganisms. In case of failing in cold chain and food consumption, foodborne illnesses could occur (Mercier, Villeneuve, Mondor, & Uysal, 2017).

At scenarios 9 to 14 the risks were not reduced in comparison to baseline scenario, showing that reducing *Salmonella* loads due to processing or by antimicrobial agents applied at chilling at slaughterhouse have no effect in risk reduction. Similar result was obtained in a Korean QMRA in which authors demonstrated that chlorination, despite reduces *Salmonella* concentration on chicken, did not reduce the risk of salmonellosis (Jeong et al., 2018). This result clarifies that any solely intervention to reduce *Salmonella*-contaminated carcasses,

despite reduce microbial load, would not necessarily reduce the risk of acquiring the disease, as already reported by other researches (Jeong et al., 2018). Despite the absence in risk reduction, antimicrobial agents contributed to the decrease of *Salmonella* contamination on carcasses. Low contamination on chicken meat carcasses is necessary to trade.

The last scenario, scenario 15, was the most effective in reducing risk of salmonellosis. At this scenario, the risk was reduced by 50.95% i.e. 3.96 in 1000 exposures. In addition, the number of cases of infection was only 38.92%. This represents the number of Brazilian population that will have contact with *Salmonella* due to chicken meat consume, being this percentage 39.02% lower than baseline (77.94%). As expected, at this scenario it was obtained the greatest risk reduction. This result was closely that the obtained at scenario 5 and 7, where the *Salmonella* prevalence was reduced by 50% and prevalence and concentration by 50%, respectively. At scenario 7, the percentage reduction in infection was 50.05% and at scenario 5 the reduction was 50.09%. For the other scenarios tested, the risk was not reduced above 1.70%. These results demonstrated that, the reduction in contamination due to processing or by antimicrobial agents applied at slaughter industry, would not directly reduce the risk of salmonellosis in Brazil. Therefore, this reduction in *Salmonella* concentration contributes to the decrease of *Salmonella* contamination on carcasses.

3.2 QMRA of *Salmonella* in chicken meat consumed at food service

Baseline scenario of chicken meat consumed at food services considered frozen chicken meat slaughtered under official inspection in Brazil. Initial *Salmonella* prevalence of 4.04% and concentration of 1.6 log CFU/g were assumed as baseline scenario. Time and temperature data were obtained from Hessel et al. (2019). In this scenario, it was assumed that scalding, spray washing and chiller steps reduced *Salmonella* counts during processing, while, at portioning, cross-contamination occurs from hands to chicken and from surfaces to chicken. Frozen chain, from slaughter industry to thawing at food services was not broken, so no *Salmonella* multiplication was considered. At food services, the thawing procedure followed time and temperature obtained from Brazilian food services. Thermal inactivation during cooking of chicken meat was assumed to completely inactivate viable *Salmonella*,

thus the numbers of *Salmonella* on chicken meat just before consumption comes from cross-contamination post cooking (Figure 1 and Table 2).

The risk of infection by *Salmonella* due to chicken meat consumed at food services in Brazil was, 0.007959 (7.95 cases in 1000 exposures), ranging from 0,003939 to 0,040614 (Table 4, Figure 2Ba). The number of cases of infection per year in the population exposed was 163,809,233.27 (77.94% of Brazilian population). In our study, we assumed that the entire Brazilian population ate at food services, since no report of frequency consumption of chicken meat at food services in the country was found. It noticed that the model of risk of infection by *Salmonella* due to consumption of chicken meat at food services, despite consider frozen chicken meat and lower modules, was slightly higher than at home (8.09 cases in 1000 exposure vs 7.09 cases in 1000 exposure). Despite the slight difference in risk, the number of cases of infection per year in the population exposed was equal for both places.

Spearman's rank correlation identifies the predictive parameters most highly correlated with the contamination of *Salmonella* per serving (Figures 2Bb). The mean number of infections was the most sensitive to serving size (Spearman's rank correlation = 0.97), *Salmonella* prevalence and use the same cutting board at food services also contributes to increase the risk (Spearman's rank correlation = 0.112 and 0.08, respectively).

Proper washing hands at food services and industries and the use of the same cutting board at industry contributes to decrease the risk (Spearman's rank correlation = -0.0112 for all). Indeed, GHP are among the most important factors resulting in foodborne illnesses and risk assessment have been demonstrated these procedures as one of the most important to increase the risk (Smadi & Sargent, 2012).

Similar to the baseline result, risk scenarios were similar to chicken meat consumed at home and food services. Table 3 shows in scenario 1 the initial *Salmonella* prevalence of 14.04%. The risk of infection was 1.84 in 100 cases, increased by 132.27% than baseline scenario. At scenario 2, 3, 5, 6, 9 to 14, risk result was lower than consumer at home. Scenario 4, 7, 8 and 15 the risk was equal to the baseline.

The category variability and uncertainty were described for each parameter included in the model at Tables 1 and 2. Each parameter was designed as C, if was calculated; F, when fixed; V, when considered as variability and U, for uncertainty. Uncertainty is the variance occurring as a consequence of limited information in the dataset, variability is intrinsic variance inherent to the living systems (CAC/GL, 2007; Vásquez, Busschaert, Haberbeck, Uyttendaele, & Geeraerd, 2014). The *Salmonella* prevalence was considered as uncertainty in our model, since this parameter may vary among farm environments and feed production facilities due to processing, storage and transportation. Our exposure assessment considered the whole chicken produced under official inspection in Brazil. However, consumers are exposed to different chicken cuts and preparations, which may not be included in our model. For variability, the initial *Salmonella* concentration, reduction or increase due to slaughter steps, probability of cross-contamination, temperatures and time were considered. Currently, one of the biggest challenges to carrying out a QMRA is the collection of real data on the whole flowchart of chicken production, because trade is branched and involves many companies located in different regions. Thus, we sustain the necessity of more studies regard real processing data to support to reduce variability and uncertainty in the model.

4. Conclusion

To the best of our knowledge, this is the first QMRA of *Salmonella* on chicken meat in Brazil. The results obtained in this study demonstrated that *Salmonella* represent measurable risks on chicken meat in Brazil, being singly higher when consumed at home or at food services. Our results suggest that the best strategy to reduce salmonellosis in Brazil is by the reduction in *Salmonella* initial prevalence. This indicates that the pathogen must be controlled at farm level. Some of these strategies include technological advances and improved preventive strategies associated to hygiene and control measures at various production levels. In addition, reduction due to antimicrobial agents applied at slaughter, despite showed no effect in risk reduction, is essential to fair trade. Finally, the highest risk reduction was observed when all factors which might contribute to reduce the risk of salmonellosis were applied and used together, demonstrating that risk mitigation strategies need to

be managed by different stakeholders of chicken meat chain in order to improve food safety.

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Authors' Contributions

In this research, all authors contributed effectively. Claudia Titze Hessel, João Pedro Pessoa and Susana de Oliveira Elias conducted the data collection, organization, analysis, interpretation and wrote the manuscript. Mateus Silva de Lima and Leonardo Werlang Isolan contribute to the data collection. Eduardo Cesar Tondo supervised the project and reviewed the manuscript.

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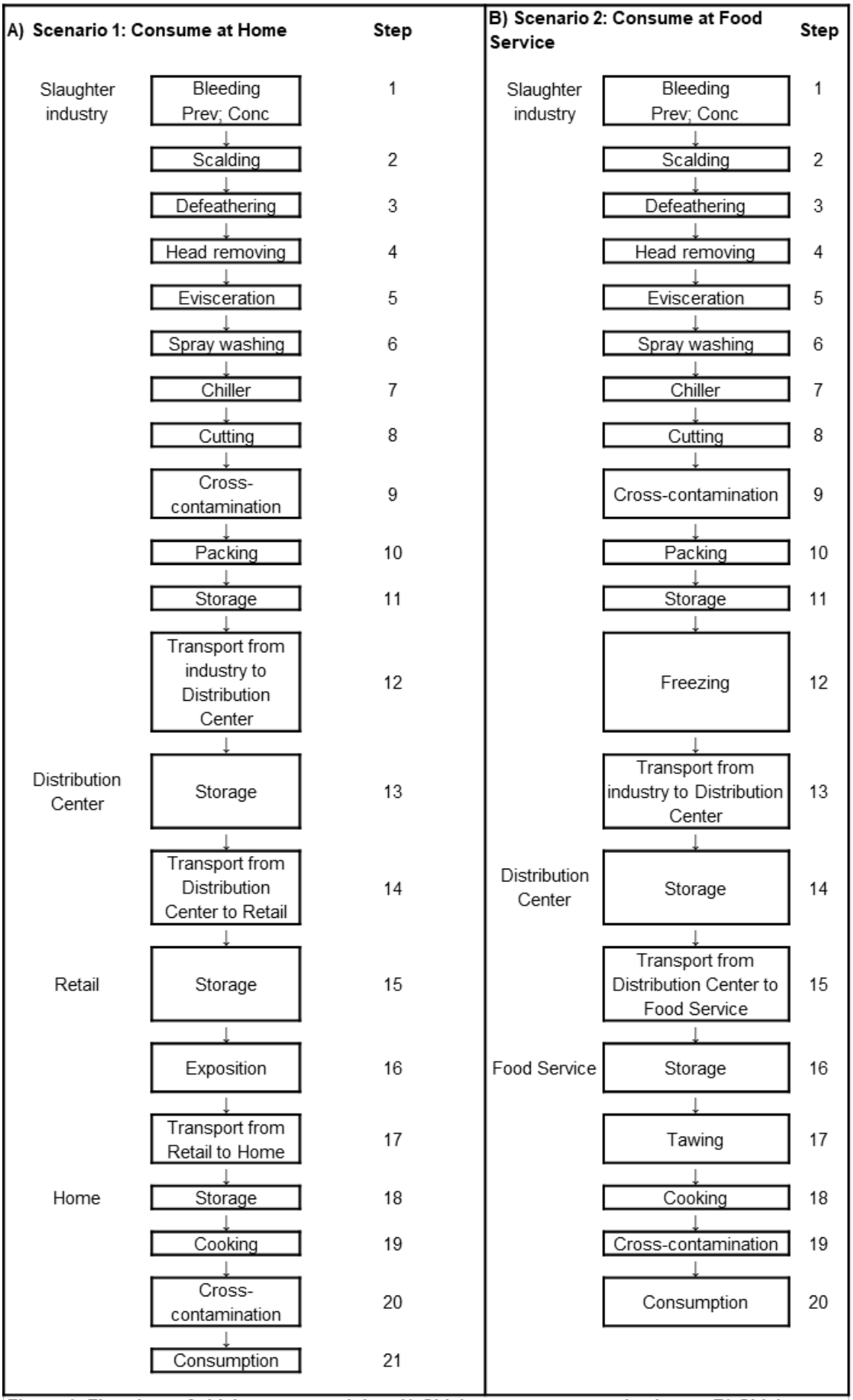

Figure 1: Flowchart of chicken meat modules. A) Chicken meat consumed at home; B) Chicken meat consumed at food services.

Table 1: Parameters and their distributions in the Risk Assessment model of chicken meat consumed at home.

Local	Step	Variable	Symbol	Category[a]	Distribution, value, or formula	Unit	Reference
Slaughter industry	Bleeding	Prevalence	Pi	U	RiskBeta(3124;74046)	%	De Lima et al., 2018
		Concentration	Ci	V	RiskPert(0,0027;1,6;6,28)	log10MPN/g	Rivera-Perez, 2014; Kotula, 1995;Borges 2019
		Temperature at bleeding	T1	V	RiskPert(20;30;40)	°C	Hessel et al., 2020b (submitted)
		Time at bleeding	t1	V	RiskPert(0,066029;0,073301;0,083301)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth	Lg1	C	((0,0204*(T1+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during bleeding	G1	C	t1*Lg1	log CFU/g	Calculated
		Level after bleeding	L1	C	Ci+G1	log CFU/g	Calculated
	Scalding	Reduction by scalding	R1	V	RiskPert(0,65;1.38;2.17)	log CFU/g	Table 3
		Level after scalding	L2	C	L1-R1	log CFU/g	Calculated
	Defeathering	Temperature during defeathering	T3	V	RiskPert(30;45;58)	°C	Hessel et al., 2020b (submitted)
		Time of defeathering	t3	V	RiskLaplace(0,0248;0,1197)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth	Lg3	C	((0,0204*(T3+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during defeathering	G3	C	t3*Lg3	log CFU/g	Calculated
		Level after defeathering	L3	C	L2+G3	log CFU/g	Calculated
	Head removing	NA					
	Evisceration	Temperature during evisceration	T4	V	RiskPert(35;40;50)	°C	Hessel et al., 2020b (submitted)
		Time of evisceration	t4	V	RiskPert(0,032112;0,1667;0,2667)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)

	Logarithmic growth	Lg4	C	((0,0204*(T4+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
	Growth during evisceration	G4	C	t4*Lg4	log CFU/g	Calculated
	Level after evisceration	L4	C	L3+G4	log CFU/g	Calculated
Spray washing	Reduction bt washing	R2	V	RiskPert(0,05;0,4;0,6)	log CFU/g	Table 3
	Level after spray washing	L5	C	L4-R2	log CFU/g	Calculated
Chiller	Reduction bt washing	R3	V	RiskPert(0,9;1,2;1,3)	log CFU/g	Table 3
	Level after chiller	L6	C	L5+G6	log CFU/g	Calculated
Cutting	<u>Transfer from hands to chicken</u>					Smadi & Sargent, 2012
	Probability that hands are not washed	HW_Prop	V	RiskPert(0,0009;0,0017;0,09)	Proportion	Smadi & Sargent, 2012
	Were hands washed? (1=yes; 0=no)	HW	V	RiskBinomial(1;1-(HW Prop))		Smadi & Sargent, 2012
	Proportion transferred from hands to chicken (Bacterial transfer rate)	Prop_HC	V	RiskPert(0,001; 0,089; 0,529)	Proportion	Smadi & Sargent, 2012
	Number on chicken via hands	Num_CC1	C	SE(HW_Prop=1;0;G6*HW)	log cfu/g	Smadi & Sargent, 2012
	<u>Transfer from surfaces to chicken</u>					Smadi & Sargent, 2012
	Probability that contaminated utensils used in chicken without washing	Brd_use_Prob	V	RiskPert(0,115;0,2342;0,345)	Proportion	Smadi & Sargent, 2012
	Were boards used for other foods? (1 = y, 0 = n)	Brd_use	V	RiskBinomial(1;Brd_use_Prob)		Smadi & Sargent, 2012
	Proportion transferred from boards to cooked chicken (bacterial transfer rate)	Prob_BC	V	RiskPert(0,105; 0,194; 0,424)	Proportion	Smadi & Sargent, 2012
	Number on cooked chicken from raw chicken via board (or utensils)	Num_CC2	C	SE(Brd_use=0;0;G6*Prob_BC)	log cfu/g	Smadi & Sargent, 2012
	Total number of *Salmonella* via cross-contamination	Num_XC	C	Num_CC1+Num_XC	log cfu/g	Smadi & Sargent, 2012
	Temperature during cutting	T7	V	RiskNormal(10,52444;0,53525)	°C	Hessel et al., 2020b (submitted)
	Time in cutting	t7	V	RiskLognorm(1,5419;12,358;RiskShift(0,0015687))	h	Hessel et al., 2020b (submitted)
	Parameter b growth model	b	F	0,0204	raiz de Log CFU/day/°C	Pessoa et al., 2020 (submitted)
	Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
	Logarithmic growth	Lg7	C	((0,0204*(T7+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
	Growth during cutting	G7	C	t7*Lg7	log cfu/g	Calculated
	level after cutting	L7	C	L6+G7	log cfu/g	Calculated
Packing	Temperature during packing	T8	V	RiskNormal(10,52444;0,53525)	°C	Hessel et al., 2020b (submitted)

		Time in packing	t8	V	RiskLognorm(1,5419;12,358;RiskShift(0,0015687))	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	raiz de Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth	Lg8	C	((0,0204*(T8+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during packing	G8	C	T8*Lg8	log cfu/g	Calculated
		level after packing	L8	C	L7+G8	log cfu/g	Calculated
	Storage	Temperature during packing	T9	V	RiskWeibull(5,0043;9,2368;RiskShift(-6,0288))	°C	Hessel et al., 2020b (submitted)
		Time in packing	t9	V	RiskPert(0;12;48)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	raiz de Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth	Lg9	C	((0,0204*(T9+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during packing	G9	C	t9*Lg9	log cfu/g	Calculated
		level afterpacking	L9	C	L8+G9	log cfu/g	Calculated
Transport from slaughter industry to distribution center	Transport 1	Temperature during transport 1	T10	V	RiskLogistic(3,00165;0,94038)	°C	Hessel et al., 2020b (submitted)
		Time of transportat 1	t10	V	RiskPert(0,08; 0,83; 8,5)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth	Lg10	C	((0,0204*(T10+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during transport 1	G10	C	t10*Lg10	log CFU/g	Calculated
		Level after transport 1	L10	C	L9+G10	log CFU/g	Calculated
Distribution Center	Storage at Distribution Center	Temperature at storage at distribution center	T11	V	RiskLogistic(1,7396;1,8843)	°C	Hessel et al., 2020b (submitted)
		Time at storage at distribution center	t11	V	RiskPert(1; 12; 48)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth at storage at distribution center	Lg11	C	((0,0204*(T11+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during at storage at distribution center	G11	C	t11*Lg11	log CFU/g	Calculated

		Level after at storage at distribution center	L11	C	L10+G11	log CFU/g	Calculated
Transport from distribution center to retail	Transport 2	Temperature at transport 2	T12	V	RiskNormal(0,9964;5,2524)	°C	Hessel et al., 2020b (submitted)
		Time of transport 2	t12	V	RiskPert(0,08; 0,83; 8,5)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth at transport 2	Lg12	C	((0,0204*(T12+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during transport 2	G12	C	t12+Lg12	log CFU/g	Calculated
		Level after transport 2	L12	C	L11+G12	log CFU/g	Calculated
Retail	Storage at Retail	Temperature at storage at retail	T13	V	RiskNormal(2,0506;3,1182)	°C	Hessel et al., 2020b (submitted)
		Time at storage at retail	t13	V	RiskPert(0; 48; 120)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth at storage at retail	Lg13	C	((0,0204*(T13+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during storage at retail	G13	C	t13*Lg13	log CFU/g	Calculated
		Level after storage at retail	L13	C	L12+G13	log CFU/g	Calculated
	Exposition at Retail	Temperature at exposition at retail	T14	V	RiskLognorm(34,477;9,8909;RiskShift(-28,636))	°C	Hessel et al., 2020b (submitted)
		Time at exposition at retail	t14	V	RiskPert(0; 48; 120)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth at exposition at retail	Lg14	C	((0,0204*(T14+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during exposition at retail	G14	C	t14*Lg14	log CFU/g	Calculated
		Level after exposition at retail	L14	C	L13+G14	log CFU/g	Calculated
Transport from retail to home	Transport 3	Temperature at transport 3	T15	V	RiskBetaGeneral(3,7711;2,4378;4,2357;38,275)	°C	Hessel et al., 2020b (submitted)
		Time of transport 3	t15	V	RiskDiscrete({0,5;1;2;4};{722;254;19;3})	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)

		Logarithmic growth at transport 3	Lg15	C	((0,0204*(T15+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during transport 3	G15	C	t15*Lg15	log CFU/g	Calculated
		Level after transport 3	L15	C	L14+G15	log CFU/g	Calculated
Home	Storage at home	Temperature at storage at home	T16	V	RiskPert(3,04;6;10,8)	°C	Hessel et al., 2020b (submitted)
		Time of storage at home	t16	V	RiskDiscrete({60;4;12;24;48;72};{141;205;191;184;132;135})	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T16	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth at storage at home	Lg16	C	((0,0204*(T16+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during storage at home	G16	C	t16*Lg16	log CFU/g	Calculated
		Level after storage at home	L16	C	L15+G16	log cfu/g	Calculated
	Cooking	Level at cooking	L17	F	0	log cfu/g	Hessel et al., 2020b (submitted)
	Cross-contamination	<u>Transfer from raw chicken to hands</u>					
		Propotion transferred from raw chicken to hands (bacterial transfer rate)	Prop_CH2	V	RiskPert(0,011;0,065;0,261)	Proportion	Smadi & Sargent, 2012
		Number on hands	Num_H2	C	L16*Prop_CH2	log CFU/g	Calculated
		<u>Transfer from hands to cooked chicken</u>					
		Probability that hands are not washed after handling raw meat	HW_Prop2	V	RiskPert(0,0009;0,0017;0,09)	Proportion	Hessel et al., 2019
		Were hands washed? (1=yes; 0=no)	HW2	C	RiskBinomial(1;1-HW_Prop2)		Calculated
		Proportion transferred from hands to cooked chicken (Bacterial transfer rate)	PropHC	V	RiskPert(0,001; 0,089; 0,529)	Proportion	Smadi & Sargent, 2012
		Number on cooked chicken from raw chicken via hands	Num_CC3	C	SE(HW2=1;0;HW2*Num_H2)	log CFU/g	Calculated
		<u>Transfer from raw chicken to cutting board (or plate)</u>					
		Proportion transferred from raw chickento cutting board (Bacterial transfer rate)	Prop_CB2	V	RiskPert(0,03;0,075;0,309)	Proportion	Smadi & Sargent, 2012
		Number on board	Num_B2	C	Num_CC3*Prop_CB2	log CFU/g	Calculated
		<u>Transfer from cutting board (or plate) to cooked chicken</u>					
		Probability that same board (or utensils) used for raw meat is used for cooked chicken without washing	Brd_use_Prob	V	RiskPert(0,115;0,2342;0,345)	Proportion	Hessel et al., 2019
		Were boards used for other foods? (1 = y, 0 = n)	Brd_use	C	RiskBinomial(1;Brd_use_Prob)	Proportion	Calculated

	Proportion transferred from boards to cooked chicken (bacterial transfer rate)	Prob_BC	V	RiskPert(0,105; 0,194; 0,424)	Proportion	Hessel et al., 2019
	Number on cooked chicken from raw chicken via board (or utensils)	Num_CC4	C	SE(Brd_use=0;0;Num_Br2*Brd_use)	log CFU/g	Calculated
	Total number of *Salmonella* via cross-contamination	Num_XC	C	Num_CC3+Num_CC4	log CFU/g	Calculated
Consumption of chicken, determination of dose-response relationship, probability of illness and number of cases	Serving size	g	V	RiskPert(60;200;480)	S	Hessel et al., 2019
	Level of pathogen (non-log)	CFU/g	V	10^Num_XC	CFU	Calculated
	Dose per serving	CFU	V	S*CFU	D	Calculated
	Parameter alpha	No units	F	0,1324	α	FAO/WHO 2002
	Parameter beta	No units	F	51,45	β	FAO/WHO 2002
	Probability of infection single dose	%	C	1-(1+D/β)^-α	Pisd	Calculated
	Risk of infection per serving	Risk	C	RiskOutput()+1-(1-Pisd*Pi)	RiS	Calculated
	Population Brasil	Inhabitants	F	210.147.125	Pop	IBGE, 2019
	% of population eating chicken meat	%	F	96,79	%eat	Hessel et al., 2019
	Population eating chicken meat in Brazil	Inhabitants	C	Pop*%eat/100	Peat	Calculated

[a] C, calculation; F, fixed; V, variability; U, uncertainty

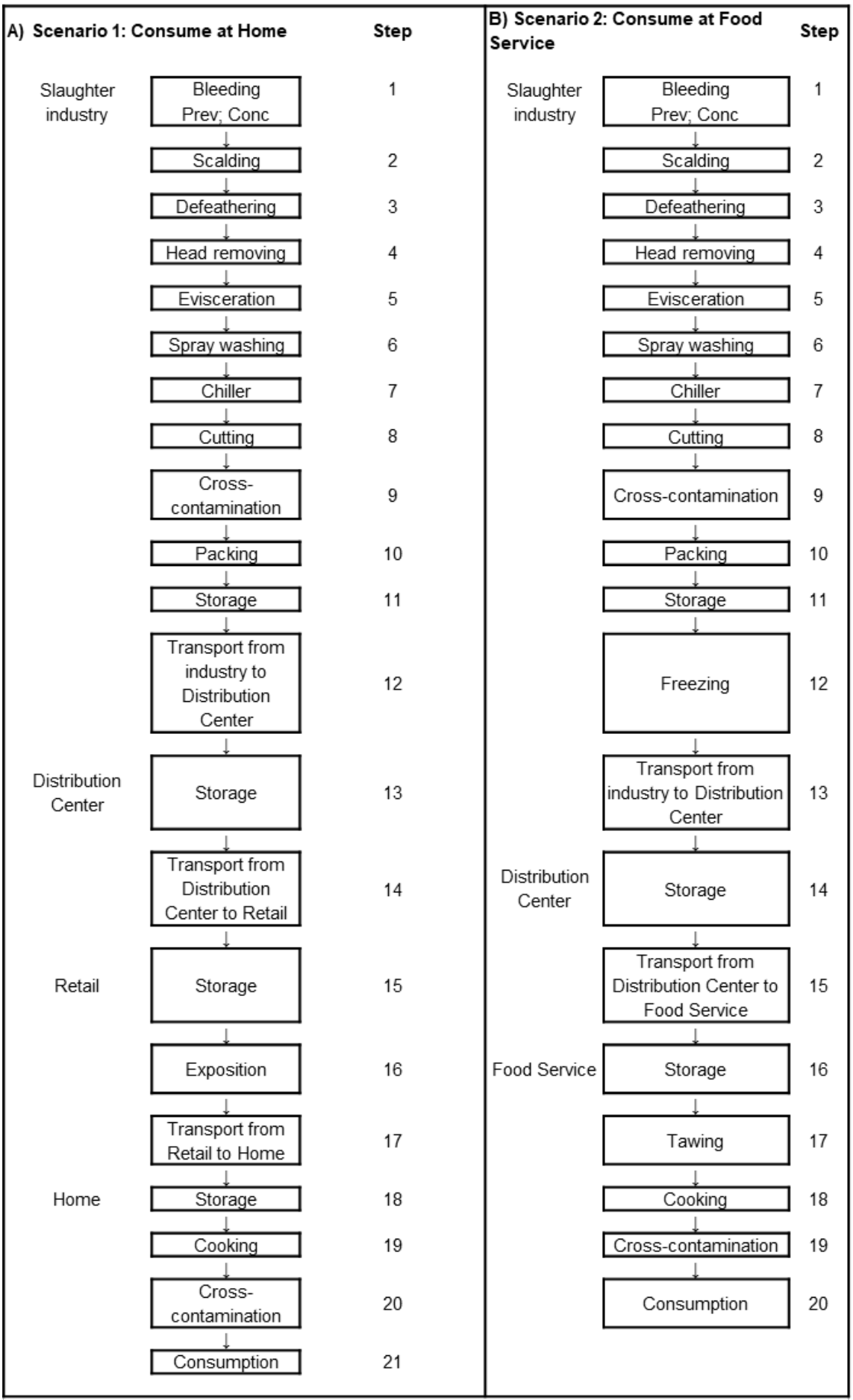

Figure 1: Flowchart of chicken meat modules. A) Chicken meat consumed at home; B) Chicken meat consumed at food services.

Table 1: Parameters and their distributions in the Risk Assessment model of chicken meat consumed at home.

Local	Step	Variable	Symbol	Category[a]	Distribution, value, or formula	Unit	Reference
Slaughter industry	Bleeding	Prevalence	Pi	U	RiskBeta(3124;74046)	%	De Lima et al., 2018
		Concentration	Ci	V	RiskPert(0,0027;1,6;6,28)	log10MPN/g	Rivera-Perez, 2014; Kotula, 1995;Borges 2019
		Temperature at bleeding	T1	V	RiskPert(20;30;40)	°C	Hessel et al., 2020b (submitted)
		Time at bleeding	t1	V	RiskPert(0,066029;0,073301;0,083301)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth	Lg1	C	((0,0204*(T1+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during bleeding	G1	C	t1*Lg1	log CFU/g	Calculated
		Level after bleeding	L1	C	Ci+G1	log CFU/g	Calculated
	Scalding	Reduction by scalding	R1	V	RiskPert(0,65;1.38;2.17)	log CFU/g	Table 3
		Level after scalding	L2	C	L1-R1	log CFU/g	Calculated
	Defeathering	Temperature during defeathering	T3	V	RiskPert(30;45;58)	°C	Hessel et al., 2020b (submitted)
		Time of defeathering	t3	V	RiskLaplace(0,0248;0,1197)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth	Lg3	C	((0,0204*(T3+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during defeathering	G3	C	t3*Lg3	log CFU/g	Calculated
		Level after defeathering	L3	C	L2+G3	log CFU/g	Calculated
	Head removing	NA					
	Evisceration	Temperature during evisceration	T4	V	RiskPert(35;40;50)	°C	Hessel et al., 2020b (submitted)
		Time of evisceration	t4	V	RiskPert(0,032112;0,1667;0,2667)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)

	Logarithmic growth	Lg4	C	((0,0204*(T4+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
	Growth during evisceration	G4	C	t4*Lg4	log CFU/g	Calculated
	Level after evisceration	L4	C	L3+G4	log CFU/g	Calculated
Spray washing	Reduction bt washing	R2	V	RiskPert(0,05;0,4;0,6)	log CFU/g	Table 3
	Level after spray washing	L5	C	L4-R2	log CFU/g	Calculated
Chiller	Reduction bt washing	R3	V	RiskPert(0,9;1,2;1,3)	log CFU/g	Table 3
	Level after chiller	L6	C	L5+G6	log CFU/g	Calculated
Cutting	<u>Transfer from hands to chicken</u>					Smadi & Sargent, 2012
	Probability that hands are not washed	HW_Prop	V	RiskPert(0,0009;0,0017;0,09)	Proportion	Smadi & Sargent, 2012
	Were hands washed? (1=yes; 0=no)	HW	V	RiskBinomial(1;1-(HW Prop))		Smadi & Sargent, 2012
	Proportion transferred from hands to chicken (Bacterial transfer rate)	Prop_HC	V	RiskPert(0,001; 0,089; 0,529)	Proportion	Smadi & Sargent, 2012
	Number on chicken via hands	Num_CC1	C	SE(HW_Prop=1;0;G6*HW)	log cfu/g	Smadi & Sargent, 2012
	<u>Transfer from surfaces to chicken</u>					Smadi & Sargent, 2012
	Probability that contaminated utensils used in chicken without washing	Brd_use_Prob	V	RiskPert(0,115;0,2342;0,345)	Proportion	Smadi & Sargent, 2012
	Were boards used for other foods? (1 = y, 0 = n)	Brd_use	V	RiskBinomial(1;Brd_use_Prob)		Smadi & Sargent, 2012
	Proportion transferred from boards to cooked chicken (bacterial transfer rate)	Prob_BC	V	RiskPert(0,105; 0,194; 0,424)	Proportion	Smadi & Sargent, 2012
	Number on cooked chicken from raw chicken via board (or utensils)	Num_CC2	C	SE(Brd_use=0;0;G6*Prob_BC)	log cfu/g	Smadi & Sargent, 2012
	Total number of *Salmonella* via cross-contamination	Num_XC	C	Num_CC1+Num_XC	log cfu/g	Smadi & Sargent, 2012
	Temperature during cutting	T7	V	RiskNormal(10,52444;0,53525)	°C	Hessel et al., 2020b (submitted)
	Time in cutting	t7	V	RiskLognorm(1,5419;12,358;RiskShift(0,0015687))	h	Hessel et al., 2020b (submitted)
	Parameter b growth model	b	F	0,0204	raiz de Log CFU/day/°C	Pessoa et al., 2020 (submitted)
	Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
	Logarithmic growth	Lg7	C	((0,0204*(T7+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
	Growth during cutting	G7	C	t7*Lg7	log cfu/g	Calculated
	level after cutting	L7	C	L6+G7	log cfu/g	Calculated
Packing	Temperature during packing	T8	V	RiskNormal(10,52444;0,53525)	°C	Hessel et al., 2020b (submitted)

		Time in packing	t8	V	RiskLognorm(1,5419;12,358;RiskShift(0,0015687))	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	raiz de Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth	Lg8	C	((0,0204*(T8+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during packing	G8	C	T8*Lg8	log cfu/g	Calculated
		level after packing	L8	C	L7+G8	log cfu/g	Calculated
	Storage	Temperature during packing	T9	V	RiskWeibull(5,0043;9,2368;RiskShift(-6,0288))	°C	Hessel et al., 2020b (submitted)
		Time in packing	t9	V	RiskPert(0;12;48)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	raiz de Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth	Lg9	C	((0,0204*(T9+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during packing	G9	C	t9*Lg9	log cfu/g	Calculated
		level afterpacking	L9	C	L8+G9	log cfu/g	Calculated
Transport from slaughter industry to distribution center	Transport 1	Temperature during transport 1	T10	V	RiskLogistic(3,00165;0,94038)	°C	Hessel et al., 2020b (submitted)
		Time of transportat 1	t10	V	RiskPert(0,08; 0,83; 8,5)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth	Lg10	C	((0,0204*(T10+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during transport 1	G10	C	t10*Lg10	log CFU/g	Calculated
		Level after transport 1	L10	C	L9+G10	log CFU/g	Calculated
Distribution Center	Storage at Distribution Center	Temperature at storage at distribution center	T11	V	RiskLogistic(1,7396;1,8843)	°C	Hessel et al., 2020b (submitted)
		Time at storage at distribution center	t11	V	RiskPert(1; 12; 48)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth at storage at distribution center	Lg11	C	((0,0204*(T11+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during at storage at distribution center	G11	C	t11*Lg11	log CFU/g	Calculated

		Level after at storage at distribution center	L11	C	L10+G11	log CFU/g	Calculated
Transport from distribution center to retail	Transport 2	Temperature at transport 2	T12	V	RiskNormal(0,9964;5,2524)	°C	Hessel et al., 2020b (submitted)
		Time of transport 2	t12	V	RiskPert(0,08; 0,83; 8,5)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth at transport 2	Lg12	C	((0,0204*(T12+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during transport 2	G12	C	t12+Lg12	log CFU/g	Calculated
		Level after transport 2	L12	C	L11+G12	log CFU/g	Calculated
Retail	Storage at Retail	Temperature at storage at retail	T13	V	RiskNormal(2,0506;3,1182)	°C	Hessel et al., 2020b (submitted)
		Time at storage at retail	t13	V	RiskPert(0; 48; 120)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth at storage at retail	Lg13	C	((0,0204*(T13+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during storage at retail	G13	C	t13*Lg13	log CFU/g	Calculated
		Level after storage at retail	L13	C	L12+G13	log CFU/g	Calculated
	Exposition at Retail	Temperature at exposition at retail	T14	V	RiskLognorm(34,477;9,8909;RiskShift(-28,636))	°C	Hessel et al., 2020b (submitted)
		Time at exposition at retail	t14	V	RiskPert(0; 48; 120)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth at exposition at retail	Lg14	C	((0,0204*(T14+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during exposition at retail	G14	C	t14*Lg14	log CFU/g	Calculated
		Level after exposition at retail	L14	C	L13+G14	log CFU/g	Calculated
Transport from retail to home	Transport 3	Temperature at transport 3	T15	V	RiskBetaGeneral(3,7711;2,4378;4,2357;38,275)	°C	Hessel et al., 2020b (submitted)
		Time of transport 3	t15	V	RiskDiscrete({0,5;1;2;4};{722;254;19;3})	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)

		Logarithmic growth at transport 3	Lg15	C	((0,0204*(T15+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during transport 3	G15	C	t15*Lg15	log CFU/g	Calculated
		Level after transport 3	L15	C	L14+G15	log CFU/g	Calculated
Home	Storage at home	Temperature at storage at home	T16	V	RiskPert(3,04;6;10,8)	°C	Hessel et al., 2020b (submitted)
		Time of storage at home	t16	V	RiskDiscrete({60;4;12;24;48;72};{141;205;191;184;132;135})	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T16	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth at storage at home	Lg16	C	((0,0204*(T16+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during storage at home	G16	C	t16*Lg16	log CFU/g	Calculated
		Level after storage at home	L16	C	L15+G16	log cfu/g	Calculated
	Cooking	Level at cooking	L17	F	0	log cfu/g	Hessel et al., 2020b (submitted)
	Cross-contamination	<u>Transfer from raw chicken to hands</u>					
		Propotion transferred from raw chicken to hands (bacterial transfer rate)	Prop_CH2	V	RiskPert(0,011;0,065;0,261)	Proportion	Smadi & Sargent, 2012
		Number on hands	Num_H2	C	L16*Prop_CH2	log CFU/g	Calculated
		<u>Transfer from hands to cooked chicken</u>					
		Probability that hands are not washed after handling raw meat	HW_Prop2	V	RiskPert(0,0009;0,0017;0,09)	Proportion	Hessel et al., 2019
		Were hands washed? (1=yes; 0=no)	HW2	C	RiskBinomial(1;1-HW_Prop2)		Calculated
		Proportion transferred from hands to cooked chicken (Bacterial transfer rate)	PropHC	V	RiskPert(0,001; 0,089; 0,529)	Proportion	Smadi & Sargent, 2012
		Number on cooked chicken from raw chicken via hands	Num_CC3	C	SE(HW2=1;0;HW2*Num_H2)	log CFU/g	Calculated
		<u>Transfer from raw chicken to cutting board (or plate)</u>					
		Proportion transferred from raw chickento cutting board (Bacterial transfer rate)	Prop_CB2	V	RiskPert(0,03;0,075;0,309)	Proportion	Smadi & Sargent, 2012
		Number on board	Num_B2	C	Num_CC3*Prop_CB2	log CFU/g	Calculated
		<u>Transfer from cutting board (or plate) to cooked chicken</u>					
		Probability that same board (or utensils) used for raw meat is used for cooked chicken without washing	Brd_use_Prob	V	RiskPert(0,115;0,2342;0,345)	Proportion	Hessel et al., 2019
		Were boards used for other foods? (1 = y, 0 = n)	Brd_use	C	RiskBinomial(1;Brd_use_Prob)	Proportion	Calculated

	Proportion transferred from boards to cooked chicken (bacterial transfer rate)	Prob_BC	V	RiskPert(0,105; 0,194; 0,424)	Proportion	Hessel et al., 2019
	Number on cooked chicken from raw chicken via board (or utensils)	Num_CC4	C	SE(Brd_use=0;0;Num_Br2*Brd_use)	log CFU/g	Calculated
	Total number of *Salmonella* via cross-contamination	Num_XC	C	Num_CC3+Num_CC4	log CFU/g	Calculated
Consumption of chicken, determination of dose-response relationship, probability of illness and number of cases	Serving size	g	V	RiskPert(60;200;480)	S	Hessel et al., 2019
	Level of pathogen (non-log)	CFU/g	V	10^Num_XC	CFU	Calculated
	Dose per serving	CFU	V	S*CFU	D	Calculated
	Parameter alpha	No units	F	0,1324	α	FAO/WHO 2002
	Parameter beta	No units	F	51,45	β	FAO/WHO 2002
	Probability of infection single dose	%	C	1-(1+D/β)^-α	Pisd	Calculated
	Risk of infection per serving	Risk	C	RiskOutput()+1-(1-Pisd*Pi)	RiS	Calculated
	Population Brasil	Inhabitants	F	210.147.125	Pop	IBGE, 2019
	% of population eating chicken meat	%	F	96,79	%eat	Hessel et al., 2019
	Population eating chicken meat in Brazil	Inhabitants	C	Pop*%eat/100	Peat	Calculated

[a] C, calculation; F, fixed; V, variability; U, uncertainty

Table 2: Parameters and their distributions in the Risk Assessment model of chicken meat consumed at food service

Local	Step	Variable	Symbol	Category[a]	Distribution, value, or formula	Unit	Reference
Slaughter industry	Bleeding	Prevalence	Pi	U	RiskBeta(3124;74046)	%	De Lima et al., 2018
		Concentration	Ci	V	RiskPert(0,0027;1,6;6,28)	log10MPN/g	Rivera-Perez, 2014; Kotula, 1995;Borges 2019
		Temperature at bleeding	T1	V	RiskPert(20;30;40)	°C	Hessel et al., 2020b (submitted)
		Time at bleeding	t1	V	RiskPert(0,066029;0,073301;0,083301)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth	Lg1	C	((0,0204*(T1+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during bleeding	G1	C	t1*Lg1	log CFU/g	Calculated
		Level after bleeding	L1	C	Ci+G1	log CFU/g	Calculated
	Scalding	Reduction by scalding	R1	V	RiskPert(0,65;1.38;2.17)	log CFU/g	Table 3
		Level after scalding	L2	C	L1-R1	log CFU/g	Calculated
	Defeathering	Temperature during defeathering	T3	V	RiskPert(30;45;58)	°C	Hessel et al., 2020b (submitted)
		Time of defeathering	t3	V	RiskLaplace(0,0248;0,1197)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth	Lg3	C	((0,0204*(T3+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during defeathering	G3	C	t3*Lg3	log CFU/g	Calculated
		Level after defeathering	L3	C	L2+G3	log CFU/g	Calculated
	Head removing	NA					

Evisceration	Temperature during evisceration	T4	V	RiskPert(35;40;50)	°C	Hessel et al., 2020b (submitted)
	Time of evisceration	t4	V	RiskPert(0,032112;0,1667;0,2667)	h	Hessel et al., 2020b (submitted)
	Parameter b growth model	b	F	0,0204	√ Log CFU/day/°C	Pessoa et al., 2020 (submitted)
	Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
	Logarithmic growth	Lg4	C	((0,0204*(T4+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
	Growth during evisceration	G4	C	t4*Lg4	log CFU/g	Calculated
	Level after evisceration	L4	C	L3+G4	log CFU/g	Calculated
Spray washing	Reduction bt washing	R2	V	RiskPert(0,05;0,4;0,6)	log CFU/g	Table 3
	Level after spray washing	L5	C	L4-R2	log CFU/g	Calculated
Chiller	Reduction bt washing	R3	V	RiskPert(0,9;1,2;1,3)	log CFU/g	Table 3
	Level after chiller	L6	C	L5+G6	log CFU/g	Calculated
Cutting	<u>Transfer from hands to chicken</u>					
	Probability that hands are not washed	HW_Prop	V	RiskPert(0,0009;0,0017;0,09)	Proportion	Smadi & Sargent, 2012
	Were hands washed? (1=yes; 0=no)	HW	V	RiskBinomial(1;1-(HW Prop))		Calculated
	Proportion transferred from hands to chicken (Bacterial transfer rate)	Prop_HC	V	RiskPert(0,001; 0,089; 0,529)	Proportion	Hessel et al., 2019
	Number on chicken via hands	Num_CC1	C	SE(HW_Prop=1;0;G6*HW)	log cfu/g	Calculated
	<u>Transfer from surfaces to chicken</u>					
	Probability that contaminated utensils used in chicken without washing	Brd_use_Prob	V	RiskPert(0,115;0,2342;0,345)	Proportion	Hessel et al., 2019
	Were boards used for other foods? (1 = y, 0 = n)	Brd_use	V	RiskBinomial(1;Brd_use_Prob)		Calculated
	Proportion transferred from boards to cooked chicken (bacterial transfer rate)	Prob_BC	V	RiskPert(0,105; 0,194; 0,424)	Proportion	Hessel et al., 2019
	Number on cooked chicken from raw chicken via board (or utensils)	Num_CC2	C	SE(Brd_use=0;0;G6*Prob_BC)	log cfu/g	Calculated
	Total number of *Salmonella* via cross-contamination	Num_XC	C	Num_CC1+Num_XC	log cfu/g	Calculated
	Temperature during cutting	T7	V	RiskNormal(10,52444;0,53525)	°C	Hessel et al., 2020b (submitted)
	Time in cutting	t7	V	RiskLognorm(1,5419;12,358;RiskShift(0,0015687))	h	Hessel et al.,

							2020b (submitted)
		Parameter b growth model	b	F	0,0204	raiz de Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth	Lg7	C	((0,0204*(T7+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during cutting	G7	C	t7*Lg7	log cfu/g	Calculated
		level after cutting	L7	C	L6+G7	log cfu/g	Calculated
	Packing	Temperature during packing	T8	V	RiskNormal(10,52444;0,53525)	°C	Hessel et al., 2019b (submitted)
		Time in packing	t8	V	RiskLognorm(1,5419;12,358;RiskShift(0,0015687))	h	Hessel et al., 2019b (submitted)
		Parameter b growth model	b	F	0,0204	raiz de Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth	Lg8	C	((0,0204*(T8+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during packing	G8	C	T8*Lg8	log cfu/g	Calculated
		level after packing	L8	C	L7+G8	log cfu/g	Calculated
	Freezing	NA					Hessel et al., 2019b (submitted)
	Storage	NA					Hessel et al., 2019b (submitted)
Transport from slaughter industry to distribution center	Transport 1	NA					Hessel et al., 2019b (submitted)
Distribution Center	Storage at Distribution Center	NA					Hessel et al., 2019b (submitted)

Transport from distribution center to food service	Transport 2	NA					Hessel et al., 2019b (submitted)
Food service	Storage at food service	NA					Hessel et al., 2019b (submitted)
	Tawing at Food Service	Temperature during Tawing at Food Service	T9	V	RiskLogistic(3,9565;3,0287)	°C	Hessel et al., 2020b (submitted)
		Time of Tawing at Food Service	t9	V	RiskPert(0;4;24)	h	Hessel et al., 2020b (submitted)
		Parameter b growth model	b	F	0,0204	raiz de Log CFU/day/°C	Pessoa et al., 2020 (submitted)
		Parameter T0 growth model	T0	F	1,647	°C	Pessoa et al., 2020 (submitted)
		Logarithmic growth	Lg9	C	((0,0204*(T9+T0))^2)	log CFU/g/h	Pessoa et al., 2020 (submitted)
		Growth during Tawing at Food Service	G9	C	t9*Lg9	log cfu/g	Calculated
		Level after Tawing at Food Service	L9	C	L8+G9	log cfu/g	Calculated
	Cooking	Level at cooking	L10	F	0	log cfu/g	Hessel et al., 2020b (submitted)
	Cross-contamination	<u>Transfer from raw chicken to hands</u>					
		Propotion transferred from raw chicken to hands (bacterial transfer rate)	Prop_CH	V	RiskPert(0,011;0,065;0,261)	Proportion	Smadi & Sargent, 2012
		Number on hands	Num_H	C	L9*Prop_CH	log CFU/g	Calculated
		<u>Transfer from hands to cooked chicken</u>					
		Probability that hands are not washed after handling raw meat	HW_Prop2	V	RiskPert(0,0009;0,0017;0,09)	Proportion	Hessel et al., 2019
		Were hands washed? (1=yes; 0=no)	HW	C	RiskBinomial(1;1-HW_Prop2)		Calculated
		Proportion transferred from hands to cooked chicken (Bacterial transfer rate)	PropHC	V	RiskPert(0,001; 0,089; 0,529)	Proportion	Smadi & Sargent, 2012
		Number on cooked chicken from raw chicken via hands	Num_CC1	C	SE(HW=1;0;HW*Num_H)	log CFU/g	Calculated

	<u>Transfer from raw chicken to cutting board (or plate)</u>					
	Proportion transferred from raw chicken to cutting board (Bacterial transfer rate)	Prop_CB	V	RiskPert(0,03;0,075;0,309)	Proportion	Smadi & Sargent, 2012
	Number on board	Num_B	C	Num_CC1*Prop_CB	log CFU/g	Calculated
	<u>Transfer from cutting board (or plate) to cooked chicken</u>					
	Probability that same board (or utensils) used for raw meat is used for cooked chicken without washing	Brd_use_Prob	V	RiskPert(0,115;0,2342;0,345)	Proportion	Hessel et al., 2019
	Were boards used for other foods? (1 = y, 0 = n)	Brd_use	C	RiskBinomial(1;Brd_use_Prob)		Calculated
	Proportion transferred from boards to cooked chicken (bacterial transfer rate)	Prob_BC	V	RiskPert(0,105; 0,194; 0,424)	Proportion	Hessel et al., 2019
	Number on cooked chicken from raw chicken via board (or utensils)	Num_CC2	C	SE(Brd_use=0;0;Num_Br2*Brd_use)	log CFU/g	Calculated
	Total number of *Salmonella* via cross-contamination	Num_XC	C	Num_CC1+Num_CC2	log CFU/g	Calculated
Consumption of chicken, determination of dose-response relationship, probability of illness and number of cases	Serving size	g	V	RiskPert(60;200;480)	S	Hessel et al., 2019
	Level of pathogen (non-log)	CFU/g	V	10^Num_XC	CFU	Calculated
	Dose per serving	CFU	V	S*CFU	D	Calculated
	Parameter alpha	No units	F	0,1324	α	FAO/WHO 2002
	Parameter beta	No units	F	51,45	β	FAO/WHO 2002
	Probability of infection single dose	%	C	1-(1+D/β)^-α	Pisd	Calculated
	Risk of infection per serving		C	RiskOutput()+1-(1-Pisd*Pi)	RiS	Calculated
	Population Brasil	Inhabitants	F	210.147.125	Pop	IBGE, 2019
	% of population eating chicken meat	%	F	96,79	%eat	Hessel et al., 2019
	Population eating chicken meat in Brazil	Inhabitants	C	Pop*%eat/100	Peat	Calculated

[a] C, calculation; F, fixed; V, variability; U, uncertainty

Table 3: Summary of *Salmonella* spp. reduction due to chicken meat slaughter steps and treatment and its distribution inputed in the model.

Slaughter Step	*Salmonella* spp.reduction (log CFU/g)	Reference	Distribution
Scalding	1.35	Lillard, 1989	RiskPert(0,65;1,38;2,17)
	0.65	Goksoy, 2004	
	0.90	Althaus, 2017	
	1.12	Xiao et al., 2019	
	1.38	Xiao et al., 2019	
	2.17	Xiao et al., 2019	
	0.46	Borges et al, 2019	
Spray washing	0.55	Lillard 1989	RiskPert(0,05;0,4;0,6)
	0.39	Geonaras & Von Holy, 2000	
	0.50	Goksoy 2004	
	0.60	Gonzalez-Miret 2006	
	0.05	Althaus 2017	
Chilling	- 0.90	Geonaras & Von Holy, 2000	RiskPert(0,9;1,2;1,3)
	-1.20	MAPA, 2019	
	-1.30	Industry*	
Treatment	***Salmonella* spp.reduction (log CFU/g)**	**Reference**	**Distribution**
Acetic acid			RiskPert(0,2;0,8;1,4)
	0,18	Jiménez, Caliusco, Tiburzi, Salsi, and Pirovani (2007)	
	0,2	Jiménez, Caliusco, Tiburzi, Salsi, and Pirovani (2007)	
	0,12	Jiménez, Caliusco, Tiburzi, Salsi, and Pirovani (2007)	
	0,2	Jiménez, Caliusco, Tiburzi, Salsi, and Pirovani (2007)	
	1,4	Fabrizio et al. (2002)	
	0,8	Fabrizio et al. (2002)	
Lactic acid			RiskPert(0,18;2,7;2,2)
	2	Hwang and Beuchat 1995	
	0,8	Anang et al. (2007)	
	1,7	Anang et al. (2007)	
	2,2	Yang et al. (1998)	
	0,18	Xiong et al. (1998)	
Cetylpyridinium chloride			RiskPert(0,001;1;1,9)
	0,001	Yang et al. (1998)	
	0,06	Wang et al. (1997)	
	0,03	Wang et al. (1997)	
	1,5	Xiong et al. (1998)	
	1,9	Xiong et al. (1998)	
	1	Kim and Slavik (1996)	
	1,6	Kim and Slavik (1996)	
	0,9	Kim and Slavik (1996)	
	1,7	Kim and Slavik (1996)	
Trisodium phosphate			RiskPert(0,03;1,4;2,4)
	1,7	Hwang and Beuchat (1995)	

	1,4	Whyte et al. (2001)	
	2,3	Mullerat et al. (1994)	
	2,2	Kim and Slavik (1994)	
	2,1	Xiong et al. (1998)	
	2,2	Xiong et al. (1998)	
	0,03	Wang et al. (1997)	
	0,05	Wang et al. (1997)	
	1,4	Fabrizio et al. (2002)	
	0,9	Fabrizio et al. (2002)	
	0,6	Li et al. (1994)	
	0,9	Li et al. (1994)	
Combined treatments			RiskPert(0,8;1,7;7)
Sodium carbonate + hot water			
	1,8	Rodriguez de Ledesma et al. (1996)	
Sodium carbonate + electricity			
	1	Li et al. (1994)	
Sodium chlorite + electricity			
	0,9	Li et al. (1994)	
	1	Li et al. (1994)	
Sodium hypochlorite + acidic electrolyzed			
	0,8	Northcutt et al. (2007)	
Trisodium phosphate + electricity			
	1,6	Li et al. (1994)	
	1,9	Li et al. (1994)	
Trisodium phosphate + hot water			
	1,9	Rodriguez de Ledesma et al. (1996)	
Chlorine + acetic acid			
	2	Fabrizio et al. (2002)	
Chlorine + trisodium phosphate			
	2	Fabrizio et al. (2002)	
Lauric acid + potassium hydroxide			
	3,4	Hinton and Cason (2008)	
Levulinic acid + sodium dodecyl sulfate			
	7	Zhao et al. (2009)	
Salmide® + EDTA			
	1,7	Mullerat et al. (1994)	
	2,7	Mullerat et al. (1994)	
Salmide® + sodium lauryl sulfate			
	1,2	Mullerat et al. (1994)	
	1,7	Mullerat et al. (1994)	
Salmide® + trisodium phosphate			
	3	Mullerat et al. (1994)	

* Data provided by industry records.

Table 4: Summary of *Salmonella* spp. increase due to chicken meat slaughter steps.

Slaughter Step	***Salmonella* spp. increase (log CFU/g)**	Reference	Distribution
Defeathering	0.07 0.68	Lillard, 1989 Goksoy, 2004	RiskPert(0,68;0,8;0,88)
Evisceration	0.09 0.31	Geonaras & Von Holy, 2000 Althaus 2017	RiskPert(0,09;0,15;0,31)

Table 5: Outputs of the QMRA model depicting the risk of infection of *Salmonella*, number of cases of infection and its reductions due to consumption of chicken meat at home.

	Scenarios	Risk of infection per serving	Percentage reduction in infection	Number of cases of infection in the population exposed	Percentage reduction in the population exposed
		Mean		Mean	
	Baseline	0.008092	-	163,809,233.27	-
1	Prevalence of *Salmonella* in chicken of 14,04%	0.018797	-132.30	380,551,972.46	-132.31
2	Prevalence of *Salmonella* in chicken of 17,76%	0.03542	-337.71	501,959,072.25	-206.42
3	Prevalence of *Salmonella* in chicken of 49%	0.0985	-1117.25	1,994,329,473.79	-1117.47
4	Reduce prevalence by 50%	0.004042	50.05	81,803,985.36	50.06
5	Cross-contamination at defeathering and evisceration at slaughter industry	0.008097	-0.062	163,809,233.27	0
6	Reduce concentration by 50%	0.008092	0	163,809,233.27	0
7	Reduce prevalence and concentration by 50%	0.004039	50.09	81,803,985.36	50.06
8	Cold chain since industry at maximum temperature of 7ºC	0.0079546	1.70	163,809,233.27	0
9	Reduce contamination at scalding and chiller	0.008085	0.09	163,809,233.27	0
10	Reduce contamination by acetic acid	0.008089	0.04	163,809,233.27	0
11	Reduce contamination by lactic acid	0.008087	0.06	163,809,233.27	0
12	Reduce contamination by cetylpyridinium chloride	0.008091	0.01	163,809,233.27	0
13	Reduce contamination by trisodium phosphate	0.00809	0.025	163,809,233.27	0
14	Reduce contamination by combined treatments	0.008085	0.09	163,809,233.27	0
15	Prevalence of *Salmonella* in chicken reduced by 50% and reduce concentration by 50%, cold chain since industry at maximum temperature of 7ºC and reduce contamination at scalding, spray washing and chiller	0.0039697	50.95	81,803,985.36	50.06

Each scenario was run in @Risk using 100,000 iterations with generator seed fixed at 1.

Table 6: Outputs of the QMRA model depicting the risk of infection of *Salmonella*, number of cases of infection and its reductions due to consumption of chicken meat at food service.

	Scenarios	Risk of infection per serving	Percentage reduction in infection	Number of cases of infection in the population exposed	Percentage reduction in the population exposed
		Mean		Mean	-
	Baseline	0.007959	-	163,809,233.27	-
1	Prevalence of *Salmonella* in chicken of 14,04%	0.018486	-132.27	380,551,972.46	-132.31
2	Prevalence of *Salmonella* in chicken of 17,76%	0.03484	-330.54	545,600,658.817	-233.07
3	Prevalence of *Salmonella* in chicken of 49%	0.09692	-1117.75	1,011,629,508.52	-517.57
4	Reduce prevalence by 50%	0.003975	50.06	81,803,985.36	50.06
5	Cross-contamination at defeathering and evisceration at slaughter industry	0.00796	-0.01	163,809,233.27	0
6	Reduce concentration by 50%	0.007952	0.09	163,809,233.30	0
7	Reduce prevalence and concentration by 50%	0.003972	50.09	81,803,985.36	50.06
8	Cold chain since industry at maximum temperature of 7ºC	0.007861	1.23	163,809,233.27	0
9	Reduce contamination at scalding and chiller	0.007951	0.10	163,809,233.27	0
10	Reduce contamination by acetic acid	0.007953	0.076	163,809,233.27	0
11	Reduce contamination by lactic acid	0.007955	0.05	163,809,233.27	0
12	Reduce contamination by cetylpyridinium chloride	0.007954	0.06	163,809,233.27	0
13	Reduce contamination by trisodium phosphate	0.007951	0.10	163,809,233.27	0
14	Reduce contamination by combined treatments	0.007949	0.12	163,809,233.27	0
15	Prevalence of *Salmonella* in chicken reduced by 50% and reduce concentration by 50%, cold chain since industry at maximum temperature of 7ºC and reduce contamination at scalding, spray washing and chiller	0.0039211	50.73	81,803,985.36	50.06

Each scenario was run in @Risk using 100,000 iterations with generator seed fixed at 1.

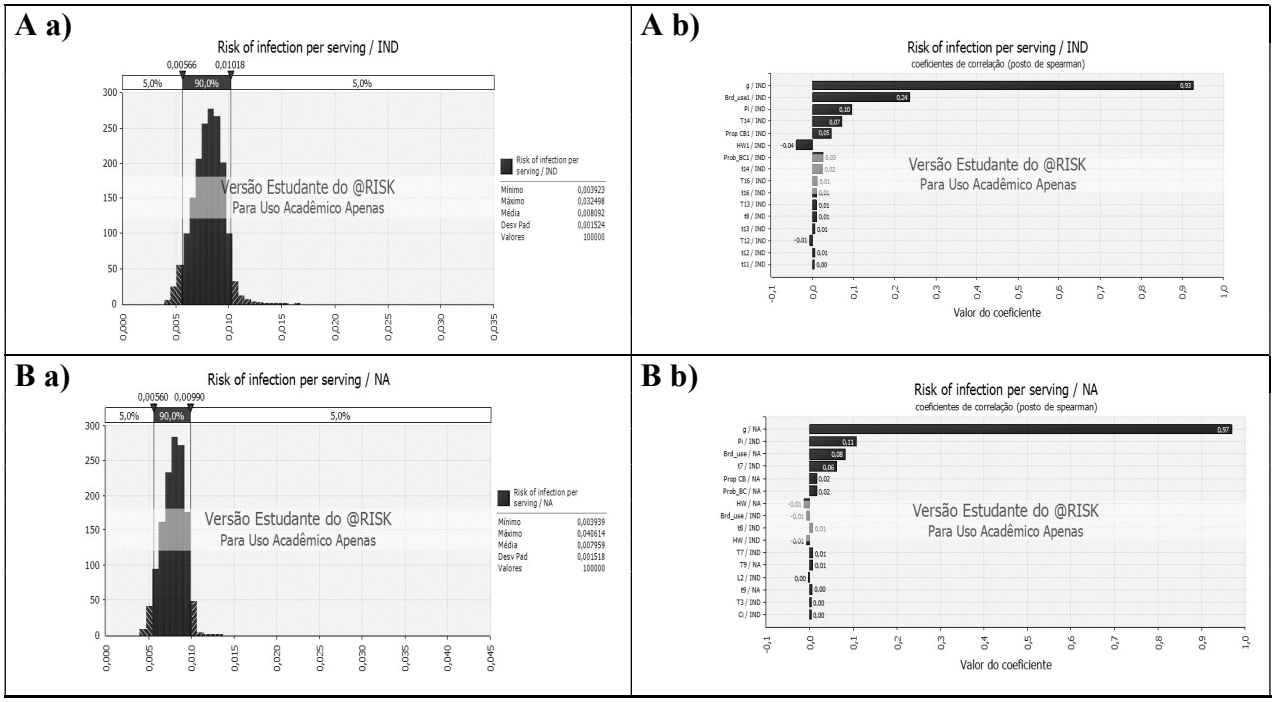

Figure 2: A a) Risk of infection of *Salmonella* due to consumption of chichen meat at home b) Tornado graphic showing the sensitivity analysis for *Salmonella* contamination in chichen meat per serving (dose) at home. Spearman's correlation coefficients were obtained from @Risk sensitivity analyses and were shown next to each bar. B: a) Risk of infection of *Salmonella* due to consumption of chichen meat at food services b) Tornado graphic showing the sensitivity analysis for *Salmonella* contamination in chicken meat per serving (dose) at food services. Spearman's correlation coefficients were obtained from @Risk sensitivity analyses and were shown next to each bar.

5.5. Artigo científico 5:

Quantitative Microbial Risk Assessment of *Salmonella* in eggs in Brazil

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Abstract:
A quantitative microbial risk assessment of salmonellosis due to consumption of eggs contaminated with *Salmonella* spp. In brazil was conducted. The model comprised 10 modules from egg industry until consumption at food services and 13 modules until consumption at home. Scenarios were simulated using prevalence and concentration found in literature, also time and temperature data were collected through questionnaires applied in food chain. Models were run using @risk software by monte carlo simulation. Sensitivity analysis of the baseline model and ten scenarios were performed. These analyses indicated that reduction in initial prevalence, is the most important action to decrease the risk of infection by *Salmonella* due to egg consumption in brazil in both models, food service and home. The use of cold chain from farm to consumption also reduced this risk. The results obtained by our study demonstrated that *Salmonella* represent measurable risks in eggs in brazil and the risk of infection at home is similar to at food service. Then, our results suggest that the risk of infection of *Salmonella* due to egg consumption can be best mitigated by reducing the initial prevalence of bacteria at farm.
Keywords: QMRA; probabilistic assessment; salmonellosis; foodborne disease; public health; poultry products.

1. Introduction

Poultry products are an important source of protein and are consumed at global level, across diverse cultures, traditions and religions. The demand of this products, specially eggs, is expected to continue increasing due to population growth and rising of consumption (FAO, 2019). The Brazilian market of eggs grows annually. In 2017, the country produced 39.9 billion units and the exportation raised 78% (ABPA, 2019; EMBRAPA, 2019; USDA, 2019).

Eggs comprise a large group of commodities and can be eaten solely or as ingredients in many food products. These foods are mainly commercialized as eggs, as egg products (liquid, frozen or dehydrated; whole meal, egg yolk) or cooked egg products (chilled or frozen). Despite the expressive consumer growth, eggs and egg products are associated with significant foodborne outbreaks and *Salmonella* spp. is the most common etiological agent involved (Brasil, 2018; Chai, Cole, Nisler, & Mahon, 2017; EFSA, 2018; Ferrari et al., 2019; Jarvis et al., 2016; Luciana Bill Mikito Kottwitz et al., 2010; Pijnacker et al., 2019; USDA, 2019; World Health Organization, 2002). Several factors raise concerns about foodborne outbreaks caused by *Salmonella* such as the acid, thermal, antimicrobial and sanitizing resistance. Especially in relation to antimicrobial resistance in Brazil, the resistance of *Salmonella* isolates in poultry is apparently higher when compared to other food isolates (De Oliveira, Brandelli, & Tondo, 2006; Luciana B.M. Kottwitz et al., 2013; Malheiros, Brandelli, NoreÑa, & Tondo, 2009).

Risk assessment is a valuable alternative when surveillance data are nonexistent or sparse, and the development of a quantitative microbial risk assessment (QMRA) offers a scientific basis approach for risk management, providing ranks of the most effective risk management options (Enger, Nelson, Clasen, Rose, & Eisenberg, 2012; Hoelzer, Pouillot, Egan, & Dennis, 2012; Membré & Guillou, 2016). The objective of this study is to perform a QMRA of salmonellosis due to consumption of eggs contaminated with *Salmonella* spp. in Brazil.

2 Materials and methods

2.1. Data collection

2.1.1. *Salmonella* prevalence and concentration

A literature research was conducted to survey *Salmonella* prevalence and concentration. A search was carried out using the terms ‘‘eggs’’ OR ‘‘ovo” AND “*Salmonella*” AND “prevalence” OR “prevalência” AND “concentration” OR “contagem” AND “Brazil” OR “Brasil” in the PubMed and in the Web of Science platforms. No date restrictions were applied. Endnote version X6 (Thomson Reuters) was used to collect publications. All articles selected were checked for duplicates, using Endnote and Mendeley (https://www.mendeley.com/). Publications were collected and included when they were published in English, Spanish or Portuguese and relevant search terms appeared in the title, abstract, or keywords. The resulting data were then summarized and pooled. Furthermore, expert’s opinion from the field (egg industries, surveillance body, and the Ministry of Agriculture and Livestock) were contacted to identify the existence of unpublished work.

Table 1 shows results of the literature research conducted to survey *Salmonella* prevalence and concentration in eggs in Brazil. The general prevalence of *Salmonella* spp. in samples collected in markets / stores it was 23.1% and contamination in slaughterhouses / processing plants was 23.2%. Carcass samples had a lower prevalence (19.7%) when compared to the prevalence of *Salmonella* in chicken meat cuts (23%). There was a small difference in the prevalence of *Salmonella* from frozen (21.1%) and chilled / fresh (20.9%) meat samples. Brazilian studies represented 17% of the analyzed data and *Salmonella* spp. the average prevalence was 15% (range 0 to 44.6%), similar to the general prevalence in Latin America (15.2%, value range 0 to 44.6%) and Africa (15.3 %, value range from 4.44% to 50%). Data from the USA and Canada had the highest prevalence of *Salmonella*, with 38.98% (values ranging from 21% to 85%). European Union (EU) studies were more numerous than other parts of the world (31%) and their data show a prevalence of *Salmonella* of 18.82%, lower than the prevalence found in Asian studies (25.9%, values varying 0 to 93). %) The total of *Salmonella* spp. the prevalence in the world was 21.02% (values ranging from 0 to 93%). Different serotypes were reported in the studies, of which S. Enteritidis was the most identified in Brazil, Latin America, Asia and the EU. Only three studies verified the concentration per gram (1.6-110 cfu / g), per carcass (2.1-2.5 log MPN) or per carcass in the production line (2.11 log MPN after harvest for < 1,08 log MPN after cooling). All of these data reinforce the importance of monitoring and general good

hygiene practices in the microbiological control of chicken producers worldwide; therefore, the objective of this systematic review is to compile data on the prevalence and concentration of *Salmonella* in chicken meat.

Other countries report similar prevalence of Brazil, such as 0.08% (Uruguay), 2.93% (Colombia), 4.83% (India) and 4.85% (Iraq) (Betancor et al., 2010; Mogollón Vergara, Rodríguez Gutiérrez, & Verjan García, 2016; Singh, Yadav, Singh, & Bharti, 2010; Zubair, Al-Berfkani, & Issa, 2017). Our result indicates that eggs in Brazil might be contaminated with *Salmonella* despite source of purchase and its prevalence follows prevalence found in other countries.

The literature research performed did not find any study of *Salmonella* concentration in eggs in Brazil. Indeed, the scarcity of prevalence and concentration data of contamination in developed or developing countries was already reported (de Oliveira Elias, Noronha, & Tondo, 2019). However, these data are indispensable to carry out QMRA, which are necessary for quantifying the potential risk involving food consumption and improving the evidence base for food safety regulations and public health policies (de Oliveira Elias, Noronha, & Tondo, 2019). Further, for the QMRA, it was assumed the data from the “Risk assessments of *Salmonella* in eggs and broiler chickens” of FAO/WHO 2002.

2.1.2. Time and temperature

Based on the document “Risk assessments of *Salmonella* in eggs and broiler Chickens”, a generic flow diagram from industry to consumption of egg was drawn. A draft of figure 1 was sent to professionals from each field for evaluation. Comments concerning subsequent steps were implemented in the final version (Figure 1). To access the time and temperature data from different members of the food chain, the contact was made to partners and different stakeholders to invite as collaborator. Once agreed the participation in the project, a form was sent by e-mail, with the required data to be filled.

A form was sent by e-mail to the egg industry under Sanitary Inspection, in the State of Rio Grande do Sul (RS). Questions regarding time-process, temperature records of processing and transport were asked. For food services and supermarkets temperatures, records from Corporate Catering Food Services (CCFS) were scanned

and received by *e-mail*. For residence, it was considered the data published by Hessel et al. (2019). To access the environmental temperature the data were obtained from the National Institute of Meteorology (INMET, n.d.). The maximum and minimum official temperature registered by the weather station, from each capital state, was compiled between 01 January 2017 and 29 November 2018.

The data compiled were analyzed and organized in Excel® tables reporting the date and reference, being each module belonging to one column. Then data were fitted into distributions (BetaGeneral, ChiSqd, Expon, Logistic, LogLogistic, LogNorm, Normal, Pareto, Pert, Student, Triangular, Uniform and Weibull) using the software @Risk (Palisade corporation, version 6.3.1). For each study, the different distributions provided were ranked according to the root mean squared error (RMSE) as a measure of goodness of fit.

Table 2 shows time and temperature data and statistical parameters obtained by distribution fitting of egg modules. As observed the entire egg chain, from farm until home or food service, occurs under environmental temperature. Properly, according to Brazilian legislation, eggs which have not been preserved by any process (i.e. dehydration or pasteurization), may be produced and sold under environmental temperature (MAPA, 1990).

Cooking and distribution modules at food service showed adequate average temperature according to Brazilian legislation, which rule temperature higher than 70 and 60 ºC for each step, respectively (BRASIL, 2004).

This temperature achievement is necessary to inactivate foodborne pathogens may present in eggs. In this field, according to Portaria 78/2009 in the state of Rio Grande do Sul is prohibit preparations with raw egg, without thermal treatment, in food services, in order to prevent foodborne outbreaks mainly caused by *Salmonella* spp. (SEVS, 2009).

2.1.3. Predictive model

The predictive microbiology can predict the growth response of the microorganism in relation to variations in factors such as temperature, storage conditions, humidity and pH. This tool is very useful to resolve doubts related to food contamination and has emerged as an essential element of food microbiology promoting the quality and safety of food, supporting risk analysis, evaluating the

shelf-life, combining actions on decision-making, developing new products and processes (Oliveira et al., 2013).

The behavior of *Salmonella* in eggs was predicted using ComBase software (https://www.combase.cc/index.php/en/). It was assumed that *Salmonella* cells survive and grow in whole eggs. This assumption was made because studies indicate that *Salmonella* contamination in eggs occurs by transovarian routes or by external membrane penetration. Thus, *Salmonella* might be present in the albumen or growth in the egg yolk (Gast & Beard, 1992; Humphrey, 1994; Latimer, Jaykus, Morales, Cowen, & Crawford-Brown, 2002; Latimer et al., 2008).

Static *Salmonella* in egg food models were performed with an initial level of 2 log CFU/g and the pH and water activity (aw) values of 7.8 and 0.997, respectively (Latimer et al., 2002, 2008; Schoeni, Glass, McDermott, & Wong, 1995). Minimum and maximum temperatures were chosen according to the limits preconized by ComBase Food Models, although real scenario extrapolates that. The predictive primary models were built at temperatures of 10, 15, 20, 25, 30, 35, 40 and 42°C. The maximum growth rate (μ) (log conc./h) at each temperature was used to obtain the secondary model. The predictive secondary model was built using the square root equation described by Ratkowsky et al. (1982) to describe μ (growth rate) as a function of the temperature.

The R^2 for the secondary model of *Salmonella* in eggs was 0.97 (Table 3). This result indicates that a good fit occurred between the Combase Food Models data and the square root model calculated in the present study, once that the regression line approximated the Combase data. Besides this, the RMSE value showed that Ratkowsky model was adequate to predict the behavior of *Salmonella* growth on eggs at 10 until 42ºC, based on Combase Food Model.

2.2. Exposure assessment model design

The risk assessment model comprised 13 modules from egg production in producer farms until consumption at homes and 10 modules if egg consumption was at food services (Figure 1). Table 4 summarizes the Excel® spreadsheet used for the risk calculations. The first and second columns represents the local and step of egg production considered in the model, respectively. The next columns describe the variables used in calculations (Variable, Unit, Category and Symbol). The column

Distribution, value, or formula is either a number, a simple formula, or an @RISK distribution representing the value of the cells. The last column (reference) represents the source of the information used to determine the value of the variables. The source can be surveyed data in the study, a literature citation or calculated from other cells in the spreadsheet.

2.2.1 Model *inputs*

2.2.1.1 Egg production and storage at farm

In the first module of QMRA model is presented the prevalence and concentration of *Salmonella*. These data were extracted from the literature research performed in our study. Few studies were found describing this data in the fields in Brazil, thus the current QMRA model assumes that these data represent prevalence of *Salmonella* as found in eggs at farm in Brazil (Table 1). It was used the median of prevalence studies at farms (2.36%), it means a total of 1188 tested samples, and 28 eggs contaminated with *Salmonella*. Since our literature research did not report the *Salmonella* concentration in eggs in Brazil, it was assumed the data from the “Risk assessments of *Salmonella* in eggs and broiler chickens” of WHO 2002. This value was transformed in log CFU/g to agree with our model units.

In relation to time and temperatures it was used the data collected from members of the food chain showed in Table 2. The increase in pathogen concentration was modeled using the predictive model generated in COMBASE. Using this approach, no growth of *Salmonella* on eggs was assumed if product temperature was below 10ºC and higher 42ºC. Thus, in the QMRA model, when a temperature below 10ºC was selected during iterations, zero growth was assigned and no increase in the initial concentration (module 1) was assumed (Table 4).

2.2.1.2 Transports, processing at egg industry, storages, and exposure at retail

The time and temperatures data were showed in Table 2. Besides, the growth of *Salmonella* and its respective level after storage was calculated as described in Section 2.1.1.1

2.2.1.3 Cooking at home

For this module it was used the study of de Paula et al., 2005 to collect the data of thermal inactivation of *Salmonella enteritidis* by cooking egg. It was assumed that egg cooked until hard yolk completely inactivate *Salmonella*. Indeed, temperatures above 60 ºC inactivates foodborne pathogens that may present in eggs, (De Paula, Mariot, & Tondo, 2005; Forsythe, 2007), in this scenario, there was a virtually predicted zero risk probability of salmonellosis due to cooked egg consumption. Thus, the concentration after cooking process was calculated by subtracting the concentration after home or food service storage of pathogen and subsequent reduction after cooking step (1.35 log CFU/ml).

2.2.1.4 Consumption

The typical serving size of egg as consumed by the Brazilian population was studied by Hessel et al (2019). The level of pathogen was calculated by summing or subtracting its concentration at the end of each module of the QMRA model (Table 4). The dose of pathogen per serving was calculated by multiplying amounts of eggs consumed and the level of pathogen (Table 4). The exposure (number of servings of eggs intake per week) was obtained from Hessel et al (2019).

For the risk calculation the Beta-Poisson model from WHO/FAO was used and for conservativeness, the upper limit of parameters α and β were 0.1324 and 51.40, respectively (World Health Organization and Food and Agriculture Organization of the United Nations, 2002). This model have been used to evaluate human dose-responses for *Salmonella* (Haas, Rose, & Gerba, 2014; Holcomb et al., 1999; World Health Organisation, 2002). In addition, by adopting Beta-Poisson model, the dose-response relationship assumed is that one single *Salmonella* cell is capable to infect and cause disease. This model was used to evaluate human dose responses for *Salmonella* in several QMRA published (Smadi & Sargent, 2012; Haas, Rose, & Gerba, 2014; Holcomb et al., 1999; World Health Organisation, 2002).

The outputs of the QMRA model were the risk of infection per week (probability of infection per week due to consumption of eggs) and number of cases (number of people that consumed eggs and get infected per week) in the exposed population (Table 4). The determination of number of cases of infection due to *Salmonella* was calculated considering the population of Brazil (IBGE…) and assuming that approximately 97% of population eats eggs (Hessel et al., 2019).

The QMRA model was built in an Excel spreadsheet (Microsoft, Redmond, WA) and simulated using @Risk software version 7.5 (Palisade Corporation). Hundred thousand iterations were performed using Latin Hypercube sampling to increase the reliability of the software to reproduce the defined distributions. Every simulation of the current model represented a randomly chosen egg from the time it was produced at farm thought all steps of chain until consumption. Spearman's correlation coefficients were used for sensitivity analysis of the baseline model and scenarios to determine the effect of input variables on the probability of infection per serving and on the number of infection cases in Brazil per serving.

2.3 Evaluation of different scenarios

Besides the main objective of the present study was to perform a QMRA to calculate the risk of salmonellosis due to consumption of eggs contaminated with *Salmonella* spp. in Brazil, this study also aimed to identify different risk-mitigation strategies, thereby decreasing the influence of variation (variability and uncertainty). For this, ten scenarios were tested to evaluate the impact of specific risk management improvements on the final risk of infection by *Salmonella* in the overall Brazilian population. The effectiveness of each risk mitigation strategie was measured as the percentage of reduction on the predicted probability of infection due to consumption of one meal keeping all other original parameters and probability distributions constant.

The first scenario (baseline) represents the current knowledge regarding Brazilian egg chain and *Salmonella*, based on the assessment performed in this research, from farm to consumption. Scenario 1 consider different cooking practice on handling eggs, to be adopted by food handlers at food services or at home, it was used the study of de Paula et al., 2005, it means a reduction of 2.79 log CFU/ml after cooking. Scenario 2 consider the use of pasteurized liquid egg yolk (5.9 log CFU/ml of reduction). Scenarios 3-6 consider different prevalence and/or concentrations of pathogen. Scenarios 7 and 8 consider implementation and maintenance of the cold chain in egg chain from industry until cooking. Scenario 9 consider reduction in prevalence and concentrations at farm and implementation and maintenance of the cold chain in egg chain from industry until cooking. Finally, scenario 10 consider the

baseline scenario of prevalence, concentration and cooking practices, occurring in warm days (minimum temperature of 15 ºC, average of 28ºC, and highest of 40 ºC).

3. Results and Discussion

3.1 QMRA of *Salmonella* in eggs consumed at home

Senitivity analysis was performed to provide a quantitative measure of the most important parameters to reduce the risk to human health from *Salmonella* due to egg consumption. The use of this analysis is important since allows to prioritize risk mitigation strategies on the parameter that has the most impact, and to identify uncertain parameters and hence focus research efforts on areas most needed to improve model outcome (Brown, 2002; Smadi & Sargeant, 2013). Spearman's rank correlation was performed to identify predictive parameters that were most highly correlated with the contamination of *Salmonella* per serving (Figures 2A). The sensitivity of the baseline model outcomes to input values and model parameters, revealed that the mean number of infection cases as the most sensitive to *Salmonella* prevalence and number of servings. It means that people who consume eggs fewer times have a lower risk of contamination by *Salmonella*. It is important to highlight that the number of infection cases do not represent the number of illnesses, but the number of people contaminated by the microorganisms. The development of disease will depend on the immunity of the person contaminated with the pathogen, the infections dose and the severity of the strain (Pouillot et al., 2016).

Environmental temperature, time of processing, storage and transport did not influence the risk of infection. The absence of influence of temperature might be related since in Brazil egg chain occurs under environmental temperature, thus, no temperature variation was input in the model, as demonstrated in Table 2.

The outputs of the QMRA model depicting the risk of infection of *Salmonella*, number of cases of infection and its reductions due to consumption of eggs at home are shown in Table 5.The baseline risk of infection per serving was 0.059 ± 0.029 (approximately 6 cases in 100 servings) and 12,277,008 number of infections in the population (5.84% of Brazilian population). At scenario 1 the cooking point of yolk did not reduce the risk of infection. Similarly, the use of pasteurized liquid egg product did not reduce the risk of infection by *Salmonella* (Scenario 2). Also, the reduction of *Salmonella* concentration by 50% did not reduce the risk of infection (scenario 5).

Similarly, the implementation of cold chain of 15ºC (scenario 7) or the increase in environmental temperatures (scenario 10) did not interfere in the risk of infection. These results can be attributed because the contaminated egg at the yolk level, exposed for long periods, can present concentrations of *Salmonella* spp.of approximately 8 log UFC / mL of yolk (De Paula et al., 2005; Lopes et al., 2019), which will not be completely inactivated after pasteurization or partial cooking. In addition, the non-reduction in risk may be related to the infective dose of 1 CFU used in this risk assessment. These data reinforce the need for biosecurity practices in egg-producing farms, which must be very controlled in relation to contamination by *Salmonella* spp. In this sense, farms can be classified according to the level of biosafety actually implemented, and eggs from them can be more or less exposed to risky temperatures. Eggs from farms with strong biosecurity practices can be kept at higher temperatures or even at ambient temperatures, as in Brazil. On the contrary, eggs from farms without all implemented biosecurity controls, must be kept under refrigeration, in order to mitigate risks of salmonellosis.

At scenario 3 the reduction of prevalence of *Salmonella* from 2.36% (baseline) to 0.83% reduced 52% in the risk of infection per serving. A similar reduction was observed when initial prevalence was reduced to 0.83% and the concentration were reduced by half (scenario 6). When cold chain was implemented since industry at maximum temperature of 9ºC (scenario 8) the risk of infection per serving was reduced in 21%. Finally, when the prevalence of *Salmonella* in eggs was 0.83%, the concentration was reduced by 50%, and cold chain was implemented since industry at maximum temperature of 9ºC (scenario 9) the risk of infection per serving was reduced in 63%, the biggest reduction found in this study.

However, scenario 4 consider the initial *Salmonella* prevalence of 9.6%, the highest observed in the literature study. In this scenario the risk of infection per serving was 0.21, representing 266% of increase comparing to the baseline. In this case the number of cases of infection in the population exposed is 45,986,142.

3.2 QMRA of *Salmonella* in eggs consumed at food services

Spearman's rank correlation was performed to identify predictive parameters that were most highly correlated with the contamination of *Salmonella* per serving (Figures 2B). The sensitivity of the baseline model outcomes to input values and

model parameters, revealed that the mean number of infection cases as the most sensitive to *Salmonella* prevalence and number of servings. Similar to the results found in QMRA model at home.

Table 6 shows the outputs of the QMRA model due to consumption of *Salmonella* in eggs consumed at food services. The baseline risk of infection per serving was 0.059 ± 0.029 (nearly 6 cases in 100 servings) and 12,278,618 cases of infection in the population exposed (5.84% of Brazilian population)., these results are very similar to those obtained at home QMRA model. The cooking point of yolk or the use of pasteurized egg did not reduce the risk of infection (scenario 1 and 2). Similarly, the reduction of *Salmonella* concentration by 50% or the implementation of cold chain of 9ºC or 15ºC or the increase in environmental temperatures did not interfere in the risk of infection (scenario 5, 7, 8 and 10, respectively).

The reduction of prevalence of *Salmonella* from 2.36% to 0.83% (scenario 3), representing 52% of reduction in the risk of infection per serving in relation to baseline. A similar reduction was observed when initial prevalence was reduced to 0.83% and the concentration were reduced by half (scenario 6). Finally, when the prevalence of *Salmonella* in eggs was 0.83%, the concentration was reduced by 50%, and cold chain was implemented since industry at maximum temperature of 9ºC (scenario 9) the risk of infection per serving was also reduced in 52%. However, scenario 4 showed the increase of prevalence of *Salmonella* to 9.6%, the highest observed in the literature study. In this scenario the risk of infection per serving was 0.21 representing 265%, of increase to the baseline.

The QMRA model at home comprises more modules than food services, allowing more conditions to microbial multiplication. Probably, because of this the cold chain at 9ºC was more effective at home model. However, several studies report that risk practices are higher at home than food services. Thus, the QMRA could be a tool to organize management control programs of *Salmonella* and to point where the pathogen can be controlled the most by the food handlers. In addition, training and educational programs to enhance such practices should be encouraged, since food safety is not one party's responsibility; rather, it is a shared responsibility among all stakeholders (Akil & Ahmad, 2019; Hessel et al., 2019).

The same sensitivity analysis applied in baseline model, determined by Spearman's rank order correlation, was applied in the risk mitigation scenarios. In all scenarios the sensitivity of the outcomes to input values and model parameters revealed that the initial prevalence and exposure was most sensitive factor. Is important to highlight that not every exposure to *Salmonella* in eggs will result in infection or illness in humans, and not all individuals in a given population are equally susceptible to all pathogens. Therefore, the risk of foodborne disease is a combination of the likelihood of exposure to a pathogen in a food, the likelihood that exposure will result in infection or intoxication and subsequently illness and the severity of the illness (Akil & Ahmad, 2019; CDC, 2018).

The category variability and uncertainty were described for each parameter included in the model at Tables 4. Each parameter was designed as C, if was calculated; F, when fixed; V, when considered as variability and U, for uncertainty. Uncertainty is the variance occurring as a consequence of limited information in the dataset, variability is intrinsic variance inherent to the living systems (CAC/GL, 2007; Vásquez, Busschaert, Haberbeck, Uyttendaele, & Geeraerd, 2014). The *Salmonella* prevalence was considered as uncertainty in our model. Our exposure assessment considered eggs produced under official inspection in Brazil. However, consumers are exposed to different egg sources and as preparations (omelets, pie, drinks), which may not be included in our model. Regard model uncertainty, the effect of climate or management practices on the flock and egg prevalence were not included in the model. In addition, the predictive model used was not personalized for different *Salmonella* Brazilian strain and no additional quantitative cross-contamination during egg product processing modules were included. For variability, it was considered the initial *Salmonella* concentration, temperatures and time. Despite the great amount of data collected in this study, more data are always needed to improve the accuracy of risk assessment models (Sant'Ana et al., 2014). However, these uncertainties might be reduced by the development of this researches. The gathered information will become inputs in the quantitative modelling providing a robust and reliable model.

4. Conclusion

The present study was carried out to estimate the risks of infection by *Salmonella* due to consumption of eggs in Brazil. To the best of our knowledge, this

is the first study examining microbial growth and reductions of *Salmonella* during the processing, distribution and consumption of eggs in Brazil. The results obtained by our study demonstrated that *Salmonella* represent measurable risks in eggs Brazil and the risk of infection at home is similar to at food service. Our results suggest that the risk of infection of *Salmonella* due to egg consumption can be best mitigated by reducing the initial prevalence at farm.

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Authors' Contributions

In this research, all authors contributed effectively. Claudia Titze Hessel, João Pedro Pessoa and Susana de Oliveira Elias conducted the data collection, organization, analysis, interpretation and wrote the manuscript. Mateus Silva de Lima and Leonardo Werlang Isolan conducted the data collection and revised the manuscript. Eduardo Cesar Tondo supervised and revised the manuscript.

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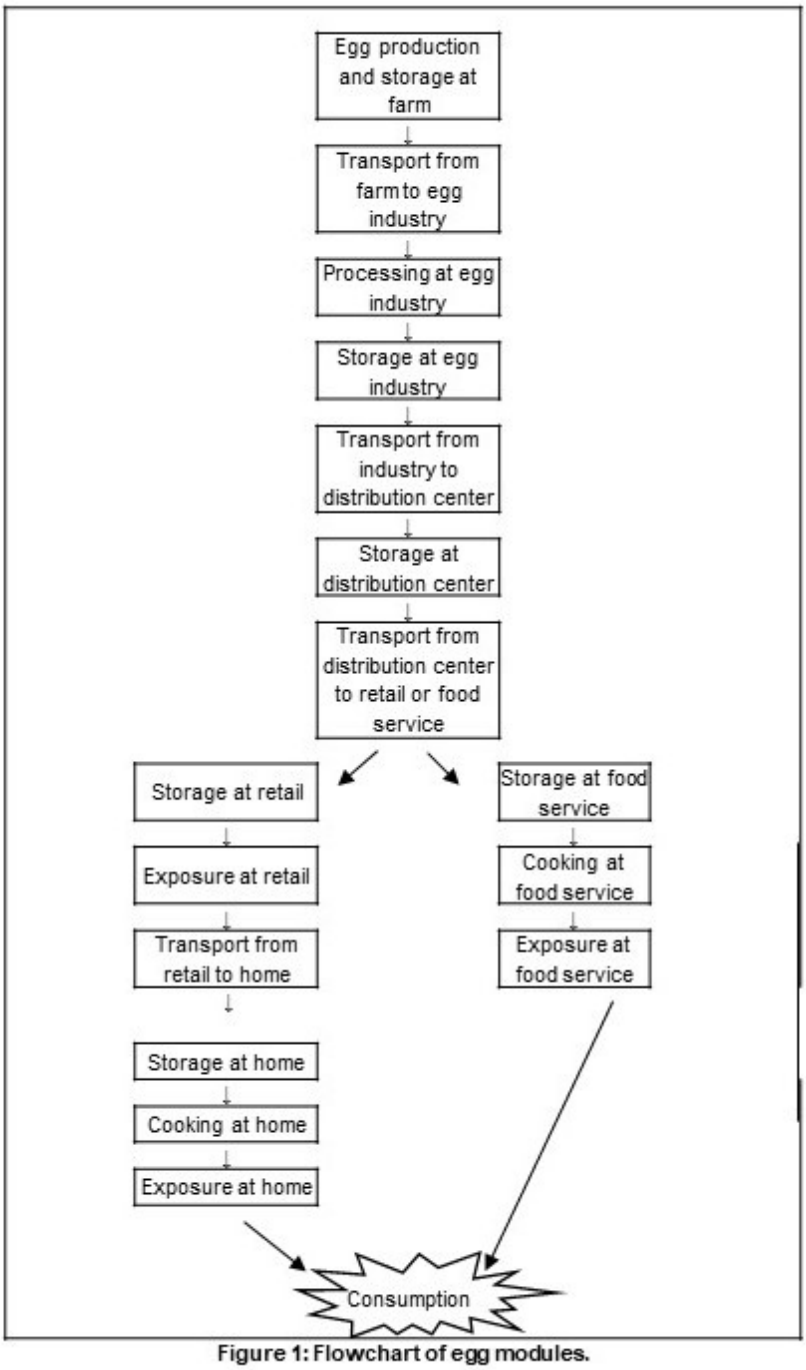

Figure 1: Flowchart of egg modules.

Table 1: *Salmonella* prevalence on eggs in Brazil.

Prevalence of *Salmonella* (%)	Brazilian State	Source	Reference
9,6 3,2	São Paulo	Retail	Oliveira & Silva, 2000
0	Rio Grande do Sul	Retail	Baú & Carvalhal, 2001
4,98	Rio Grande do Sul	Farm	Flôres et al. 2002
1,47	São Paulo	Retail	Campello, 2012
0	Rio de Janeiro	Farm	Melo, 2015
2,36	Rio Grande do Sul	Farm	Wolschick, 2015
0,83	Rio Grande do Sul	Farm	Wolschick, 2015
1,13	Rio Grande do Sul	Farm	Wolschick, 2015
2,46	Rio Grande do Sul	Farm	Wolschick, 2015
3,92	Rio Grande do Sul	Farm	Wolschick, 2015
3,83	Rio Grande do Sul	Farm	Wolschick, 2015
0	Rondonia	Grocery fair	Rodrigues, 2016
0	Rondonia	Grocery fair	Rodrigues, 2016
2	Rondonia	Grocery fair	Rodrigues, 2016
0	Bahia	Farm	Mottin, 2016
0	Distrito Federal	Retail	Souza, 2017

Table 2: Time and temperature data and statistical parameters obtained by distribution fitting of eggs modules.

		Temperature						Time			
Local	Step	n	Minimum (ºC)	Maximum (ºC)	Median (ºC)	Average±SD (ºC)	Distribution according to @Risk	n	Minimum (h)	Maximum (h)	Median (h)	Average±SD (h)
Farm	Egg production	794	7,00	38,00	24,00	24.91 ± 6.22	Pert	NA	0,50	24,00	12,00	NA*
Industry	Storage	794	7,00	38,00	24,00	24.91 ± 6.22	Pert	NA	0,50	24,00	12,00	NA
	Transport from farm to egg industry	794	7,00	38,00	24,00	24.91 ± 6.22	Pert	NA	0,50	24,00	12,00	NA
	Processing at egg industry	794	7,00	38,00	24,00	24.91 ± 6.22	Pert	NA	1,00	2,00	1,50	NA
	Storage at egg industry	794	7,00	38,00	24,00	24.91 ± 6.22	Pert	NA	6,00	24,00	12,00	NA
	Transport from industry to distribution center	794	7,00	38,00	24,00	24.91 ± 6.22	Pert	NA	1,00	24,00	6,00	6.81 ± 5.57
Distribution Center	Storage at distribution center	794	7,00	38,00	24,00	24.91 ± 6.22	Pert	NA	6,00	24,00	12,00	NA
	Transport from distribution center to retail or food service	794	7,00	38,00	24,00	24.91 ± 6.22	Pert	NA	1,00	12,00	6,00	4.06 ± 2.85
Retail	Storage	794	7,00	38,00	24,00	24.91 ± 6.22	Pert	NA	0,00	720,00	210,00	NA
	Exposure	794	7,00	38,00	24,00	24.91 ± 6.23	Pert	NA	0,00	720,00	210,00	NA
	Transport from retail to home	794	7,00	38,00	24,00	24.91 ± 6.24	Pert	NA	0,00	6,00	2,00	NA
Home	Storage	794	7,00	38,00	24,00	24.91 ± 6.25	Pert	1181	0,00	312,00	1,00	NA
	Exposition	794	7,00	38,00	24,00	24.91 ± 6.26	Pert	1196	0,00	0,60	720,00	NA
	Storage	794	7,00	38,00	24,00	24.91 ± 6.24	Pert	NA	0,00	720,00	90,00	NA
Food Service	Cooking	47	2,10	97,50	83,20	75.02 ± 27.55	Logistic	NA	0,03	1,00	0,25	NA
	Exposure	619	4,90	99,20	71,50	71.18 ± 10.27	Logistic	NA	0,08	2,00	0,50	NA

* NA = not avaiable.

Table 3: Secondary model represented by square root equation to predict growth rate as a function of temperature of *Salmonella* in eggs.

Secondary Model	R^2	RMSE
$\sqrt{\mu}$ = 0.0136(T-0.559)	0.97	0.024

μ: growth rate (log CFU/g/h); R^2: coefficient of determination; RMSE: root mean square error; T: temperature (ºC).

Table 4: Parameters and their distributions in the Risk Assessment model.

Local	Step	Variable	Unit	Category[a]	Symbol	Distribution, value, or formula	Reference
Farm	Egg production and storage	Prevalence	%	U	Pi	RiskBeta(1;1000)	Surveyed in the study[a]
		Concentration	log10MPN/g	V	Ci	RiskPert(1;152;400)	FAO/WHO 2002
		Temperature at farm	°C	V	T1	RiskPert(7; 24; 38)	Surveyed in the study[b]
		Time at farm	h	V	t1	RiskPert(0; 12;24)	Surveyed in the study[b]
		Parameter b growth model	√ Log CFU/day/°C	F	b	0,0076	COMBASE
		Parameter T0 growth model	°C	F	T0	0,558823529	COMBASE
		Logarithmic growth	log CFU/g/h	C	Lg1	(0,0076*SE(T1-T0<0;0;(T1-T0)))^2	COMBASE
		Growth during farm	log CFU/g	C	G1	T1*Lg1	Calculated
		Level after farm	log CFU/g	C	L1	Ci+G1	Calculated
Transport from farm to industry	Transport 1	Temperature during transport 1	°C	V	T2	RiskPert(7;10;15)	Surveyed in the study[b]
		Time of transportat 1	h	V	t2	RiskPert(0; 12;24)	Surveyed in the study[b]
		Parameter b growth model	√ Log CFU/day/°C	F	b	0,0076	COMBASE
		Parameter T0 growth model	°C	F	T0	0,558823529	COMBASE
		Logarithmic growth	log CFU/g/h	C	Lg2	(0,0076*SE(T3-T0<0;0;(T3-T0)))^2	COMBASE
		Growth during	log CFU/g	C	G2	T2*Lg2	Calculated

		transport 1					
		Level after transport 1	log CFU/g	C	L2	L1+L2	Calculated
Industry	Processing at industry	Temperature at processing at industry	°C	V	T3	RiskPert(7;10;15)	Surveyed in the study[b]
		Time at processing at industry	h	V	t3	RiskPert(0; 12;24)	Surveyed in the study[b]
		Parameter b growth model	√ Log CFU/day/°C	F	b	0,0076	COMBASE
		Parameter T0 growth model	°C	F	T0	0,558823529	COMBASE
		Logarithmic growth	log CFU/g/h	C	Lg3	(0,0076*SE(T3-T0<0;0;(T3-T0)))^2	COMBASE
		Growth during processing at industry	log CFU/g	C	G3	T3*Lg3	Calculated
		Level after processing at industry	log CFU/g	C	L3	L2+L3	Calculated
	Storage at industry	Temperature at storage at industry	°C	V	T4	RiskPert(7;10;15)	Surveyed in the study[b]
		Time at storage at industry	h	V	t4	RiskPert(1; 1,5;2)	Surveyed in the study[b]
		Parameter b growth model	√ Log CFU/day/°C	F	b	0,0076	COMBASE
		Parameter T0 growth model	°C	F	T0	0,558823529	COMBASE
		Logarithmic growth	log CFU/g/h	C	Lg4	(0,0076*SE(T4-T0<0;0;(T4-T0)))^2	COMBASE
		Growth during storage at industry	log CFU/g	C	G4	T4*Lg4	Calculated
		Level after storage at	log CFU/g	C	L4	L3+L4	Calculated

		industry					
Transport from industry to distribution center	Transport 2	Temperature at transport 2	°C	V	T5	RiskPert(7;10;15)	Surveyed in the study[b]
		Time of transport 2	h	V	t5	RiskPert(1; 1,5;2)	Surveyed in the study[b]
		Parameter b growth model	√ Log CFU/day/°C	F	b	0,0076	COMBASE
		Parameter T0 growth model	°C	F	T0	0,558823529	COMBASE
		Logarithmic growth at transport 2	log CFU/g/h	C	Lg5	(0,0076*SE(T4-T0<0;0;(T4-T0)))^2	COMBASE
		Growth during transport 2	log CFU/g	C	G5	T4*Lg4	Calculated
		Level after transport 2	log CFU/g	C	L5	L3+L4	Calculated
Distribution Center	Storage at Distribution Center	Temperature at storage at distribution center	°C	V	T6	RiskPert(7;10;15)	Surveyed in the study[b]
		Time at storage at distribution center	h	V	t6	RiskPert(6; 12; 24)	Surveyed in the study[b]
		Parameter b growth model	√ Log CFU/day/°C	F	b	0,0076	COMBASE
		Parameter T0 growth model	°C	F	T0	0,558823529	COMBASE
		Logarithmic growth at storage at distribution center	log CFU/g/h	C	Lg6	(0,0076*SE(T5-T0<0;0;(T5-T0)))^2	COMBASE
		Growth during at storage at distribution center	log CFU/g	C	G6	T5*Lg5	Calculated

		Level after at storage at distribution center	log CFU/g	C	L6	L4+L5	Calculated
	Transport 3	Temperature at transport 3	°C	V	T7	RiskPert(7;10;15)	Surveyed in the study[b]
		Time of transport 3	h	V	t7	RiskPert(1; 6; 12)	Surveyed in the study[b]
Transport from distribution center to retail		Parameter b growth model	√ Log CFU/day/°C	F	b	0,0076	COMBASE
		Parameter T0 growth model	°C	F	T0	0,558823529	COMBASE
		Logarithmic growth at transport 3	log CFU/g/h	C	Lg7	(0,0076*SE(T6-T0<0;0;(T6-T0)))^2	COMBASE
		Growth during transport 3	log CFU/g	C	G7	T6*Lg6	Calculated
		Level after transport 3	log CFU/g	C	L7	L5+L6	Calculated
	Storage at Retail	Temperature at storage at retail	°C	V	T8	RiskPert(7;10;15)	Surveyed in the study[b]
		Time at storage at retail	h	V	t8	RiskPert(0; 3; 9,3)	Surveyed in the study[b]
Retail		Parameter b growth model	√ Log CFU/day/°C	F	b	0,0076	COMBASE
		Parameter T0 growth model	°C	F	T0	0,558823529	COMBASE
		Logarithmic growth at storage at retail	log CFU/g/h	C	Lg8	(0,0076*SE(T7-T0<0;0;(T7-T0)))^2	COMBASE
		Growth during storage at retail	log CFU/g	C	G8	T7*Lg7	Calculated
		Level after storage at retail	log CFU/g	C	L8	L6+L7	Calculated

	Exposition at Retail	Temperature at exposition at retail	°C	V	T9	RiskPert(7;10;15)	Surveyed in the study[b]
		Time at exposition at retail	h	V	t9	RiskPert(0; 210; 720)	Surveyed in the study[b]
		Parameter b growth model	√ Log CFU/day/°C	F	b	0,0076	COMBASE
		Parameter T0 growth model	°C	F	T0	0,558823529	COMBASE
		Logarithmic growth at exposition at retail	log CFU/g/h	C	Lg9	(0,0076*SE(T8-T0<0;0;(T8-T0)))^2	COMBASE
		Growth during exposition at retail	log CFU/g	C	G9	T8*Lg8	Calculated
		Level after exposition at retail	log CFU/g	C	L9	L7+L8	Calculated
Transport from retail to home or food service	Transport 4	Temperature at transport 4	°C	V	T10	RiskPert(7;10;15)	Surveyed in the study[b]
		Time of transport 4	h	V	t10	RiskPert(0; 210; 720)	Hessel et al, 2019
		Parameter b growth model	√ Log CFU/day/°C	F	b	0,0076	COMBASE
		Parameter T0 growth model	°C	F	T0	0,558823529	COMBASE
		Logarithmic growth at transport 4	log CFU/g/h	C	Lg10	(0,0076*SE(T9-T0<0;0;(T9-T0)))^2	COMBASE
		Growth during transport 4	log CFU/g	C	G10	T9*Lg9	Calculated
		Level after transport 4	log CFU/g	C	L10	L8+L9	Calculated
Home	Storage at home	Temperature at storage at home	°C	V	T11	RiskPert(7;10;15)	Surveyed in the

							study[b]
		Time of storage at home	h	V	t11	RiskDiscrete({0,5;1;2;3};{0,72;0,25;0,01;0,003})	Hessel et al, 2019
		Parameter b growth model	√ Log CFU/day/°C	F	b	0,0076	COMBASE
		Parameter T0 growth model	°C	F	T11	0,558823529	COMBASE
		Logarithmic growth at storage at home	log CFU/g/h	C	Lg11	(0,0076*SE(T10-T0<0;0;(T10-T0)))^2	COMBASE
		Growth during storage at home	log CFU/g	C	G11	T10*Lg10	Calculated
		Level after storage at home	log cfu/g	C	L11	L9+L10	Calculated
Food service	Storage at food service	Temperature during storage at food service	°C	V	T12	RiskPert(7;10;15)	Surveyed in the study[b]
		Time of market storage at food service	h	V	t12	RiskDiscrete({24;84;276;564;720};{0,01;0,36;0,41;0,17;0,02})	Surveyed in the study[b]
		Parameter b growth model	√ Log CFU/day/°C	F	b	0,0076	COMBASE
		Parameter T0 growth model	°C	F	T12	0,558823529	COMBASE
		Logarithmic growth at storage at food service	log CFU/g/h	C	Lg12	(0,0076*SE(T11-T0<0;0;(T11-T0)))^2	COMBASE
		Growth during storage at food service	log CFU/g	C	G12	T11*Lg11	Calculated
		Level after storage at food service	log CFU/g	C	L12	L10+L11	Calculated
Cooking		Log reduction after cook by boiling	log CFU/g	F	Rc1	1,35	De Paula, Mariot, Tondo, 2005

	Log reduction after cook by frying	log CFU/g	F	Rc2	2,79	De Paula, Mariot, Tondo, 2005
	Log reduction by pasteurization	log CFU/g	F	Rc3	5,9	Latimer et al, 2008
	Level after cooking	log CFU/g	F	L13	L11 or L12 - Rc1 or Rc2 or Rc3	Calculated
Consumption of eggs, determination of dose-response relationship, probability of illness and number of cases	Serving size	S	V	g	RiskPert(60; 120; 300)	Hessel et al, 2019
	Level of pathogen (non-log)	CFU	V	CFU/g	10^L13	Calculated
	Dose per serving	D	V	CFU	S*CFU	Calculated
	Parameter alpha	α	F	No units	0,1324	FAO/WHO 2002
	Parameter beta	β	F	No units	51,4	FAO/WHO 2002
	Probability of infection single dose	Pisd	C	%	1-(1+D/α)^-β	Calculated
	Risk of infection per serving	E	C	Servings	RiskPert(0;2;6)	Hessel et al 2019
	Population Brasil	Rim	F	Ri	RiskOutput()+1-(1-Pi*Pisd)^E	Calculated
	% of population eating eggs	In	F	Inhabitants	210.147.125	IBGE, 2019
	Population eating eggs in Brazil	%eat	C	%	97,54	Hessel et al 2019

[a] = Parameters are described in Table 1; [b] = Parameters are described in Table 2;

[a] C, calculation; F, fixed; V, variability; U, uncertainty

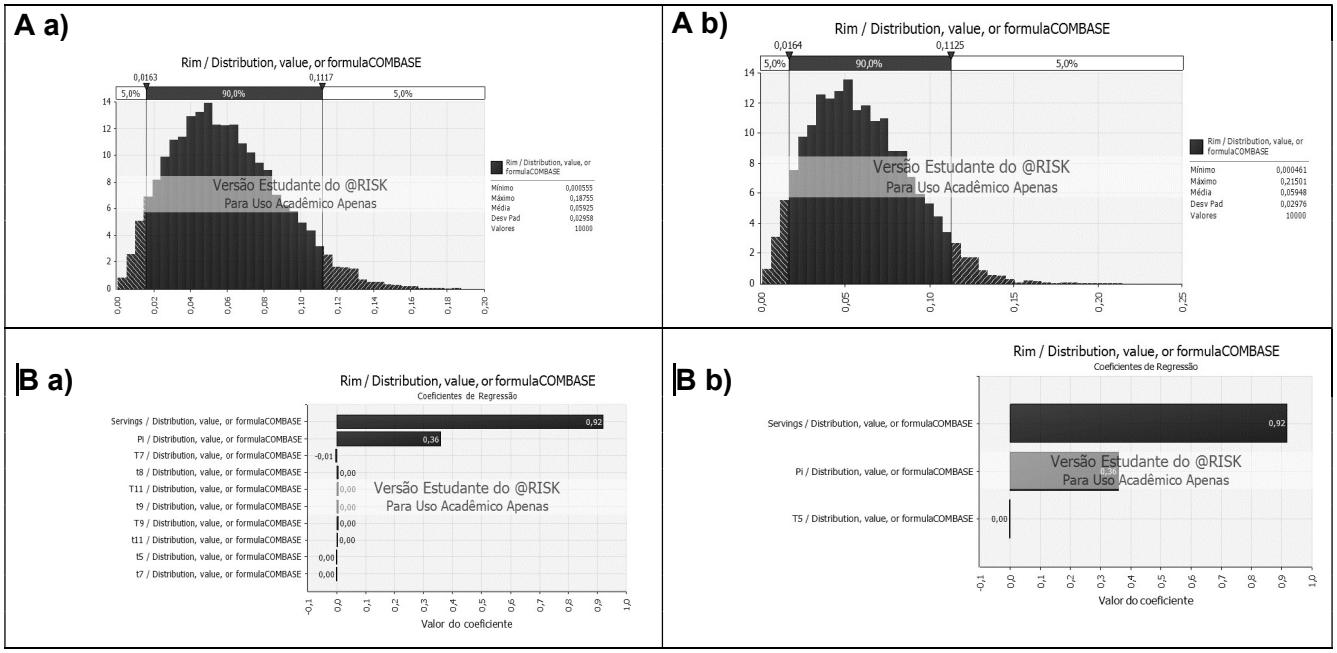

Figure 2: A a) Risk of infection of *Salmonella* due to consumption of eggs at home b) Tornado graphic showing the sensitivity analysis for *Salmonella* contamination in eggs per serving (dose) at home. Spearman's correlation coefficients were obtained from @Risk sensitivity analyses and were shown next to each bar. B: a) Risk of infection of *Salmonella* due to consumption of eggs at food services b) Tornado graphic showing the sensitivity analysis for *Salmonella* contamination in eggs per serving (dose) at food services. Spearman's correlation coefficients were obtained from @Risk sensitivity analyses and were shown next to each bar.

Table 5: Outputs of the QMRA model depicting the risk of infection of *Salmonella*, number of cases of infection and its reductions due to consumption of eggs at home.

	Scenarios	Risk of infection per week per serving	Percentage reduction in infection	Number of cases of infection per week in the population exposed	Percentage reduction in the population exposed
	Baseline	Mean 0.05925	-	Mean 12,277,008.29	-
1	Fried from1,5 to 2,5 min	0.05919	0.10	12,276,120.68	0.01
2	Pasteurized whole egg	0.05866	0.99	12,272,171.52	0.04
3	Prevalence of *Salmonella* in eggs of 0.83%	0.02839	52.08	5,879,389.65	52.11
4	Prevalence of *Salmonella* in eggs of 9.60%	0.2169	-26607	45,986,142.98	-27457
5	Reduce concentration by 50%	0.05914	0.18	12,276,852.27	0,01
6	Prevalence of *Salmonella* in eggs of 0.83% and reduce concentration by 50%	0.02829	52.25	5,879,314.22	52.11
7	Cold chain since industry at maximum temperature of 15ºC	0.05483	7.46	11,562,082.47	5.82
8	Cold chain since industry at maximum temperature of 9ºC	0.04671	21.16	9,838,119.95	19.86
9	Prevalence of *Salmonella* in eggs of 0.83%, reduce concentration by 50% and cold chain since industry at maximum temperature of 9ºC	0.02184	63.14	4,588,306.34	62.62
10	Warm days	0.05941	-27	12,278,564.30	0.01

Each scenario was run in @Risk using 100,000 iterations with generator seed fixed at 1.

Table 6: Outputs of the QMRA model depicting the risk of infection of *Salmonella,* number of cases of infection and its reductions due to consumption of eggs at food services.

Scenarios		Risk of infection per week per serving	Percentage reduction in infection	Number of cases of infection per week in the population exposed	Percentage reduction in the population exposed
		Mean		Mean	
Baseline		0.05948	-	12,278,618.76	-
1	Fried from1,5 to2,5 min	0.05952	-0.07	12,278,618.76	0.00
2	Pasteurized whole egg	0.05945	0.05	12,278,618.76	0.00
3	Prevalence of *Salmonella* in eggs of 0.83%	0.02849	52.10	5,880,168.20	52.11
4	Prevalence of *Salmonella* in eggs of 9.60%	0.21753	-265.72	45,991,841.02	-274.58
5	Reduce concentration by 50%	0.0594	0.13	12,278,618.76	0.00
6	Prevalence of *Salmonella* in eggs of 0.83% and reduce concentration by 50%	0.02851	52.07	5,880,168.20	52.11
7	Cold chain since industry at maximum temperature of 15ºC	0.05945	0.05	12,278,618.76	0.00
8	Cold chain since industry at maximum temperature of 9ºC	0.05942	0.10	12,278,618.76	0.00
9	Prevalence of *Salmonella* in eggs of 0.83%, reduce concentration by 50% and cold chain since industry at maximum temperature of 9ºC	0.02845	52.17	5,880,168.20	52.11
10	Warm days	0.05941	0.12	12,278,618.76	-0.00

Each scenario was run in @Risk using 100,000 iterations with generator seed fixed at 1.

6. DISCUSSÃO GERAL

O Brasil é segundo maior produtor de carne de frango e, atualmente, ocupa o primeiro lugar nas exportações mundiais. Além disso, a produção e o consumo de ovos cresce anualmente no país. A *Salmonella* spp. é um dos principais patógenos causadores de DTA, sendo transmitida, principalmente por esses alimentos. Por este motivo, o objetivo desta Tese foi realizar a avaliação quantitava de risco com a finalidade de calcular o risco de salmonelose, a partir do consumo de frangos e ovos produzidos sob inspeção oficial no Brasil. Embora haja a possibilidade de frangos e ovos produzidos sem inspeção terem maiores prevalências e quantidades de *Salmonella*, aumentando o risco de salmoneloses na população, a decisão de contemplar somente as indústrias sob inspeção oficial, foi baseada no fato de que estas empresas possuem maior número de informações e controles de seus processos, além de serem regularizadas para a produção de alimentos.

Para realização deste trabalho foi realizada uma parceria com o Ministério da Agricultura, Pecuária e Abastecimento (MAPA). Esse fato é importante, uma vez que o MAPA regula grande parte da produção de alimentos no Brasil, sendo portanto, o gestor de risco, em uma AR corretamente estruturada. Através desta colaboração, foram viabilizadas visitas em abatedouros-frigoríficos para conhecimento das realidades e coleta de dados de tempo e temperatura de processamento nesses locais. Além disso, também foi possível ter acesso a laudos de qualidade microbiológica de carcaças de frango, que contribuíram para a realização deste estudo. Abatedouros-frigoríficos, indústrias de ovos, serviços de alimentação, centros de distribuição e supermercados igualmente colaboraram com este estudo, através da permissão de visitas e fornecimento de planilhas de controle de temperatura e dados de processo. Especialistas de diferentes setores da cadeia de produção e distribuição de carne de aves e ovos também colaboraram com este estudo, assim como foram levantados os dados de consumo e boas práticas de manipulação de alimentos em residências brasileiras. Provavelmente, estas são algumas das mais robustas avaliações de riscos de salmonelose pelo consumo de carne de frango e ovos realizada no Brasi.

De acordo com o "*Guide for National Food Safety Authorities*", publicado pela FAO /OMS (2007), a Avaliação de Riscos deve ser baseada em dados científicos de

qualidade, detalhados e a representatividade deve ser proveniente de fontes apropriadas e organizada de maneira sistemática (CAC/GL, 2007). Por este motivo, para identificação e caracterização do perigo foi realizada uma revisão sistemática sobre prevalência e concentração de *Salmonella* spp. em carne de frango e ovos, utilizando dados disponíveis na literatura científica mundial. Para carne de frango, 1086 artigos foram identificados com os termos de busca e 182 foram selecionados, após aplicação dos critérios de inclusão definidos no estudo. A prevalência média de *Salmonella* spp. em frangos, no mundo, foi de 21,02%, enquanto que, no Brasil, a prevalência média de *Salmonella* spp. em carne de frangos foi de 14,96%, sendo que o principal sorovar identificado foi *S.* Enteritidis. Este resultado foi semelhante à prevalência geral encontrada na América Latina (15,2%) e na África (15,3%), seguidos da prevalência média de estudos europeus (18,82%). Estudos realizados em países asiáticos e América do Norte (Estados Unidos e Canadá) reportaram as maiores prevalências de *Salmonella* spp. (25,9% e 38,98%, respectivamente). Além disso, observou-se que a prevalência do patógeno em carcaças coletadas nos mercados foi similar à de abatedouros-frigoríficos (23,1% *vs*. 23,2%). Amostras de carcaça apresentaram menor prevalência (19,7%) quando comparadas à cortes de carne de frango (23%) e houve uma pequena diferença na prevalência de *Salmonella* spp. de amostras de carne congelada (21,1%) e refrigerada/fresca (20,9%).

Apenas três manuscritos relataram a concentração de *Salmonella* spp. em amostras de frango, as quais variaram entre 1,6 CFU/g a 2,3 ± 0.2 log MPN por carcaça.

Para ovos, nove artigos brasileiros foram encontrados, e a mediana de prevalência de *Salmonella* spp. neste produto foi de 2,10%. A pesquisa bibliográfica não encontrou nenhum estudo sobre a concentração de *Salmonella* spp. em ovos no Brasil. Portanto, para o cálculo do risco de salmonelose realizado neste trabalho, foram assumidos os dados publicados pela FAO/OMS (2002). Cinco estudos encontraram ausência de *Salmonella* spp. nos ovos, apesar da fonte de coleta. Estudos realizados nas granjas encontraram prevalência variando de 0 a 4,98%, e a mediana foi de 2,36% (WOLSCHICK, 2015). Outros países relatam prevalências de *Salmonella* spp. em ovos semelhantes as do Brasil, como 0,08% (Uruguai), 2,93% (Colômbia), 4,83% (Índia) e 4,85% (Iraque) (BETANCOR et al., 2010; SINGH;

YADAV; BHARTI, 2010; MOGOLLÓN VERGARA; RODRÍGUEZ GUTIÉRREZ; VERJAN GARCÍA , 2016; ZUBAIR; ISSA, 2017).

A partir dos estudos de prevalência de *Salmonella* spp. em carne de frango e ovos identificados na revisão sistemática realizada neste trabalho, foi possível observar que o Brasil reporta prevalências inferiores a outros países. Esses dados indicam a eficácia das medidas de controle implementadas nas indústrias sob inspeção oficial no Brasil. No entanto, sabe-se que dados de prevalência e concentração podem ser variáveis em diferentes regiões. Por este motivo, além dos dados obtidos a partir da revisão sistemática realizada, este trabalho considerou também dados de prevalência reportados pelo MAPA e de realidades de indústrias, a fim de permitir a criação de um modelo matemático mais realista, capaz de calcular o risco de salmonelose, devido ao consumo de carne de frango e e ovos no Brasil.

Para realização da avaliação da exposição, onde ocorreu a caracterização da cadeia produtiva e hábitos de consumo na população brasileira, foram realizados dois estudos. O primeiro objetivou a coleta de dados reais sobre tempos e temperaturas da cadeia produtiva de frangos e ovos, e o segundo objetivou a coleta de informações de manipulação e consumo desses alimentos, em residências e serviços de alimentação, no Brasil. No primeiro estudo, a fim de obter dados reais de tempos e temperaturas das cadeias de carne de frango e ovos no Brasil foi construído um fluxograma a partir do documento do *Codex Alimentarius* "*Guidelines for the control of Campylobacter and Salmonella spp. in chicken meat CAC/GL 78-2011*" para carne de frango e para ovos utilizou-se o *documento "Risk assessments of Salmonella in eggs and broiler chickens"*. O fluxograma de frango continha etapas, desde o abate até o consumo de carne de frango em casa ou em serviços de alimentação, enquanto o de ovos, desde a produção até o consumo nos mesmos locais. Cada fluxograma foi validado por 10 especialistas da área para ajustá-los à realidade brasileira, sendo esses membros da cadeia produtiva de frango ou ovo, ou ligados ao MAPA.

A partir da validação do fluxograma de carne de frango pelos especialistas, dois cenários de consumo foram identificados: 1) cadeia refrigerada de carne de frango vendida em supermercados e consumida em casa, contendo 21 módulos; 2) cadeia de carne de frango congelado, consumido em serviços de alimentação, contendo 20 módulos. Para a cadeia de ovos, os mesmos cenários foram identificados, sendo contemplados 13 módulos para o consumo em residências, e 10

módulos para o consumo em serviços de alimentação, ambos ocorrendo à temperatura ambiente.

Para cada módulo, dados de tempo e temperatura foram obtidos diretamente dos membros da cadeia produtiva colaboradores do projeto (abatedouros-frigoríficos de frango, indústrias de ovos, centros de distribuição, serviços de alimentação e supermercados). O contato inicial com estes locais foi realizado através de *e-mail* ou telefone.

Para coleta de dados de tempo e temperatura de processamento de carne de frango, em abatedouros-frigoríficos, um questionário foi enviado por *e-mail* às indústrias inspecionadas pelo MAPA no Estado do Rio Grande do Sul. Foram realizadas perguntas referentes a temperaturas e tempos de processamento e solicitado o envio de planihas de controle de temperaturas. Foram recebidos dados de 15 abatedouros-frigoríficos registrados entre janeiro de 2012 a maio de 2018. Duas visitas em plantas industriais de frango, localizadas no Rio Grande do Sul, também foram realizadas para conhecimento da realidade e coleta de dados de tempo e temperatura *in loco*. Para coleta de dados de tempo e temperatura de ovos foi utilizado o mesmo modelo de coleta de dados aplicado à carne de frango.

A temperatura de transporte dos abatedouros-frigoríficos de frangos para o centro de distribuição foi avaliada através de um termopar contendo quatro sensores. Cada sensor foi colocado em diferentes pacotes de carne de frango e o dispositivo foi ligado pouco antes do caminhão deixar o abatedouro-frigorífico. Depois que o dispositivo foi ligado, a temperatura foi registrada a cada 30 segundos. O termopar foi desligado quando o caminhão chegou ao seu destino e a porta do mesmo foi aberta. O mesmo procedimento foi realizado a partir do centro de distribuição até o supermercado. As medições foram realizadas entre maio a novembro de 2018, em três dias diferentes.

O tempo de transporte dos abatedouros-frigoríficos de frangos para o centro de distribuição, indústrias de ovos para centro de distribuição e centros de distribuição de frangos e ovos até os supermercados ou serviços de alimentação foi estimado a partir de dados fornecidos pelo GoogleMaps®. As localizações de origem e destino foram fornecidas pelos colaboradores do projeto. Para cada rota foram assumidos três momentos de trajeto, com horários de partida às 7h, 12h e 18h.

Os dados de serviço de alimentação foram coletados a partir de planilhas de controle de temperatura de equipamentos de armazenamento refrigerado e de

cocção de 141 restaurantes industriais. Os restaurantes pertencem à maior empresa de produção de refeições do Brasil e estão localizados nas cinco macrorregiões brasileiras. Foram compilados dados entre maio e novembro de 2018.

Os dados de tempo e temperatura de carne de frangos e ovos em supermercados, bem como de validade desses produtos foram obtidos diretamente nesses locais, no Estado do Rio Grande do Sul. Planilhas de controle de temperatura de armazenamento no estoque e na exposição aos consumidores foram disponibilizadas, contendo registros entre maio e novembro de 2018.

Para obtenção de dados de temperatura ambiente no Brasil, foi acessada a página do Instituto Nacional de Meteorologia (INMET, n.d.). As temperaturas ambientais máximas e mínimas, no período de 01 de janeiro de 2017 a 29 de novembro de 2018, registradas pelas estações meteorológicas em cada um dos 27 Estados brasileiros foram compiladas.

Todos os dados de tempo e temperatura foram compilados e organizados em planilhas de Excel®, onde cada etapa da produção (módulo) correspondeu a uma coluna. Em seguida, os dados de temperatura foram ajustados às distribuições de probabilidade (BetaGeneral, ChiSqd, Expon, Logistic, LogLogistic, LogNorm, Normal, Pareto, Pert, Student, Triangular, Uniform e Weibull), através da utilização do software @Risk (Palisade corporation, versão 6.3.1). As distribuições foram selecionadas a partir das referências de Roccato, Uyttendaele e Membré (2017) e Alfama et al. (2019). Para definição do melhor ajuste, utilizou-se o erro quadrático médio da raiz de cada módulo.

Para carne de frango, foram compilados 60.166 registros de tempo e temperatura, considerando todos os módulos da cadeia produtiva, do abate ao consumo. Em geral, a média de temperaturas da cadeia resfriada foi adequada (< 5ºC), embora tenham sido observadas alguns desvios na manutenção da temperatura. A cadeia congelada demostrou temperaturas máximas de -14,50 ºC, o que está de acordo com a legislação brasileira. A recomendação oficial é manter o frango congelado a uma temperatura não superior a -12 ± 2 ° C (MAPA, 1998). As temperaturas de cocção e de cadeia quente em serviços de alimentação também demonstraram adequação à legislação (>70 ºC na cocção e >60 ºC, na distribuição quente, respectivamente) (BRASIL, 2004).

Para ovos, foram compilados 14.159 dados de tempo e temperatura, nos cenários e módulos avaliados. A cadeia de ovos no Brasil, da produção à cocão,

ocorre à temperatura ambiente. De fato, de acordo com a legislação brasileira, os ovos que não são desidrados ou pasteurizados, podem ser produzidos e comercializados em temperatura ambiente (MAPA, 1990). Dados de cocção e de cadeia quente nos serviços de alimentação foram igualmente adequados à legislação brasileira (>70 ºC e >60 ºC, respectivamente) (BRASIL, 2004).

As temperaturas coletadas foram ajustadas, principalmente, às distribuições Beta Geral e Logística. Para ajuste de dados de tempo, foi definida a distribuição Pert, uma vez que essa permite a determinação de valores com base nas estimativas de valores mínimos, máximos e mais prováveis para um evento. Esta distribuição é usualmente utilizada para descrever parâmetros de tempo em avaliações de risco (SANT'ANA et al., 2012; SMADI; SARGEANT, 2013; JARVIS et al., 2016). A utilização de dados em termos de distribuições de probabilidades torna possível observar a variabilidade dos parâmetros que influenciam as contagens microbianas e sua probabilidade de ocorrência (MEMBRÉ; GUILLOU, 2016). Essas informações são importantes para desenvolver uma QMRA precisa, pois tempos e temperaturas podem influenciar na multiplicação bacteriana.

A análise dos dados de tempo e temperatura da cadeia de carne de frango e ovos demonstrou que os mesmos estavam em conformidade com os parâmetros recomendados estabelecidos pela legislação brasileira. Esses dados reforçam a eficácia das medidas de controle implementadas na cadeia produtiva desses alimentos no Brasil.

Atualmente, se considera um dos maiores desafios para a realização de uma avaliação de riscos quantitativa a coleta de dados reais das etapas do fluxograma do campo à mesa de alimentos. Neste estudo, obteve-se uma grande quantidade de dados da cadeia de frangos e ovos no Brasil, devido a parceria realizada com o MAPA e com diferentes membros da cadeia produtiva desses alimentos. Esses dados foram utilizados como *inputs* no modelo e, sendo esses reais e aplicáveis ao cenário brasileiro, tornam a predição de risco mais fidedigna e com menor incerteza. No entanto, reconhece-se que os mesmos representam apenas uma amostra da realidade da produção de um país continental como o Brasil. Por este motivo, mais dados devem ser gerados e analisados, o que levará a melhoria de predições por modelos de cálculo de risco.

No segundo estudo da etapa de avaliação da exposição, a fim de acessar o perfil de consumo e as boas práticas de manipulação de carne de frango e ovos pela

população brasileira, foi desenvolvido e aplicado um questionário. Não foram encontrados estudos científicos brasileiros que caracterizassem o comportamento da população em relação ao consumo e à manipulação desses alimentos.

Para construção do questionário foram elaboradas perguntas sobre os temas a partir de artigos científicos e algumas pertinentes ao cenário brasileiro e a esta Tese. Após a construção do questionário, a adequação e a aplicabilidade do instrumento foram verificadas por profissionais da área de segurança de alimentos. Além disso, foi realizado um teste piloto com 10 pessoas, escolhidas por conveniência, com diferentes perfis socioeconômicos (sexo, idade, grau de instrução e renda familiar). Após os ajustes, a versão final do questionário consistiu em 61 questões, que foram aprovadas pelo Comitê de Ética da Universidade Federal do Rio Grande do Sul.

O questionário continha perguntas sobre práticas de manipulação de frangos e ovos durante a compra, o armazenamento, a preparação e o consumo em casa. O questionário foi composto por quatro seções: 1) Perguntas sobre dados socioeconômicos, 2) Perguntas sobre carne de frango, 3) Perguntas sobre ovos e 4) Perguntas sobre boas práticas de manipulação e percepção de riscos. O questionário foi divulgado através de mídias sociais e por listas de *e-mail,* e enviado através do GoogleForms® para diferentes consumidores em todo o Brasil, entre março e abril de 2018. O comportamento dos respondentes foi mensurado, utilizando o *Weighted Harmonic Outbreak Prevention Index* (WHOPI), desenvolvido por Elias et al. (2015).

Ao todo, 1217 questionários foram recebidos e analisados. O WHOPI dos entrevistados não foi correlacionados com o perfil socioeconômico e coma percepção de riscos. As principais não conformidades em boas práticas identificadas foram relacionadas temperatura de transporte do frango do supermercado até as residências, ponto da gema de ovos e à higienização de utensílios. 90,70% dos entrevistados declararam sempre lavar as mãos antes, durante e após o preparo de alimentos, e 9% apenas algumas vezes. Em relação ao uso de diferentes utensílios para o preparo de diferentes alimentos, 76,50% dos entrevistados declararam fazê-lo, enquanto 23,40% decalraram utilizar o mesmo utensílio para diferentes preparações, sem lavá-los. A lavagem adequada dos utensílios de cozinha entre a preparação de alimentos crus e cozidos ou alimentos prontos para consumo foi identificada como a principal forma de eliminar contaminação cruzada dentro da

cozinha (KUSUMANINGRUM et al., 2016). Os dados obtidos de boas práticas foram utilizados nos módulos de contaminação cruzada, do modelo de cálculo de risco.

Para as práticas de manipulação e consumo de frango, 81,20% dos entrevistados decalraram comprar carne de frango refrigerada e, entre eles, menos de 2% compraram frango refrigerado em feiras de rua ou diretamente de produtores. O local mais comum escolhido para comprar frango foram os supermercados ou açougues (98,60%). No momento da compra, 45,50% respondentes conferriam as informações de rótulo sobre o produto. Após a compra de carne de frango, o principal comportamento declarado foi o transporte de carne por menos de 30 minutos até as casas, onde a carne foi armazenada sob refrigeração (72,30%). Nenhum comportamento predominante foi identificado em casa em relação ao tempo entre o armazenamento e o cozimento, variando de 1 hora a mais de 48 horas de armazenamento refrigerado. A maioria dos entrevistados declarou preparar a carne de frango assada ou frita (90,40%), acompanhada de algum tipo de molho (83%). Por este motivo, neste estudo, a carne de frango foi considerada sempre processada termicamente, associado ao comportamento brasileiro de não consumir frango cru. Uma vez preparada, a carne de frango foi exposta à temperatura ambiente, antes de ser servida, por menos de 30 minutos até 1 hora (47,90% e 42,30%, respectivamente). Quando servida, a carne de frango permaneceu de menos de 30 minutos a 1 hora em temperatura ambiente (42,10% e 38,80%, respectivamente). A maioria dos entrevistados (92,60%) afirmou reutilizar carne de frango, após armazenamento refrigerado (91,10%), em um recipiente com tampa (97%), por menos de 24 horas (68,4%). O consumo de carne de frango reutilizada foi majoritariamente de uma só vez (66,10%). Além disso, 80,90% dos entrevistados compraram carne de frango congelada. Em relação ao procedimento de degelo, quase metade dos entrevistados utilizou microondas ou geladeiras para descongelar carne de frango (49,20%), além de 32,10% em temperatura ambiente e 13,60% em água de frango crua. Além disso, a maioria dos entrevistados declarou desconger o frango de 2 a 6 horas (31,50%), seguido por menos de 1 hora (28,70%) e de 6,1 e 12 horas (16,80%). Quando perguntados sobre onde consumiam carne de frango, 46,10% dos respondentes declararam comer apenas em casa e 44% dentro e fora de casa.

Em relação às práticas de manipulação e consumo de ovos, a maioria dos respondentes declarou comprar ovos (95,30%) de quitandas ou mercados locais

(67,80%), enquanto 5,20% adquiriram ovos diretamente de produtores e 24% dos locais mencionados anteriormente. No momento da compra de ovos, 51,50% dos entrevistados registraram que observavam a origem do ovo, a data de validade e o rótulo, indicando o selo da fiscalização sanitária pelo MAPA, na embalagem. Em casa, 90,20% das pessoas declararam que os ovos foram armazenados em geladeiras e 9,40% em temperatura ambiente, permanecendo, principalmente, entre 8 e 15 dias (41,80%) e entre 1 e 7 dias (36,70%), até o preparo. Quanto ao preparo, 97,10% dos entrevistados prepararam ovos por cozimento ou fritura, até obter gemas completamente cozidas (76,50%), enquanto o restante declarou preparar gemas ou omeletes cruas ou mal cozidas (10,70%). As omeletes foram consideradas como preparações contendo gema de ovo não completamente cozida e, por esse motivo, foram analisadas juntamente às gemas mal passadas. O principal comportamento observado foi o preparo dos ovos por menos de 30 minutos antes de servir (92,40%), expondo-os à temperatura ambiente (86,40%) e consumidos em até 30 minutos (88,90%). Apenas 25% dos entrevistados relataram reutilizar ovos. No caso de reutilização, a maioria foi armazenada sob refrigeração (93,30%), em um recipiente tampado (89,90%), e consumida de uma só vez (87,54%), até 1 dia (87,50%), após o armazenamento. Além disso, 75,70% dos entrevistados consumiram ovos apenas em casa, sendo que 56,30% afirmaram comer gema cozida, 33,60% de gema crua ou mal passada e 10,10%, com ambos pontos de dureza da gema.

O perfil de consumo de carne de frango no Brasil demostrou que 96,79% dos respondentes declararam consumir carne de frango, pelo menos 2 vezes na semana (45,57%), em uma refeição diária (74,40%) e na quantidade de, pelo menos, 90 gramas (46,98%). Em relação a ovos, 97,54% dos entrevistados declararam consumir ovos, sendo 10,79% em preparações com gema mole. A frequencia de consumo de ovos foi de 2 à 3 vezes por semana (37,86%) e de 1 ou 2 unidades (47,31% e 46,12%, respectivamente). A partir dos dados de consumo obtidos no questionário, foi calculado o consumo diário em função de distribuição de probabilidade. A distribuição de probabilidade definida foi Discreta, devido a natureza dos dados obtidos no questionário.

A última etapa da avaliação de risco quantitativa é a caracterização de riscos. Nesta etapa integrou-se as informações das três partes anteriores, ou seja, da identificação do perigo, caracterização do perigo e avaliação da exposição. Nesta

etapa foi realizada a estimativa da probabilidade da ocorrência de salmonelose, através do consumo de carne de frango e ovos produzidos por inspeção oficial no Brasil. Diversos cenários foram testados para avaliar o impacto de melhorias específicas na gestão de riscos, através da redução no risco de infecção por *Salmonella* spp. na população brasileira. A eficácia de cada uma das estratégias de mitigação de risco testadas foi medida como a porcentagem de redução na probabilidade prevista de infecção, devido ao consumo de uma refeição, mantendo todos os outros parâmetros originais e distribuições de probabilidade constantes.

O modelo de avaliação de risco compreendeu 21 módulos, desde o abatedouro-frigorífico até o consumo em residências e 20 módulos, para o consumo em serviços de alimentação.

Para carne de frango consumida nas residências, o cenário real considerou a carne de frango refrigerada abatida sob inspeção oficial no Brasil e comercializada nos supermercadso. Nesse cenário foi assumida a prevalência inicial de *Salmonella* spp. de 4,04% (prevalência identificada a partir dos dados oficiais do MAPA, em 10 anos no RS) e uma concentração média de 1,6 log UFC/g na etapa de sangria no abatedouro-frigorífico. Assumiu-se que no abatedouro-frigorífico as etapas de escaldagem, lavagem com água potável de carcaças e *chiller* reduziram a concentração de *Salmonella* spp., enquanto durante o porcionamento ocorreu a contaminação cruzada a partir das mãos dos manipuladores de alimentos para as carcaças e das superfícies de utensílios para as carcaças. Também no cenário real, assumiu-se que o processamento térmico durante o cozimento da carne de frango inativou todas células de *Salmonella* spp. viáveis. Isso foi assumido porque não há o hábito de consumo de carne de frango crua no Brasil e os dados de temperaturas de cocção em serviços de alimentação demonstrarm temperaturas adequadas, acima dos 70º C preconizados pela legislação brasileira. Após a cocção, assumiu-se que ocorreu contaminação cruzada via mãos de manipuladores e superfícies, portanto, o número de *Salmonella* spp. na carne de frango imediatamente antes do consumo foi devido a contaminação cruzada ocorrida, após o cozimento. Esse cenário coloca grande importância na prevenção da contaminação cruzada dentro das cozinhas de residências e serviços de alimentação, indicando que uma estratégia de mitigação de risco de salmoneloses poderia ser a realização de campanhas educacionais para incentivar a prevenção da contaminação cruzada nas cozinhas, após a cocção adequada de carne de frango.

Para os parâmetros de contaminação cruzada foram utilizados os índices de boas práticas identificados no questionário aplicado na avaliação da exposição e o modelo de contaminação cruzada de Smadi & Sargeant (2012).

Os parâmetros de tempo e temperatura, desde o abatedouro-frigorífico até o consumo final, foram obtidos na etapa de avaliação da exposição. O modelo preditivo de multiplicação de *Salmonela* spp. foi desenvolvido por Pessoa et al. (2019). No trabalho de Pessoa et al. (2019), a multiplicação de cinco sorovares de *Salmonella* (*S*. Enteritidis, *S*. Typhimurium, *S*. Heidelberg, *S*. Saintpaul e *S*. Infantis) foi predita na carne de frango brasileira, crua. Esses sorovares foram utilizados por serem importantes para a produção avícola brasileira (BRASIL, 2012). Foi utilizado os coeficientes de correlação de Spearman para análise de sensibilidade do modelo real para determinar o efeito das variáveis inseridas na probabilidade de doença por porção e no número de casos de salmonelose no Brasil.

O risco de infecção por *Salmonella* spp. devido ao consumo de carne de frango nas residências brasileiras foi de 0,008092 (8,092 em 1.000 exposições), variando de 0,003923 a 0,032498. O número de casos de infecção na população foi de 163.809.233.27, o que representa 77,94% da população, considerando a população de 210.147.125 (IBGE, 2019). Dados epidemiológicos relatam que entre 2009 e 2018 foram registradas apenas 274 salmoneloses no Brasil (BRASIL, 2019), mostrando que, possivelmente, o risco calculado foi superestimado. Algumas possíveis razões para essa superestimação são: (1) A alta porcentagem da população exposta, relacionada ao grande consumo de carne de frango no Brasil (HESSEL et al., 2019). De fato, o risco devido ao consumo de carne de frango contaminada com *Salmonella* spp. depende do conjunto de circunstâncias existentes durante o consumo de cada refeição individual. Assim, o cálculo assumiu o risco por porção em que cada carne de frango ingerida teve o mesmo risco previsto de doença e que o risco de cada exposição foi independente de outras exposições (POUILLOT et al., 2016); (2) a contaminação de *Salmonella* spp. assumida na depenagem e evisceração, que pode não ocorrer em toda produção de carne de frango; (3) a combinação de diferentes vias de contaminação cruzada (contaminação cruzada na indústria e em casa), que nem sempre pode ocorrer; (4) O modelo considerou o pior cenário, assumindo que 1 UFC de *Salmonella* spp. seria capaz de causar salmonelose. Outros autores relataram doses infecciosas mais altas de *Salmonella* spp. (AKIL et al., 2019); (5) o número real de casos de

salmonelose ocorridos no Brasil provavelmente está subestimado, devido à subnotificação.

A correlação de Spearman revelou que contribuíram para o aumento do número médio de casos de infecção a quantidade de ingestão de *Salmonella* spp., a contaminação cruzada através das superfícies de placas de corte na indústria, a prevalência inicial de *Salmonella* spp., a temperatura no armazenamento no supermercado e a contaminação cruzada de frango cru para placas de corte nas residências. Por outro lado, a lavagem das mãos em casa e a temperatura durante o transporte do centro de distribuição para o supermercado contribuíram para diminuir o risco de infecção por *Salmonella* spp.

O maior risco de infecção por *Salmonella* spp. relacionado à quantidade de *Salmonella* spp. ingerida está relacionado às circunstâncias existentes durante o consumo de cada refeição individual. Isso significa que as pessoas que consomem menos carne de frango têm menor risco de contaminação por *Salmonella* spp., o que é bastante evidente. No entanto, é importante destacar que o número de casos de infecção não representa o número de casos de salmonelose, mas o número de pessoas contaminadas por pelo menos 1 célula de *Salmonella* spp. A quantidade de células de *Salmonella* spp. necessária para causar salmonelose, ou seja, a dose infecciosa, pode influenciar o desenvolvimento ou não da doença, porque a ocorrência de salmonelose dependerá da imunidade de cada pessoa contaminada pelo patógeno, da virulência da cepa de *Salmonella* (POUILLOT et al., 2016), entre outros fatores.

Além do cálculo de risco real, diversos cenários foram testados para avaliar o impacto de melhorias específicas na gestão de riscos, a fim de reduzir a ocorrência de salmonelose na população brasileira. Os cenários 1, 2, 3 e 4 consideraram outros dados de prevalência de *Salmonella* spp. na carne de frango no Brasil. No cenário 1, a prevalência inicial de *Salmonella* spp. foi de 14,04%, identificada a partir da revisão sistemática realizada na etapa de identificação e caracterização deste trabalho. O cenário 2 considerou a prevalência de *Salmonella* spp. de 17,76%, segundo os dados do MAPA. Esses dados foram obtidos pelo MAPA, após a estimativa da prevalência de *Salmonella* spp. nas carcaças de frango, das cinco macrorregiões brasileiras, entre 2017 e 2018. O cenário 3 considerou a prevalência de *Salmonella* spp. em frango de 49% (BORGES et al., 2019). O cenário 4 considerou a redução de 50% da prevalência inicial (4,04%), ou seja, uma

prevalência em torno de 2,0%. O cenário 5 considerou a contaminação adicional por *Salmonella* spp. durante a depenagem e evisceração, pois essas etapas são consideradas fonte de *Salmonella* spp. durante o abate de frangos. O cenário 6 considerou 50% de redução na concentração inicial de *Salmonella* spp. O cenário 7 considerou uma redução de 50% na prevalência e na concentração inicial de *Salmonella* spp. O cenário 8 considerou a manutenção da carne de frango a uma temperatura máxima de 7ºC, desde a indústria até o armazenamento doméstico. O cenário 9 considerou a redução na contagem de *Salmonella* spp. na etapa de escaldagem, lavagem das carcaças com água potável e resfriamento no *chiller*. Os cenários 10, 11, 12, 13 e 14 consideraram a redução da concentração de *Salmonella* spp., devido a utilização de sanitizantes na lavagem de carcaças dentro da indústria (ácido acético, ácido lático, cloreto de cetilpiridínio, fosfato trissódico e tratamentos combinados). Por fim, o cenário 15 considerou a redução de 50% na prevalência e nas concentrações de *Salmonella* spp. na carne de frango na indústria acrescida de uma redução de *Salmonella* spp., devido a escaldagem, lavagem de carcaças com água potável, resfriamento no *chiller* e manutenção da cadeia de frio em temperatura máxima de 7ºC até a cocção. Nesse último cenário, todos os fatores que podem contribuir para reduzir o risco de salmonelose foram considerados no modelo.

Os resultados demonstraram que no cenário 1, o risco de infecção aumentou 132,30%, enquanto que no cenário 2 o risco aumentou 337,71%, e no cenário 3, o risco aumentou em 1.117,25% em relação ao cenário real. Observou-se que o aumento da prevalência inicial de *Salmonella* spp. no frango está intimamente relacionado ao incremento do risco de salmonelose. Logo, essa é outra estratégia de mitigação do risco de salmonelose, a qual também está relacionada a primeira estratégia de mitigação identificada nesse estudo, a prevenção de contaminação cruzada dentro das cozinhas. Quanto maior a prevalência de *Salmonella* nas carcaças de frango que são preparadas nas cozinhas, maior pode ser a contaminação cruzada nesses locais.

O cenário 4, que considerou redução de 50% da prevalência inicial de *Salmonella* spp., demonstrou que o risco de infecção foi de 0,004042 (4.042 em 1.000 consumos), representando 50,05% de redução de risco, em comparação com o cenário real. Outros modelos de QMRA relataram resultados diferentes para a redução da prevalência de *Salmonella* spp.. Por exemplo, Smadi & Sargent (2012) demonstraram que uma redução de 50% da prevalência inicial de *Salmonella* spp.

no varejo canadense reduziu o risco para 0%, enquanto que em um estudo chinês, a redução da prevalência de *Salmonella* spp. na carne de frango de 41,8% para 8,8% resultou em uma redução de risco de apenas 10% (ZHU et al., 2017). A diferença de resultados entre QMRA indicam que deve-se ter cautela na comparação de resultados de avaliação de riscos, pois cada modelo utiliza *inputs* específicos, que, consequentemente, podem gerar *outputs* diferentes.

O cenário 5 simulou um aumento na contaminação por *Salmonella* spp. devido à depenagem e evisceração. O risco nesse cenário foi de 8,097 casos em 1000 exposições, semelhante ao cenário real. A falta de influência no risco pode estar relacionada ao baixo aumento de concentração de *Salmonella* spp. nessas etapas: 0,8 log UFC/g para depenagem e 0,15 log UFC/g para evisceração, números provenientes de estudos científicos. No entanto, na realizade de abatedouros-frigoríficos, esses valores são variáveis entre as indústrias e também entre os lotes de frango. Além disso, outros fatores estão relacionados ao aumento da contaminação por *Salmonella* spp. nestes locais, tais como o manuseio higiênico de carcaças, equipamentos de abate sujos, dispersão de água contaminada de etapas de escaldagem e refrigeração e dispersão de resíduos após evisceração (RIVERA-PÉREZ, 2014).

O cenário 6 avaliou a redução de 50% da concentração de *Salmonella* spp. e o risco não foi modificado quando comparado ao cenário real. Este resultado demonstra que apenas a redução das cargas de *Salmonella* spp. no início do processo industrial não reduziu o risco de salmonelose na população. No entanto, no cenário 7, em que a prevalência e a concentração foram reduzidas em 50%, o risco foi proporcionalmente reduzido em 50,09%. A redução obtida provavelmente ocorreu devido à redução da prevalência. Apesar da ausência de redução de risco pela redução na concentração de *Salmonella*, a redução na carga microbiana é essencial para reduzir a contaminação cruzada (CARRASCO et al., 2012; RAJAN et al., 2017). Portanto, essa estratégia não deve ser ignorada pelas indústrias.

O cenário 8 representa a implementação da cadeia de frio (< 7 ºC), desde a indústria até as residências. A redução de risco foi de 1,70%, comparada ao cenário real. A baixa contribuição da temperatura na redução de risco pode ser explicada pela adequação das temperaturas da cadeia de carne de frango no Brasil e manutenção da cadeia de frio, observadas na etapa de avaliação da exposição.

Nos cenários 9 a 14, os riscos não foram reduzidos em comparação ao cenário real, demostrando que a redução das contagens de *Salmonella* spp. devido a escaldagem, chiller e uso de sanitizantes na lavagem de carcaças não impactaram nos riscos de salmonelose, na população. Resultado semelhante foi obtido em um QMRA coreano, no qual os autores demonstraram que a cloração, apesar de reduzir a concentração de *Salmonella* spp. no frango, não reduziu o risco de salmonelose (JEONG et al., 2018). Similarmente ao cenário 6, apesar da ausência de redução de risco, a estratégia de redução da contaminação dentro da indústria não deve ser ignorada, pois a redução na carga microbiana é essencial para reduzir a contaminação cruzada. Essas estratégias de redução de contagens podem ser bastante importantes, inclusive para reduzirem as prevalências de *Salmonella*, principalmente considerando carcaças contaminadas com baixas concentrações do microrganismo, uma vez que, por exemplo, as reduções obtidas pelo uso de sanitizantes na lavagem de carcaças podem chegar a 3,4 log (HINTON & CASON, 2008; LORETZ et al. 2010).

O último cenário, cenário 15, foi o mais eficaz na redução do risco de salmonelose. Nesse cenário, o risco foi reduzido em 50,95%, ou seja, 3,96 casos em 1.000 exposições. Além disso, o número de casos de infecção foi de apenas 38,92%. Como esperado, nesse cenário foi obtida a maior redução de risco. Esse resultado foi próximo do obtido nos cenários 5 e 7, onde a prevalência de *Salmonella* spp. foi reduzida em 50% e a prevalência e concentração em 50%, respectivamente. No cenário 7, a redução percentual na infecção foi de 50,05% e no cenário 5, a redução foi de 50,09%. Para os demais cenários testados, o risco não foi reduzido acima de 1,70%. Esses resultados demonstraram que a redução da contaminação por processamento ou por agentes sanitizantes aplicados na indústria de abate não reduziria diretamente o risco de salmonelose no Brasil. Portanto, essa redução na concentração de *Salmonella* spp. contribui para a diminuição da contaminação por *Salmonella* spp. nas carcaças.

Para carne de frango consumida em serviços de alimentação, o cenário real considerou a carne congelada de frango sob inspeção oficial no Brasil. Conforme identificado no estudo de avaliação da exposição, a cadeia congelada não possui desvios de temperatura, os quais puderam influenciar na multiplicação de *Salmonella*. Assim, do abatedouro-frigorífico ao descongelamento, nos serviços de alimentação, assumiu-se que nenhuma multiplicação de *Salmonella* spp. ocorreu

nesses módulos. Os parâmetros de tempo, temperatura e boas práticas seguiram os dados obtidos na etapa de avaliação da exposição, e o modelo preditivo de multiplicação de *Salmonella* spp. em carne de frango adotado foi o de Pessoa et. al (2019).

O risco de infecção por *Salmonella* spp. devido à carne de frango consumida nos serviços de alimentação no Brasil foi de 0,007959 (7,95 casos em 1.000 exposições), variando de 0,003939 a 0,040614. O número de casos de infecção por ano na população exposta foi de 163.809.233,27 (77,94% da população brasileira). Neste estudo, foi assumido que toda a população brasileira consome frango em serviços de alimentação, uma vez que não foi encontrado nenhum relato de frequência de consumo de carne de frango em serviços de alimentação no país. Observou-se que o risco, apesar de considerar carne de frango congelada e menos módulos, foi um pouco menor que os riscos nas residências. Apesar da pequena diferença de risco, o número de casos de infecção por ano na população exposta foi igual nos dois locais.

A correlação de Spearman demonstrou que o número médio de salmonelose foi mais sensível ao tamanho da porção, seguido pela prevalência de *Salmonella* spp. e o uso da mesma placa de corte para carne de frango crua e preparada, nos serviços de alimentação. A lavagem adequada das mãos nos serviços e indústrias de alimentos e a substituição/lavagem da placa de corte na indústria contribuíram para diminuir o risco.

Os resultados dos cenários testados foram semelhantes ao resultado do modelo de consumo de frango nas residências. Nos cenários 1, 2, 3, 5, 6, 9 a 14, o resultado do risco foi menor do que o consumido nas residências e nos cenários 4, 7, 8 e 15, o risco foi igual ao cenário real.

Neste trabalho a prevalência de *Salmonella* spp. foi considerada uma incerteza no modelo, pois esse parâmetro pode variar entre plantéis, granjas, e abatedouros-frigoríficos. Para variabilidade, foram consideradas a concentração inicial de *Salmonella* spp., a redução ou aumento, devido às etapas de abate, probabilidade de contaminação cruzada, temperaturas e tempos de processo.

A mitigação de risco mais relevante observada nesse estudo foi a redução na prevalência inicial de *Salmonella* spp. e aprevenção da contaminação cruzada nas cozinhas onde são preparadas as carnes de frango. Isso indica que o patógeno deve ser controlado nas granjas, antes das aves serem transportadas ao abatedouro-

frigorifico. Além disso, as reduções devido ao processo de abate e agentes sanitizantes aplicados, apesar de não apresentarem efeito na redução de risco, são importantes para redução da contaminação da carcaça, podendo contribuir para a comercialização desses produtos. Por fim, a maior redução de risco foi observada quando todos os fatores que podem contribuir para reduzir o risco de salmonelose foram aplicados e utilizados em conjunto, demonstrando que as estratégias de mitigação de risco precisam ser gerenciadas por diferentes setores envolvidos na cadeia de carne de frango.

A avaliação de risco de salmonelose pelo consumo de ovos no Brasil compreendeu 13 módulos, desde a produção de ovos em fazendas de produtores até o consumo em residências e 10 módulos, considerando o consumo de ovos em serviços de alimentação.

Para o cálculo do risco, assumiu-se o consumo com gemas mal cozidas, uma vez que ovos corretamente processados termicamente não apresentam riscos de salmonelose. No cenário real a prevalência utilizada foi de 2,36%, e para concentração, como a avaliação sistemática realizada não identificou estudos brasileiros relatando a concentração de *Salmonella* em ovos no Brasil, foram assumidos os dados das FAO/OMS (2002). Esse valor foi transformado em log UFC/g para concordar com unidades utilizadas no modelo dessa Tese. Em relação ao tempo e temperatura, foram utilizados os dados na etapa de avaliação da exposição. O modelo preditivo adotado foi o disponÍvel no *software* COMBASE.

O risco inicial de infecção por porção foi de 0,059 (aproximadamente 6 casos em 100 porções) e 12.277.008 número de infecções na população. A correlação de Spearman demonstraram que a prevalência de *Salmonella* e o número de porções contribuíram para o aumento do risco de salmoneloses pelo consumo de ovos. Isso significa que as pessoas que consomem ovos menos vezes têm um risco menor de contaminação por *Salmonella* spp. É importante destacar que o número de casos de infecção não representa o número de doenças, mas o número de pessoas contaminadas pelo microrganismo. A temperatura ambiente, o tempo de processamento, armazenamento e transporte não influenciaram o risco de infecção. A ausência de influência da temperatura pode estar relacionada à ausência de variação na temperatura da cadeia de ovos, que no Brasil ocorre à temperatura ambiente.

A fim de avaliar o impacto de melhorias específicas na gestão de riscos para reduzir a ocorrência de salmonelose na população brasileira pelo consumo de ovos, diversos cenários foram testados. O cenário 1 considerou diferentes práticas de cozimento no manuseio de ovos a serem adotadas pelos manipuladores de alimentos nos serviços de alimentação ou nas residências. Para isso, foi utilizado o estudo de Paula et al. (2005) que demonstrou uma redução de 2,79 log UFC/ml de gema de ovo, após uma cocção parcial. O cenário 2 considerou o uso de gema de ovo líquida pasteurizada (5,9 log UFC/ml de redução). Os cenários 3 a 6 consideraram diferentes prevalências e/ou concentrações do patógeno. Os cenários 7 e 8 consideraram a implementação e manutenção da cadeia de frio na cadeia de ovos, da indústria até o cozimento. O cenário 9 considerou a redução na prevalência e concentrações na granja e a implementação e manutenção da cadeia de frio na cadeia de ovos da indústria até o cozimento. Finalmente, o cenário 10 considerou o cenário de linha de base de prevalência, concentração e práticas culinárias, ocorrendo em dias quentes (temperatura mínima de 15 ºC, média de 28 ºC e mais alta de 40 ºC).

No cenário 1, o ponto de cocção inadequado da gema não reduziu o risco de infecção. Da mesma forma, o uso de ovo líquido pasteurizado não reduziu o risco de infecção por *Salmonella* spp. (cenário 2). Esses resultados podem ser atribuídos porque o ovo contaminado em nível de gema, exposto por longos períodos, pode apresentar concentrações de *Salmonella* spp.de aproximadamente 8 log UFC/mL de gema (DE PAULA et al., 2005; LOPES et al., 2019), o que não será totalmente inativado, após pasteurização ou cocção parcial. Além disso, a não redução do risco pode estar relacionada a dose infectante de 1 UFC utilizada nessa avaliação de risco. Esses dados reforçam a necessidade de práticas de biosseguridade nas granjas produtoras de ovos, as quais devem ser bastante controladas em relação a contaminações por *Salmonella* spp. Nesse sentido, granjas podem ser classificadas conforme o nível de bioseguridade de fato implementado, e os ovos provenientes das mesmas podem ser mais ou menos expostos a temperaturas de risco. Ovos vindos de granjas com fortes práticas de bioseguridade podem ser mantidos em temperaturas mais altas ou mesmo em temperaturas ambientes, como ocorre no Brasil. Ao contrário, ovos provenientes de granjas sem todos os controles de bioseguridade implementados, devem ser conservados sob refrigeração, a fim de mitigar riscos de salmonelose.

No cenário 3, a redução da prevalência de *Salmonella* de 2,36% (cenário real) para 0,83% reduziu 52% no risco de infecção por porção. Uma redução semelhante foi observada quando a prevalência inicial foi reduzida para 0,83% e a concentração reduzida pela metade (cenário 6). No entanto, o cenário 4 considera a prevalência inicial de *Salmonella* spp. de 9,6%, a mais alta observada no estudo da literatura. Nesse cenário, o risco de infecção por porção foi de 0,21, representando 266% de aumento em comparação à linha de base. Nesse caso, o número de casos de infecção na população exposta foi de 45.986.142.

A redução da concentração de *Salmonella* spp. em 50% não reduziu o risco de infecção (cenário 5), assim como a implementação da cadeia de frio a 15ºC (cenário 7) ou o aumento da temperatura ambiental para 28º C (cenário 10) não interferiram no risco de infecção, uma vez que a *Salmonella* spp. pode ser desenvolver em ambas as temperaturas, assim como em temperaturas ambientais, como as consideradas no cenário real.

Por outro lado, quando a cadeia de frio foi implementada desde a indústria na temperatura máxima de 9ºC (cenário 8), o risco de infecção por porção foi reduzido em 21%. Por fim, quando a prevalência de *Salmonella* spp. nos ovos foi de 0,83%, a concentração foi reduzida em 50% e a cadeia de frio foi implementada desde a indústria a temperatura máxima de 9ºC (cenário 9) o risco de infecção por porção foi reduzido em 63%, o maior redução encontrada neste estudo.

Os resultados do modelo devido ao consumo de *Salmonella* spp. nos ovos consumidos nos serviços de alimentação demonstraram que o risco de infecção por porção foi de 0,059 (quase 6 casos em 100 porções) e 12.278.618 casos de infecção na população exposta. Esses resultados são muito semelhantes aos obtidos no modelo para o consumo em residências. A correlação de Spearman dos resultados do modelo demonstram que e o número médio de casos de infecção foi mais sensível à prevalência de *Salmonella* e ao número de porções.

Similarmente aos resultados obtidos para consumo nas residências, os cenários 1, 2, 5, 7, 8 e 10 não reduziram o risco de salmonelose. Os cenários 3, 6 e 9 reduziram o risco, e o cenário 4 demostrou aumento do mesmo.

Na avaliação de riscos de ovos, a prevalência de *Salmonella* spp. foi considerada uma incerteza no modelo, enquanto considerou-se a concentração inicial de *Salmonella* spp., temperaturas e tempo, as variabilidade do modelo. Apesar da grande quantidade de dados coletados neste estudo, sempre são

necessários mais dados para melhorar a precisão dos modelos de avaliação de riscos.

7. CONCLUSÃO E CONSIDERAÇÕES FINAIS

- ✓ A revisão sistemática demostrou que a prevalência de *Salmonella* em carne de frango no Brasil variou de 0 a 44,6%, apresentando média de 14,96%, quanto que prevalências maiores foram encontradas em diversos outros países;

- ✓ A prevalência de *Salmonella* spp. em ovos variou de 0 à 9,6%, sendo a média de 2,10%. A pesquisa bibliográfica realizada não encontrou nenhum estudo sobre a concentração de *Salmonella* spp. em ovos no Brasil;

- ✓ A média de temperaturas da cadeia resfriada, cadeia congelada e temperaturas de cocção e manutenção de cadeia quente de frango no Brasil estavam adequada à legislação brasileira (abaixo de 5ºC, abaixo de -12ºC, acima de 70 ºC e acima de 60 ºC, respectivamente);

- ✓ A cadeia de ovos no Brasil, da produção ao consumo, ocorreu em temperatura ambiente;

- ✓ As principais não conformidades de boas práticas praticadas durante o manipulação de carne de frango e ovos em residências brasileiras foram relacionadas à temperatura de transporte da carne de frango do supermercado às residências, ponto de cocção da gema (gema mole) de ovos e à higienização de utensílio;

- ✓ O padrão de consumo de carne de frango predominantemente identificado foi de pelo menos 2 vezes na semana, em uma refeição diária e na quantidade de, pelo menos, 90 gramas.

- ✓ O padrão de consumo de ovos predominantemente identificado foi de 2 à 3 vezes na semana e de 1 ou 2 ovos, por refeição.

- ✓ O risco de infecção por *Salmonella* devido ao consumo de carne de frango nas residências no Brasil foi de 8,092 em 1.000 exposições. Os fatores que mais contribuíram para o aumento do risco de salmonelose foram a ocorrência de contaminação cruzada nas cozinhas, a prevalência inicial de *Salmonella* nos frangos ao entrar no abatedouro-frigorífico e a quantidade de células de *Salmonella* ingeridas;

- ✓ O risco de infecção por *Salmonella* spp. devido à carne de frango consumida nos serviços de alimentação no Brasil foi de 7,95 casos em 1.000 exposições. Os fatores que mais contribuíram para o aumento do risco de salmonelose foram os mesmos que nas residências.

- ✓ As estratégias principais para mitigação identificadas foram a implementação de campanhas para redução de contaminação cruzada dentro das cozinhas de residências e serviços de alimentação, após cocção adequada da carne de frango e redução na prevalência inicial de *Salmonella* nos frangos ao entrar no abatedouro frigorífico;

- ✓ A redução da contaminação de *Salmonella* spp. em carcaças de frango, dentro das indústrias, não teve impacto no risco de salmonelose na população, no entanto, essas estratégias não devem ser ignoradas, pois a redução na carga microbiana é importante para e pode impactar na redução das prevalências de *Salmonella* e reduzir a possibilidade de contaminação cruzada;

- ✓ O risco de infecção por *Salmonella* devido ao consumo de ovos nas residências e serviços de alimentação foi de 6 casos em 100 porções. Contribuíram para o aumento do risco a prevalência inicial e a quantidade de *Salmonella* ingerida.

- ✓ A redução na prevalência inicial de *Salmonella* nas granjas e implementação da cadeia de frio (abaixo de 9º C) foram as estratégias mais eficazes para mitigação do risco de salmonelose pelo consumo de ovos.

- ✓ As avaliações de risco desenvolvidas nessa Tese podem auxiliar no desenvolvimento de estratégias de intervenção e gestão para mitigar os riscos de salmonelose pelo consumo de frangos e ovos no Brasil.

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