

UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO” CÂMPUS BOTUCATU
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

**INFLUÊNCIA DOS SISTEMAS DE PRODUÇÃO ANIMAL NA
PRESENÇA DE CEPAS PATOGÊNICAS DE *Escherichia coli* NA
CADEIA PRODUTIVA DA CARNE BOVINA**

LETÍCIA ROBERTA MARTINS COSTA

Botucatu – São Paulo
Janeiro / 2022

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Dissertação apresentada junto ao
Programa de Pós-graduação em
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Orientador: Prof. Dr. Juliano Gonçalves
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“O caminho do sucesso é o caminho da aprendizagem contínua.”

Surama Jurdi

LISTA DE ABREVIACÕES

A/A - Aderência agregativa
A/E - *Attaching and effacing*
AAF – Aderência agregativa por fímbrias
ABIEC - Associação Brasileira das Indústrias Exportadoras de Carnes
aEPEC - EPEC atípica
AMPc - Adenosina monofosfato cíclico
APPCC – Análise de Perigos e Pontos Críticos de Controle
APT – Água Peptonada Tamponada
BFP - *Pilus* formador de feixe
BPF – Boas Práticas de Fabricação
CDC - Centro de Controle e Prevenção de Doenças dos Estados Unidos
CFA - Colonization factor antigens
CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico
DAEC - *Escherichia coli* difusamente aderente
DEC - *Escherichia coli* diarreiogênica
EAEC - *Escherichia coli* enteroagregativa
EAF – Fator de aderência de EPEC
EHEC - *Escherichia coli* enterohemorrágica
EIEC - *Escherichia coli* enteroinvasiva
EMB – Ágar Eosina Azul de Metileno
EPEC – *Escherichia coli* enteropagotogênica
EPM – Escola Paulista de Medicina
ETEC – *Escherichia coli* enterotoxigênica
ExPEC – *Escherichia coli* extra intestinais
FMVZ – Faculdade de Medicina Veterinária e Zootecnia
Gb3 – Globotriaosilceramida
GMPc - Guanosina monofosfato cíclico
HC - Colite hemorrágica
IMViC – Indol, Vermelho de Metila, Vogues Proskauer e Citrato
LACEN - Laboratórios Centrais de Saúde Pública
LEE - *Locus of enterocycle effacement*

LPS – Lipopolissacarídeos

LT - Toxina termolábil

MILi – Motilidade, Indol e Lisina

MLST – Tipificação de sequências multilocus

PCR – Reação em cadeia da polimerase

PFGE - *Pulsed Field Gel Electrophoresis*

PIB – Produto Interno Bruto

RIISPOA - Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal

ST - Toxina estável ao calor

STEC - *Escherichia coli* produtora de toxina Shiga

Stx – Shiga toxina

Stx1 – Toxina Shiga 1

Stx2 – Toxina Shiga 2

SUH -Síndrome urêmica-hemolítica

TEC - Tonelada Equivalente Carcaça

tEPEC – EPEC típica

VTEC - *Escherichia coli* produtora de verotoxina

WGS – Sequenciamento completo do genoma

LISTA DE TABELAS

Table 1 - Description of points, samples and collection methods for the detection of <i>E. coli</i> carried out at the cattle slaughterhouse.....	38
Table 2 - <i>E. coli</i> pathotypes and their virulence markers used for identification	39
Table 3 - List of primers for identifying <i>E. coli</i> pathotypes.	39
Table 4- List of primers for identification of <i>hlyA</i> , <i>stx1</i> / <i>stx2</i> genes in STEC positive samples.....	40
Table 5- Primers according to Clermont et al. (2013) for the determination of phylogenetic groups.de DEC.....	41
Table 6- List of primers for the identification of DEC serogroups	42
Table 7- Frequency of generic <i>Escherichia coli</i> according to the sample collected in a cattle slaughterhouse in the state of Minas Gerais.	43
Table 8 - Frequency of pathogenic <i>Escherichia coli</i> according to the type of sample collected at a cattle slaughterhouse in Minas Gerais.....	43
Table 9 - Frequency of diarrheagenic <i>Escherichia coli</i> pathotypes from animal feces collected at a cattle slaughterhouse in the state of Minas Gerais.	44
Table 10 - Frequency of diarrheagenic <i>Escherichia coli</i> phylogroups from animal feces collected at a cattle slaughterhouse in the state of Minas Gerais.	44
Table 11 - Frequency of pathogenic <i>Escherichia coli</i> serogroups from animal feces collected at a cattle slaughterhouse in the state of Minas Gerais.	45

LISTA DE FIGURAS

Figura 1 – Representação da interação de cada patotipo de DEC com a célula-alvo.	13
Figura 2 - Mecanismo de patogenicidade de EPEC na célula alvo.....	14
Figura 3 - Mecanismo de patogenicidade de EIEC na célula alvo.	15
Figura 4 - Mecanismo de patogenicidade de EAEC na célula alvo.....	16
Figura 5 - Mecanismo de patogenicidade de ETEC na célula alvo.	17
Figura 6 - Mecanismo de patogenicidade de DAEC na célula alvo.....	18
Figura 7 - Mecanismo de patogenicidade de STEC na célula alvo.	21
Figura 8. Atual interpretação dos resultados de PCR para os grupos filogenéticos de <i>E. coli</i> de acordo com Clermont et al., 2013.....	22

SUMÁRIO

RESUMO	1
ABSTRACT	2
CAPÍTULO 1	3
1 INTRODUÇÃO.....	4
2 REVISÃO BIBLIOGRÁFICA	6
2.1 Mercado brasileiro de carne bovina e sua importância para a economia internacional.....	6
2.2 Sistemas de Produção de Bovinos no Brasil.....	6
2.2.1 Sistema Extensivo	7
2.2.2 Sistema Intensivo	7
2.3 Segurança dos alimentos e sua relação com os produtos agroalimentares.....	8
2.4 Doenças de origem alimentar	10
2.5 <i>Escherichia coli</i>	10
2.5.1 <i>E. coli</i> enteropatogênica (EPEC)	13
2.5.2 <i>E. coli</i> enteroinvasiva (EIEC)	14
2.5.3 <i>E. coli</i> enteroagregativa (EAEC).....	15
2.5.4 <i>E. coli</i> enterotoxigênica (ETEC).....	16
2.5.5 <i>E. coli</i> difusamente aderente (DAEC)	17
2.5.6 <i>E. coli</i> produtora de Shiga-toxina/Enterohemorrágica(STEC/EHEC)	18
2.6 Grupos filogenéticos de <i>E.coli</i>	21
3 REFERÊNCIAS BIBLIOGRÁFICAS	23
CAPÍTULO 2	34
Artigo Científico.....	34
NORMAS PARA PUBLICAÇÃO.....	52

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RESUMO

Escherichia coli produtora de Shiga Toxina (STEC) é um dos principais patógenos envolvidos em surtos alimentares. Sendo os bovinos, reservatórios naturais de STEC, e através da pele ou conteúdo fecal podem ser uma fonte de contaminação cruzada no ambiente industrial. O objetivo deste trabalho foi avaliar a influência dos sistemas de produção de bovinos de corte na presença de cepas patogênicas de *Escherichia coli*. Coletou-se um total de 485 amostras nos pontos: pós-sangria (A), pós-evisceração (B), pós-lavagem final (C), cortes cárneos (D), fezes bovinas (F), superfícies (S), fezes humanas (H) e água residual (AR), de um abatedouro-frigorífico bovino. Caracterizou-se as amostras quanto aos patótipos, filogrupos e sorogrupos, analisando-as através do teste qui-quadrado ($p < 0,05$), comparando os sistemas de criação: extensivo e intensivo. 126 amostras foram confirmadas como *E. coli*, sendo o pontos F(100%) e H(80%) os pontos com maior positividade. Apenas no ponto F identificou-se DEC, sendo 28% dos animais do sistema intensivo e 38% do extensivo positivos para STEC, e 4% do sistema extensivo positivos para EIEC, não havendo diferença estatística entre as criações. O filogrupo B1 foi o mais detectado em ambos os sistemas, com 24% de positividade no sistema intensivo e 68% no extensivo. Os sorogrupos, O113 e O104 foram detectados em 4% dos animais criados em sistema intensivo e 6% dos do sistema extensivo. Diante disso, STEC foi o patótipo mais detectado e B1 o filogrupo mais encontrado entre as amostras. Sorogrupos O113 e O104 foram detectados, enfatizando a relevância do estudo para saúde pública.

Palavras-chave: Diarreio gênicas, saúde pública, surtos alimentares.

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ABSTRACT

Shiga Toxin-producing *Escherichia coli* (STEC) is one of the main pathogens involved in food outbreaks. Cattle, natural reservoirs of STEC, through the skin or fecal content can be a source of contamination for the carcass and final product in the industrial environment. The objective of this work was to evaluate the influence of beef cattle production systems on the presence of pathogenic strains of *Escherichia coli*. A total of 485 samples were collected at the following points: post-bleeding (A), post-evisceration (B), post-final washing (C), meat cuts (D), bovine feces (F), surfaces (S), human feces (H) and wastewater (AR) from a bovine slaughterhouse. The samples were characterized in terms of pathotypes, phylogroups and serogroups, analyzing them using the chi-square test ($p < 0.05$), comparing the rearing systems: extensive and intensive. 126 samples were confirmed as *E. coli*, with points F (100%) and H (80%) being the points with the highest positivity. Only at point F was STEC identified, with 28% of the animals from the intensive system and 38% from the extensive system positive, and 4% from the extensive system positive for EIEC, with no statistical difference between the creations. Phylogroup B1 was the most detected in both systems, with 24% positivity in the intensive system and 68% in the extensive system. The serogroups, O113 and O104 were detected in 4% of animals raised in an intensive system and 6% of those in an extensive system. Therefore, STEC was the most detected pathotype and B1 the most phylogroup most found among the samples. Serogroups O113 and O104 were detected, emphasizing the relevance of the study to public health.

Keywords: Diarrhea, food outbreaks, public health.

CAPÍTULO 1

1 INTRODUÇÃO

O Brasil destaca-se no âmbito agropecuário, devido ao seu importante papel de potência produtora de alimentos, sendo eles produtos de origem animal ou grãos, fortalecendo o agronegócio brasileiro e favorecendo o desenvolvimento econômico do país (EMBRAPA, 2020).

A pecuária bovina brasileira contribui positivamente para este cenário, devido a avanços tecnológicos dos sistemas de produção aliados a fatores como a grande extensão territorial, capacidade de produzir animais com baixos custos de produção (BARCELLOS et al., 2020), condições climáticas favoráveis e grande oferta de mão de obra de baixo custo. Diante disso, foca-se então na produtividade e consequentemente, competitividade e abrangência no mercado internacional (EMBRAPA, 2020).

A atividade pecuária pode ser dividida em dois grupos, sendo a pecuária de leite, responsável pela produção de leite e seus derivados e a pecuária de corte, realizada com o intuito de criar animais destinados à produção de carne para o consumo humano. Para isso, utilizam-se sistemas de produção que sejam sustentáveis e lucrativos (BARCELLOS et al., 2020). Um deles é o sistema extensivo, no qual os animais são criados e alimentados unicamente de pastagens (CÉZAR et al, 2005). Outro sistema conhecido é o intensivo, o qual se diferencia por utilizar o confinamento na fase de terminação dos machos (CÉZAR et al, 2005).

Os consumidores representam o elo de todos os processos que ocorrem na cadeia de produção, e estão cada vez mais conscientes e exigentes quanto à qualidade e segurança dos alimentos (DIAS et al., 2014; TONDO, et al., 2015). No caso da carne bovina, a segurança dos alimentos envolve todo o sistema de produção, iniciando-se na fazenda até a mesa do consumidor (DE ARAÚJO et al., 2017).

A medicina veterinária preventiva é um importante aliado da segurança dos alimentos, através de ações relacionadas a patógenos de fácil dispersão e alto risco biológico (SOARES & ROSINHA, 2019). Os perigos biológicos são os que representam o maior risco à inocuidade dos alimentos. Dentre eles, estão as bactérias e suas toxinas, principais responsáveis pelas toxinfecções alimentares, parasitas, protozoários e vírus (TONDO, 2020).

Escherichia coli é um dos principais patógenos envolvidos em surtos alimentares no mundo, pertence à família *Enterobacteriaceae*, e possui como principal habitat a microbiota intestinal dos animais e humanos (ACHA & SZYFRES, 2001). Nos seres humanos, *E. coli* pode ser classificada como comensal, que possuem como habitat o intestino humano, e, portanto, não são patogênicas, e, podem ser patogênicas, ou seja, são capazes de causar vários tipos de infecções, além de possuírem vários patotipos (FOCACCIA, 2005).

No grupo dos patogênicos, está *E. coli* diarreiogênica (DEC), que pode ser subdividida em seis subgrupos, também chamados de patotipos. São eles, *E. coli* enterotoxigênica (ETEC), *E. coli* enteroinvasiva (EIEC), *E. coli* enteroagregativa (EAEC), *E. coli* produtora da toxina Shiga (STEC/EHEC), *E. coli* difusamente aderente (DAEC), e *E. coli* enteropatogênica (EPEC) (NATARO & KAPER, 1998).

DEC estão associadas a surtos relacionados ao consumo de alimentos, entretanto, STEC/EHEC em particular, são importantes para a saúde pública, uma vez que são responsáveis por causar doença severa em humanos, desde diarreia até síndrome hemolítico-urêmica (OLIVEIRA, 2008). Possuem como principal reservatório os animais de produção, principalmente os ruminantes, e devido ao consumo de carne crua ou malpassada contaminada por esse patógeno, pode ocasionar infecção humana (NASCIMENTO et al., 2014).

O sorotipo O157:H7, integra o subgrupo STEC/EHEC e é o mais associado a surtos alimentares, com quadros de infecções do trato gastrointestinal em humanos. Após anos de pesquisas e avanços na biologia molecular, outros importantes sorogrupos relacionados às infecções do trato gastrointestinal em humanos foram descobertos, e são conhecidos como “*big six*”, composto pelos grupos O26, O103, O111, O45, O121e O145 (JOHNSON et al., 2006; CAPRIOLI et al., 2005).

2 REVISÃO BIBLIOGRÁFICA

2.1 Mercado brasileiro de carne bovina e sua importância para a economia internacional

Atualmente o Brasil ocupa relevantes posições no setor da carne bovina, é o maior exportador, segundo maior produtor e terceiro maior consumidor de carne bovina no mundo. Além disso, o rebanho bovino brasileiro corresponde a 187,55 milhões de cabeças, o que coloca o Brasil como o maior detentor de rebanho comercial do mundo (ABIEC, 2021). De acordo com a Associação Brasileira das Indústrias Exportadoras de Carnes (ABIEC, 2021), foi registrado em 2020 o abate de 41,5 milhões de cabeça e, como resultado a produção de carne de 10,32 milhões Toneladas Equivalentes Carcaças (TEC) acarretando um crescimento de 8% nas exportações de carne bovina.

De toda a carne produzida, aproximadamente 26,07% é destinada à exportação, sendo os principais produtos exportados: a carne in natura, carnes industrializadas e miúdos (ABIEC, 2021). Os principais países de destino da carne bovina brasileira são Hong Kong, China e Egito (ABIEC, 2021). O mercado interno, retém 73,93% de toda a produção, gerando um consumo per capita de 36,39 kg/ano.

A relevância da pecuária no Brasil, fortifica-se anualmente. No ano de 2020 o país finalizou o ano com um Produto Interno Bruto (PIB) de R\$7,4 trilhões, cerca de 4,1% a menos que em 2019. Entretanto, mesmo diante dessa queda, o PIB da pecuária contribuiu positivamente, com um crescimento de 8,4% para 10% do PIB total. Além disso, investimentos em insumos para produção, genética, faturamento de animais comercializados pelas indústrias e varejo, foram responsáveis pela movimentação de R\$ 747,05 bilhões na pecuária de corte em 2020, cerca de 20,8% a mais que 2019, salientando a influência do setor na economia brasileira (ABIEC, 2021).

2.2 - Sistemas de Produção de Bovinos no Brasil

Define-se sistemas de produção como a associação entre tecnologias,

práticas de manejo, raça do animal, genética, propósito da criação e a região onde a atividade é executada (CEZAR et al., 2005; BARCELLOS et al., 2020).

Apenas através da integração de todos esses fatores, é possível desenvolver a implantação de um sistema de produção sustentável e lucrativo (BARCELLOS et al., 2020).

Diante a grande variedade destes fatores no Brasil, compreende-se que o sistema de produção de bovinos não é único e segmenta-se de acordo com os regimes alimentares dos rebanhos prevalentes no país (CEZAR et al., 2005).

2.2.1 Sistema Extensivo

O sistema extensivo caracteriza-se pela utilização unicamente de pastagens cultivadas e nativas como fonte de alimento energético e proteico para os animais, limitando a suplementação alimentar ao fornecimento mineral (fósforo, sódio, selênio, zinco, cobre e etc) (CEZAR et al., 2005).

Aproximadamente 80% dos sistemas produtivos de carne bovina brasileira, são extensivos e desenvolvem as etapas da cria a engorda (CEZAR et al., 2005). Uma vez que, o país possui uma diversidade de solos e clima favorável para implantar pastagens de qualidade, criação e adaptação de diferentes espécies bovinas e seus cruzamentos.

Os animais são criados soltos em grandes áreas de pastagem, e fazem parte das instalações apenas infraestruturas essenciais, tais como o curral, cochos e bebedouros usufruindo ao máximo os recursos naturais, com economia de equipamentos, instalações e mão-de-obra (DE ALENCAR, 2003).

2.2.2 Sistema Intensivo

Sistema de produção intensivo, diferencia-se devido a terminação dos animais em confinamento, além de uma alimentação capaz de proporcionar uma engorda econômica, durante um período específico (CEZAR et al., 2005).

Este sistema tem o intuito de aumentar a produtividade e consequentemente o lucro do pecuarista, através de melhorias relacionadas ao desenvolvimento de tecnologia e assistência de instrumentos técnicos, além do oferecimento de uma alimentação que associa o consumo de pastagem

cultivada com suplementação e confinamento (MARION, 2012).

O confinamento de bovinos vem crescendo na pecuária brasileira devido a fatores tais como, o aumento de tecnologias disponíveis, disponibilidade de grãos, redução no tempo de terminação dos animais, planejamento de abate durante o decorrer de todo o ano (GOMES et al., 2015).

Em contrapartida, existem algumas adversidades que merecem atenção, como por exemplo a incidência de doenças causadas pelo contato com dejetos, brigas entre os animais, e a necessidade de maior mão de obra, elevando os custos de implantação (GIACOMET et al., 2013).

2.3 Segurança dos alimentos e sua relação com os produtos agroalimentares

O principal foco da agroindústria, e mais especificamente da pecuária nacional, são os produtos agroalimentares (TOLEDO, BATALHA, AMARAL, 2000), que vão além da porteira, englobando a indústria de insumos, produção de bovinos, processamento e distribuição até que alcance os mais diversos nichos de consumidores (EMBRAPA, 2020). Considerado um dos alimentos mais nutritivos, a carne bovina, possui alto valor protéico devido a quantidade e composição dos aminoácidos presentes, é de alta digestibilidade e ainda fornece os aminoácidos essenciais para o desenvolvimento dos seres humanos (BRIDI, 2014).

Em vista disso, produzir alimentos em condições sanitárias adequadas, seguindo procedimentos e práticas como as Boas Práticas de Fabricação (BPF), Análise de Perigos e Pontos Críticos de Controle (APPCC), são ações primordiais. Desde que, sejam bem definidas permitindo o alcance de qualidade com redução de perdas, desperdícios e, conseqüentemente de custos (LUSA et al., 2016).

Considera-se que os animais, a partir da pele, pelos, conteúdo gastrintestinal, região orofaríngea (SAMULAK et al., 2011), são importantes fontes de microrganismos para o produto final, conseqüentemente, os microrganismos estão distribuídos amplamente no ambiente dos abatedouros frigoríficos (FONTOURA et al., 2020). A esfolagem e a evisceração são etapas do

processamento com maior impacto na contaminação microbiana (FONTOURA et al., 2020).

Na esola existe a possibilidade de contaminação cruzada de forma direta entre a pele, pelos e cascos e a musculatura e indireta por meio de facas ou mãos dos manipuladores (BARCO et al., 2015). Na evisceração, o risco é de extravasamento de conteúdo intestinal, por ruptura das alças intestinais, o que compromete a qualidade microbiana das carcaças devido ao aumento da quantidade e variedade de microrganismos advindos do conteúdo intestinal (SERRAINO et al., 2012).

Ainda, naturalmente, a carne é suscetível à contaminação microbiológica (LANNA, 2013), devido a suas características intrínsecas, tais como composição química, elevada atividade de água e pH próximo à neutralidade, que a torna um ótimo meio para multiplicação de microrganismos (FONTOURA et al., 2020).

Portanto, adotar boas práticas de higiene durante todas as etapas da produção da carne bovina, desde o abate até o produto final é elementar para evitar a contaminação microbiana das carcaças, garantindo qualidade e proteção da saúde pública (FAO, 2005).

Com o objetivo de garantir uma padronização do processo de abate, o Ministério da Agricultura Pecuária e abastecimento – MAPA, instituiu em 1952 o primeiro Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal (RIISPOA) através do Decreto nº 30.691, que foi revogado e substituído pelo Decreto nº 9.013/2017. O RIISPOA, regulamenta as normas sobre fiscalização e inspeção industrial e sanitária dos produtos de origem animal (BRASIL, 2017).

Portanto, se faz necessário uma constante conscientização do consumidor, indústria e governantes em relação aos perigos dos alimentos. Assim, uma produção adequada e de qualidade a níveis de segurança alimentar são fatores essenciais aos consumidores, o qual a qualidade deixa de ser um diferencial competitivo e transforma-se em uma exigência para permanência no mercado (COLETTI, 2012).

2.4 Doenças de origem alimentar

As doenças de origem alimentar são nomeadas, de acordo com o Ministério da Saúde, de doenças transmitidas por alimentos (DTA'S)(MS, 2019). As DTA'S são aquelas ocasionadas pelo consumo de alimentos e/ou água, contaminados (MS, 2019) por microrganismos patogênicos (NUNES et al., 2017) tais como as bactérias, vírus, fungos, protozoários e até mesmo pela ingestão de substâncias químicas como metais pesados, agrotóxicos e produtos químicos(NUNES et al., 2018).

Em vista disso, DTA's são capazes de ocasionar infecções, intoxicações ou toxinfecções alimentares de forma crônica ou aguda, caracterizadas por surto ou casos isolados (SIRTOLI & COMARELLA, 2018).

Mundialmente, a ocorrência de DTA's tem crescido significativamente, consequência da necessidade de alimentos em grande volume devido ao crescimento da população. Outros fatores que corroboram para tal situação, é a maior oferta e procura de alimentos destinados ao pronto consumo coletivo (*ready to eat foods*), assim como o consumo de alimentos em locais públicos, e até mesmo o fácil deslocamento da população, tanto no âmbito nacional quanto internacional(DE PAULA et al., 2014).

Segundo o Ministério da Saúde, existem mais de 250 doenças de origem alimentar (SVS/MS, 2019). No Brasil, em 2018, 503 surtos de doenças de origem alimentar foram notificados, com 6.803 doentes, 731 hospitalizados e 9 pessoas chegaram a óbito. O principal patógeno responsável pelos surtos, foi *Escherichia coli*, que ocasionou 27,5% dos surtos, desses, 5,3% estavam associados ao consumo de carne bovina (SVS/MS, 2019).

2.5 *Escherichia coli*

Theodor Escherich, pediatra e microbiologista, no ano de 1885, enquanto realizava um estudo sobre micróbios intestinais infantis descobriu uma bactéria de crescimento rápido, nomeada primeiramente como *Bacterium coli commune*, e que em 1958 recebeu então o nome de *Escherichia* (ESCHERICH, 1988; FRIEDMANN, 2014).

O gênero *Escherichia*, pertence à família Enterobacteriaceae e dispõe de várias espécies, tais como *Escherichia blattae*, *Escherichia fergusonii*, *Escherichia hermanii*, *Escherichia vulneris* e *Escherichia coli*, sendo a *E. coli* a de maior interesse (TRABULSI & ALTERTHUM, 2008).

Caracteriza-se *E. coli* como bastonetes, Gram-negativos, não esporulados, mesófilos devido a sua ótima temperatura de crescimento entre 35° e 37°C e que, devido a presença de flagelos peritríqueos podem ser móveis (TORTORA et al., 2012; QUINN et al., 2005). Diante a sua capacidade de obter energia através de respiração ou fermentação, são microrganismos aeróbios facultativos capazes de fermentar sorbitol, xilose, glicose, maltose, manose, manitol e arabinose levando a produção de ácido e glicose (QUINN et al., 2005; FORSYTHE, 2013).

No que se diz respeito ao isolamento desse microrganismo, utiliza-se meios de cultura compostos por lactose, tais como o ágar MacConkey, onde *E. coli* produz colônias de cor rosa e o ágar EMB formando colônias com brilho metálico (QUINN et al., 2005). Também pode ser utilizado meios que contenham componentes fluorogênicos, como os caldos Lauril Tryptose e Caldo EC, para que seja possível avaliar a presença de D-glucoronidase (NATARO & KAPER, 1998).

Com o intuito de avaliar o perfil de crescimento do patógeno através de suas propriedades bioquímicas, realiza-se testes utilizando meios de cultura como EPM (TOLEDO, FONTES & TRABULSI, 1982a), MILi e Citrato de Simons (TOLEDO, FONTES & TRABULSI, 1982b), que permitem avaliar as reações de hidrólise da ureia, presença de gás, glicose, sulfeto de hidrogênio, triptofano, motilidade, indol, lisina e citrato ou IMViC – Indol, Vermelho de Metila, Vogues Proskauer e Citrato (MARTINS et al., 1992; QUINN et al., 2005).

E. coli faz parte da microbiota intestinal de mamíferos, como os ruminantes, e é considerado o microrganismo anaeróbio facultativo predominante da microbiota intestinal humana (KAPER & NATARO, 1998). Entretanto, pode ser encontrada, embora em menor frequência, na microbiota intestinal de pássaros, répteis e peixes, assim como no solo, água, plantas e alimentos (HARTL & DYKHUIZEN, 1984; LEIMBACH et al., 2013).

Nas primeiras horas de vida humana, a bactéria coloniza o trato gastrointestinal e desde então *E. coli* e hospedeiro geram benefício mútuo.

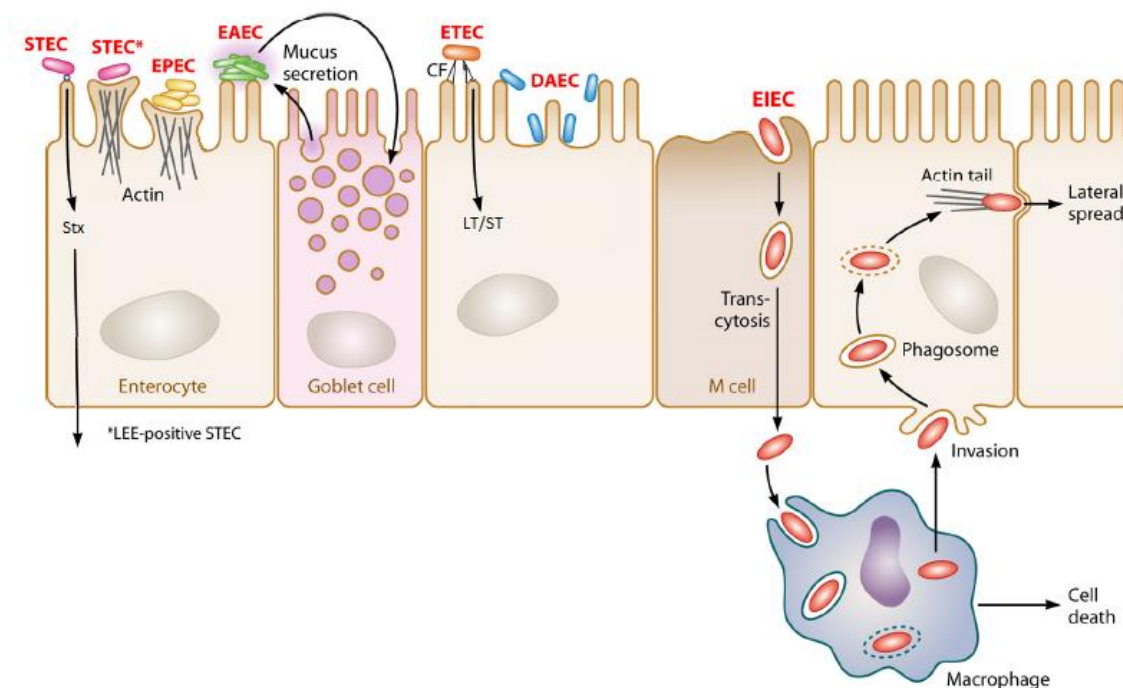
Normalmente, mantém-se confinada inofensivamente ao lúmen intestinal, exceto em casos de hospedeiro imunossuprimido ou rompimento de barreiras gastrointestinais, quando mesmo as espécies não patogênicas são capazes de causar infecção (KAPER & NATARO, 1998).

Contudo por meio da aquisição de fatores de virulência como enterotoxinas (STa, STb, LT1 e LT2), adesinas, lipopolissacarídeos (LPS), toxinas como as do tipo Shiga (*stx1* e *stx2*), hemolisinas (α e β), fatores necrosantes citotóxicos (CNF1 e CNF2) (HIRSH & ZEE, 2013), a bactéria torna-se mais adaptativa a novos sítios e conseqüentemente capaz de ocasionar um amplo espectro de doenças (KAPER et al., 2004).

Fundamentalmente classificadas em três grupos, as cepas de *E. coli* podem estar relacionadas a infecções extraintestinais (ExPEC), capazes de atingir trato urinário e meninge, á manifestações clínicas entéricas ocasionadas por *E. coli* diarreiogênica (DEC) e as comensais (NATARO & KAPER, 1998; MENG et al., 2007).

Classifica-se *E. coli* diarreiogênica (DEC) em seis patotipos (Figura 1) que distinguem-se quanto a fatores de virulência, mecanismos de patogenicidade, colonização na célula alvo, sintomas clínicos e danos nos hospedeiros. São eles, *E. coli* produtora de Shiga- toxina/Enterohemorrágica (STEC/EHEC), *E. coli* enterotoxigênica (ETEC), *E. coli* enteroinvasiva (EIEC), *E. coli* enteropatogênica (EPEC) típica ou atípica, *E. coli* difusamente aderente (DAEC) e *E. coli* enteroagregativa (EAEC) (NATARO E KAPER, 1998; CROXEN et al., 2013).

Figura 1 – Representação da interação de cada patotipo de DEC com a célula-alvo.



Legenda: Padrões de adesão dos patótipos de DEC ao epitélio do hospedeiro. EPEC (representada em amarelo) e STEC (representada em rosa) são patógenos extracelulares que se ligam ao epitélio intestinal e apagam microvilosidades, formando lesões A / E características. Devido à presença de pili formadores de feixe, a EPEC é capaz de formar microcolônias, resultando em um padrão de aderência localizada. ETEC (representada em laranja) usa fatores de colonização para fixação ao intestino do hospedeiro. EAEC (representada em verde) forma biofilmes na mucosa intestinal, e as bactérias aderem umas às outras, bem como à superfície celular, para formar um padrão de aderência agregativo (AA) conhecido como "tijolo empilhado". DAEC (representada em azul) é dispersa sobre as superfícies das células intestinais, resultando em um padrão de aderência difusa (DA). EIEC (representada em vermelho) são patógenos intracelulares que penetram o epitélio intestinal através das células M para obter acesso à submucosa. E, escapam dos macrófagos submucosos por indução da morte celular dos macrófagos seguida por invasão basolateral de colonócitos e disseminação lateral. Fonte: adaptado de Croxen et al. (2013) e Kaper, Nataro e Mobley (2004).

2.5.1 *E. coli* enteropatogênica (EPEC)

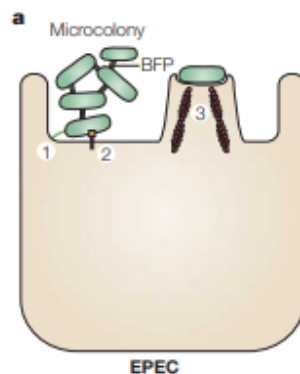
O termo *E. coli* enteropatogênica (EPEC) foi usado para descrever cepas de *E. coli* relacionadas a uma série de surtos envolvendo crianças com diarreia nas décadas de 1940 e 1950 (BRAY, 1945; KAPER et al., 2004). Durante muitos anos o mecanismo de patogenicidade da EPEC era desconhecido, e só era identificado baseado na sorotipagem O:H (KAPER et al., 2004).

Entretanto, após avanços nos estudos, atualmente é um dos patótipos com a patogênese mais compreendida (KAPER et al., 2004). O mecanismo de patogenicidade (Figura 2) ocorre quando a bactéria, por meio da proteína

intimina (codificada pelo gene *eae*), se liga às células epiteliais do intestino delgado, destroem as microvilosidades, provocando então a lesão histopatológica característica *attaching and effacing* (A/E) formando pedestais (CHEN & FRANKEL, 2005). O gene *eae*, é codificado pela ilha de patogenicidade LEE (*locus of enterocycle effacement*) sendo o responsável por induzir a lesão A/E e consequentemente diarreia aquosa, náuseas e desequilíbrio de nutrientes (HE et al., 2015).

Este patotipo pode ser classificado em dois grupos, EPEC típica (tEPEC) e EPEC atípica (aEPEC). tEPEC são caracterizadas por possuírem o plasmídeo EAF (EPEC *adherence fator*), responsável por codificar fímbrias do tipo IV conhecidas como pilus formador de feixe (BFP) que permitem a aderência do microrganismo na célula, enquanto aEPEC não possui esse plasmídeo (KAPER, 1996; FRANKEL et al., 1998). As cepas tEPEC são patogênicas para humanos e não são detectadas em animais, já as cepas atípicas são patogênicas para ambos (TOZZOLI et al., 2014).

Figura 2 - Mecanismo de patogenicidade de EPEC na célula alvo.



Legenda: *E. coli* enteropatogênica (EPEC) – indução da lesão denominada *attaching and effacing* (A/E). (1) Adesão ao enterócito, (2) Translocação proteica por sistema de secreção do tipo III, (3) alteração do citoesqueleto e destruição das microvilosidades. Fonte: Adaptado de KAPER et al. (2004).

2.5.2 *E. coli* enteroinvasiva (EIEC)

O mecanismo de patogenicidade de EIEC (Figura 3) ocorre através da invasão da mucosa do cólon humano, mediada pela expressão de genes cromossômicos e de plasmídeo (KAPER et al., 2004).

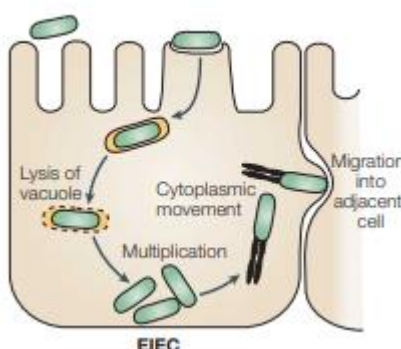
Logo após a invasão nas células epiteliais do cólon, as bactérias são

capazes de se replicar intracelularmente e se disseminam entre as células adjacentes, levando a destruição da barreira epitelial intestinal.

Consequentemente, um quadro de disenteria é desencadeado, caracterizado normalmente por presença de sangue e muco (O'BRIEN & LaVECK, 1983).

As cepas de EIEC são fenotipicamente e genotipicamente semelhantes às de *Shigella* spp., o que pode afetar dados epidemiológicos dificultando as investigações das infecções causadas por EIEC, o que justifica os poucos surtos notificados relacionados a este patotipo (CROXEN et al., 2013). A transmissão pode ocorrer de pessoa para pessoa, através da ingestão de água ou alimentos contaminados (CROXEN et al., 2013).

Figura 3 - Mecanismo de patogenicidade de EIEC na célula alvo.



Legenda: *E. coli* enteroinvasiva (EIEC) – invasão dos enterócitos por lise do fagolisossoma e movimentação para células adjacentes com o auxílio de microfilamentos de actina. Fonte: Adaptado de KAPER et al. (2004).

2.5.3 *E. coli* enteroagregativa (EAEC)

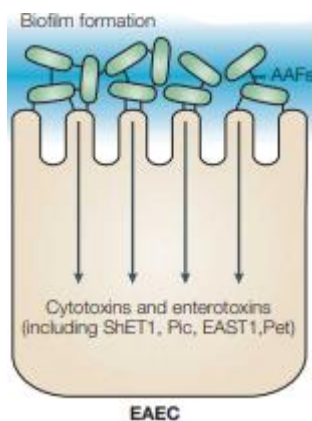
O patotipo EAEC tem sido apresentado como uma das causas mais comuns de diarreia aguda persistente, principalmente em crianças de países de baixa renda (BOISEN et al., 2020). Além disso, é relatado como a segunda causa mais recorrente da diarreia dos viajantes (PABALAN et al., 2013; HARRINGTON et al., 2006).

Caracterizadas devido a sua capacidade de aderir á células HEp-2, formando um padrão conhecido como aderência agregativa (AA), o qual ocorre o empilhamento de bactérias também chamado de padrão “tijolo empilhado”

(KAUR et al., 2010; NATARO et al., 1987). O padrão AA, é mediado por fímbrias de aderência agregativa (AAF) regulados pelo Regulador de Aderência Agregativa (*AggR*) (NATARO et al., 1994; ESTRADA-GARCIA & NAVARRO - GARCIA, 2012).

O mecanismo de patogenicidade de EAEC (Figura 4), envolve a colonização e aderência das bactérias na mucosa intestinal e devido a sua capacidade de aumentar a produção e secreção de muco, as bactérias se auto aglutinam, formando biofilme no epitélio do intestino, seguido pela secreção de enterotoxinas e citotoxinas. Consequentemente, pode levar a casos de diarreia aquosa acompanhada de muco e sangue, náusea, vômito e febre baixa (VILLASECA et al., 2005; CROXEN et al., 2013).

Figura 4 - Mecanismo de patogenicidade de EAEC na célula alvo



Legenda: *E. coli* enteroagregativa (EAEC) – adesão aos enterócitos e agregação dos microrganismos, com formação de um espesso biofilme e produção de toxinas como EAST1 e Pet. Fonte: Adaptado de KAPER et al. (2004).

2.5.4 *E. coli* enterotoxigênica (ETEC)

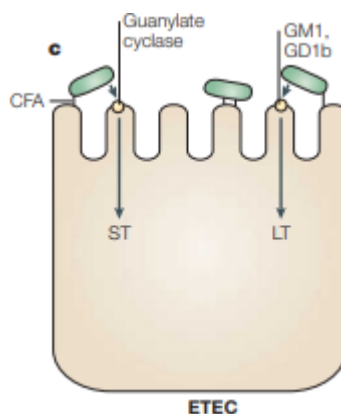
E. coli enterotoxigênica também conhecida como *E. coli* produtora de enterotoxinas, é responsável por colonizar a mucosa do intestino delgado através de pilis ou fímbrias que podem possuir várias formas e são chamadas CFA (*colonization factor antigens*), sendo seu principal mecanismo de patogenicidade (Figura 5) (RODRÍGUEZ-ANGELES, 2002).

Possuem como principal fator de virulência a síntese de uma ou ambas enterotoxinas denominadas como toxina termolábil (LT) responsável por aumentar o nível intracelular de AMPc, e toxina estável ao calor (ST),

responsável por aumentar o nível intracelular de GMPc encontrados nas células intestinais, causando a saída de água e íons e consequentemente desequilíbrio eletrolítico (FOSTER & SMITH, 2009).

O quadro clínico é caracterizado por diarreia aguda, normalmente não sanguinolenta e em alguns caso pode haver febre e vômito. As cepas de ETEC estão mais envolvidas com diarreia em crianças, principalmente durante os primeiros meses de vida, com uma frequência de isolamento entre 10 e 30%. Em menor frequência pode acometer crianças maiores de dois anos e adultos, podendo ser assintomática além de ser um dos patógenos responsáveis por causar diarreia do viajante (NATARO & KAPER, 1998; CROXEN et al., 2013; NAGY & FEKETE, 2005).

Figura 5 - Mecanismo de patogenicidade de ETEC na célula alvo.



Legenda: *E. coli* enterotoxigênica (ETEC) – Aderência a receptores específicos dos enterócitos, com produção de toxinas termolábeis (ST) e/ou termoprodutivas (LT). Fonte: Adaptado de KAPER et al. (2004).

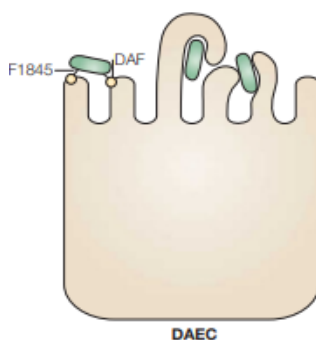
2.5.5 *E. coli* difusamente aderente (DAEC)

O patotipo DAEC é caracterizado por cepas que possuem um padrão de aderência difusa em células HeLa ou HEp-2 cultivadas, capazes de causar um efeito citopático, no qual as bactérias encobrem, através de projeções celulares semelhantes a dedos, de maneira uniforme toda a superfície da célula (Figura 6) (ABBAS et al., 2016). O mesmo padrão de aderência pode ser encontrado em cepas de EPEC. Cerca de 75% das cepas DAEC produzem adesinas codificadas por operons *afa* / *dra* / *daa* (KAPER et al., 2004), e portanto, a presença de tais genes permite a diferenciação entre DAEC e EPEC (LE BOUGUÉNEC &

SERVIN, 2006).

DAEC foi apontado como causa de diarreia em vários estudos, além de estarem associadas a infecções do trato urinário, diarreia em crianças de 18 meses a 5 anos, complicações na gravidez, contudo também podem ser assintomáticas tanto em crianças quanto em adultos (SERVIN, 2005).

Figura 6 - Mecanismo de patogenicidade de DAEC na célula alvo.



Legenda: *E. coli* difusamente aderente (DAEC) – aderência a receptores dos enterócitos, como o DAF, e indução do crescimento de projeções celulares que envolvem as bactérias. Fonte: Adaptado de KAPER et al. (2004).

2.5.6 *E. coli* produtora de Shiga-toxina/Enterohemorrágica (STEC/EHEC)

E. coli produtora de Shiga-toxina (STEC) também conhecidas como *E. coli* produtora de verotoxina (VTEC), possuem como principal fator de virulência a produção de Shiga-toxina denominada também como verocitotoxina, em virtude de serem citopáticas a células Vero (NATARO & KAPER, 1998; NGUYEN & SPERANDIO, 2012).

São conhecidos mais de 200 sorotipos de *E. coli* capazes de produzir *stx*, entretanto nem todos são patogênicos, uma vez que possuir uma ou mais toxinas do tipo *stx* não garante sua expressão. Portanto, utiliza-se o termo STEC para qualquer cepa capaz de produzir o gene *stx* (KAPER, 2004).

Primeiramente utilizado com o intuito de nomear amostras O157:H7, o termo *Escherichia coli* enterohemorrágica (EHEC), atualmente é considerado um subgrupo de STEC, e é empregado para cepas positivas e capazes de expressar o gene *stx* e que contenham a Ilha de Patogenicidade LEE (*locus of enterocyte effacement*) responsável por causar lesões A/E (*attaching and effacing*) o que podem levar a quadros de colite hemorrágica (HC) e Síndrome urêmica-hemolítica (SUH) (KAPER, 2004; SADIQ, et al., 2014).

As toxinas shiga também conhecidas como “Shiga-like”, foram assim

denominadas devido a sua semelhança estrutural e antigênica à toxina produzida pela *Shigella dysenteriae* tipo 1, causador da disenteria bacilar (O'BRIEN & LaVECK, 1983; DOYLE & BEUCHAT, 2007). São produzidas no cólon e após sua absorção pelo epitélio intestinal circulam na corrente sanguínea até encontrarem receptores Gb3, mais encontrados no rim, intestino e cérebro (GUTH, 2008). No intestino são capazes (Figura 7) de causar apoptose das células endoteliais, o que pode acarretar quadros de colite hemorrágica. Nos rins, danificam as células endoteliais obstruindo os vasos ocasionando inflamação renal, o que pode provocar a SUH com um quadro de trombocitopenia, anemia hemolítica e insuficiência renal aguda podendo ser fatal (SCHEUTZ et al., 2012; KAPER et al., 2004).

A família Stx é dividida em dois grandes subgrupos, Stx1 e Stx2, que possuem cerca de 55% de homologia entre seus aminoácidos (KAPER, 2004; FOCACCIA, 2005). O grupo Stx1 inclui Stx1a, Stx1c e Stx1d, já Stx2 é mais heterogêneo e composto por Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f e Stx2g (SCHEUTZ et al., 2012; KAPER et al., 2004), sendo o grupo mais associado com doenças graves.

Animas domésticos e selvagens são possíveis portadores assintomáticos de STEC, entretanto, os ruminantes principalmente os bovinos são considerados os principais reservatórios (CALDORIN et al., 2013).

Diferentemente dos seres humanos, os bovinos são carentes em receptores Gb3 no trato gastrointestinal (PRUIMBOOM-BREES et al., 2000), mas ainda são detectados nos rins e no cérebro. Portanto, STEC coloniza a junção reto-anal do gado, onde os efeitos da toxina Shiga são impermeáveis (NAYLOR et al., 2003).

Em vista disso, os bovinos são hospedeiros tolerantes e podem contribuir para a persistência e transmissão deste patógeno humano (NGUYEN & SPERANDIO, 2012).

A eliminação de STEC acontece principalmente através das fezes bovinas (RICE et al., 2003), e uma vez no meio ambiente, os seres humanos podem se infectar através do consumo de produtos de origem bovina contaminados, como a carne, leite e produtos lácteos (ARMSTRONG et al., 1996). Ainda, por meio de água contaminada e vegetais, representando uma grave preocupação para

saúde pública (HILBORN et al., 1999; OLSEN et al., 2002). Estudos relatam, que o contato direto com os ruminantes, ou interações com pessoas infectadas representam outras possíveis fontes de transmissão (RANGEL et al., 2005).

Grande parte dos casos envolvendo STEC, estão associados ao sorotipo O157:H7. Desde 1982, após o surto de colite hemorrágica por consumo de hambúrgueres contaminados em uma grande rede de fast-food americana, *E. coli* O157:H7, no âmbito de saúde pública, tornou-se o sorotipo enterohemorrágico mais importante (RANGEL et al., 2005).

Cerca de 10 a 15% de pessoas infectadas com *E. coli* O157 podem desenvolver síndrome urêmica hemolítica entre os dias 5 e 13 após o início da diarreia, sendo mais comum em crianças menores de 5 anos (TARR et al., 2005; CDC, 2018).

No que se diz respeito ao Brasil, existem poucos dados epidemiológicos disponíveis que associam SUH com alimentos (SOUZA et al., 2011). Entretanto estudos demonstram a ocorrência de SUH nos últimos anos, e investigações epidemiológicas realizadas em quatro estados brasileiros mostraram que as cepas O157: H7 foram isoladas em dois pacientes hospitalizados, um com SUH e outro com diarreia sanguinolenta (DOS SANTOS et al., 2015).

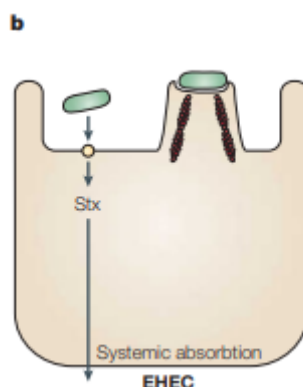
A não obrigatoriedade de pesquisar *E. coli* O157:H7 em alimentos envolvidos em surtos, a não implementação de métodos capazes de detectá-la na maioria dos Laboratórios Centrais de Saúde Pública (LACEN), são explicações plausíveis para falta de dados epidemiológicos associando a doença com surtos alimentares (DE PAULA et al., 2014). Entretanto, no que diz respeito ao controle microbiológico no abatedouro frigorífico, a Instrução Normativa nº 60, de 20 de dezembro de 2018, estabelece a obrigatoriedade do controle microbiológico em carcaças de bovinos e suínos, com o intuito de avaliar toda a higiene do processo e reduzir a prevalência de agentes patogênicos (BRASIL, 2018).

E. coli O157:H7 é considerado o principal sorotipo entre as STEC, responsável por causar surtos de enfermidades veiculadas por alimentos. Todavia, outros sorogrupos estão se tornando relevantes, como O26, O45, O103, O111, O121, O145, O113 e O104, conhecidos como não-O157, e também estão associados a doenças severas em humanos nos Estados Unidos, Europa

e regiões da América Latina (IRINO et al, 2007).

A grande interferência da STEC na saúde pública, salienta o impacto desse patotipo em muitos campos de estudo, como na Saúde Única, visto que envolve a tríade epidemiológica: hospedeiro, agente e meio ambiente (CROXENet al., 2013).

Figura 7 - Mecanismo de patogenicidade de STEC na célula alvo.



Legenda: *E. coli* produtora de Shiga toxina/EHEC – indução da lesão A/E adicionalmente à produção das toxinas Shiga (*Stx*), que são absorvidas e dão origem a complicações sistêmicas. Fonte: Adaptado de KAPER et al. (2004).

2.6 Grupos filogenéticos de *E.coli*

Com o objetivo de estudar os padrões de colonização, patogenicidade e a epidemiologia das infecções por *E. coli*, as análises filogenéticas entre estirpes de *E. coli* tem sido muito empregada (MÜŞTAK et al., 2015). Em vista disso, torna-se possível diferenciar cepas microbianas de acordo com sua semelhança genética, constatando se tais patógenos pertencem a mesma família clonal e consequentemente determinar se um grupo filogenético é mais comum em certos quadros infecciosos (CLERMONT et al., 2013).

A princípio foram definidos quatro grupos filogenéticos de *E. coli*, são eles: A, B1, B2 e D. Clermont et al. (2000), elaboraram um protocolo de PCR triplex, com o intuito de detectar os genes *chuA*, *yjaA* e *TspE4.C2* como marcadores filogenéticos (CLERMONT et al., 2000).

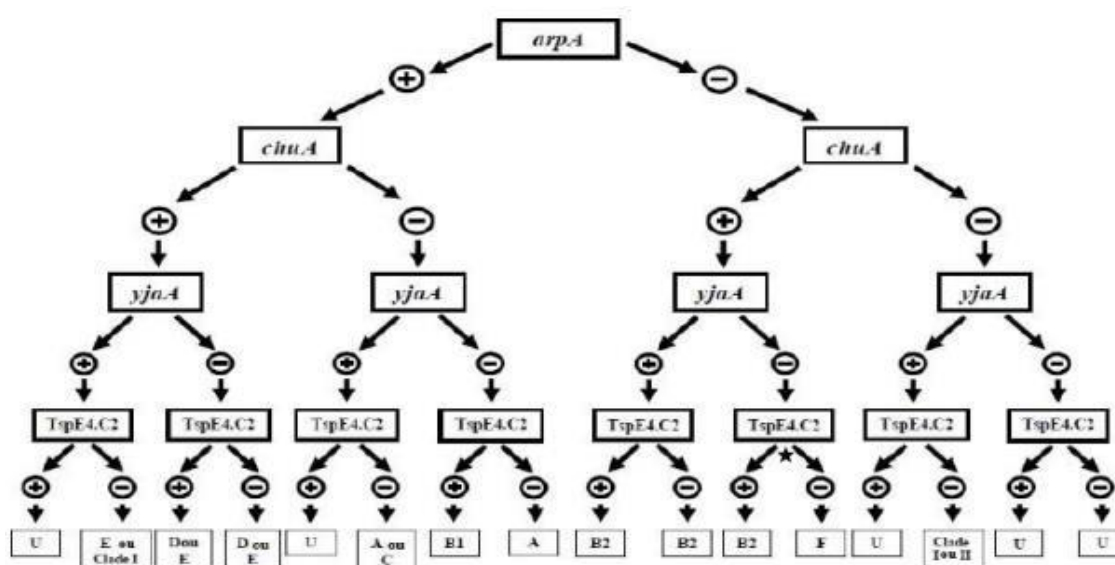
Estudos evidenciaram que os grupos B2 e D possuem o gene *chuA*, e com o intuito de diferenciar esses dois grupos entre si, utiliza-se o gene *yjaA* que está presente em todas as cepas B2 e ausentes no grupo D. Em relação aos grupos

A e B1, o gene *TspE4.C2* é o responsável pela diferenciação, uma vez que está presente no grupo B1 e ausente no grupo A (GORDON et al., 2008; CLERMONT et al., 2011).

Os resultados das 3 amplificações geraram o estabelecimento da primeira árvore decisória para determinação do grupo filogenético, método esse muito adotado em pesquisas (GORDON et al., 2008).

Devido a novos estudos e aprimoramento de técnicas da biologia molecular, novos grupos filogenéticos, apresentados na Figura 8, foram acrescentados a classificação antiga, são eles o grupo C, E, F, Clado I e G (CLERMONT et al., 2013; CLERMONT et al., 2019). Esses grupos diferem entre si no que se diz respeito a características fenotípicas e bioquímicas, perfil de resistência a antimicrobianos, tamanho do genoma, fonte de isolamento e a presença de genes de virulência (CLERMONT et al., 2019).

Figura 8. Atual interpretação dos resultados de PCR para os grupos filogenéticos de *E. coli* de acordo com Clermont et al., 2013.



Fonte: Silva., 2015

Quadros de infecções extraintestinais ocasionadas por *E. coli*, são causados comumente por isolados dos filogrupos B2, D, E e F (SCHMIDT et al., 2015). As cepas consideradas mais virulentas, normalmente classificam-se no grupo B2, e em alguns casos fazem parte do grupo D. Já os isolados considerados comensais pertencem aos grupos A e B1 (CLERMONT et al.,

2000).

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CAPÍTULO 2

Artigo Científico

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Article

Influence of animal production systems on the presence of pathogenic strains of *Escherichia coli* in the bovine production chain

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Abstract: Among the main pathogens involved in food outbreaks worldwide is diarrheagenic *Escherichia coli* (DEC). Cattle are considered natural reservoirs of DEC, and, during the slaughter stages, they can be a source of contamination for the carcass and final product. The objective of this work was to evaluate the influence of beef cattle production systems in Southeast Brazil, in the presence of diarrheagenic strains of *Escherichia coli*. A total of 485 samples were collected at the following points: post-bleeding (A), post-evisceration (B), post-final washing (C), meat cuts (D), bovine feces (F), surfaces (S), feces human (H) and wastewater (AR), from a cattle slaughterhouse, analyzing animals from two production systems: intensive and extensive. The samples were characterized in terms of pathotypes, phylogroups, and serogroups and analyzed using the chi-square test ($p < 0.05$). 126 samples were positive for *E. coli*, with points F (100%) and H (80%) being the points with the highest positivity. Only at point F was DEC identified ($n=35$), with 28% of the animals in the intensive system and 38% in the extensive system positive for STEC, and 4% of the animals in the extensive system positive for EIEC, with no statistical difference between the production systems. Regarding phylogroups, B1 was the most detected in both systems, with 24% positivity in the intensive system and 68% in the extensive system. Serogroups O113 and O104 were detected, both in 4% of animals raised in an intensive system and in 6% of those in an extensive system. In view of the results, even with the identification of STEC and EIEC in the animals' feces, there was no difference between the rearing systems, with regard to pathotypes, phylogroups, and serogroups.

Keywords: Diarrheogenics, food outbreaks, public health.

Introduction

Escherichia coli is one of the main pathogens involved in food outbreaks in the world, it is part of the *Enterobacteriaceae* family, and its main habitat is the intestinal microbiota of animals and humans (ACHA & SZYFRES, 2001). Its potential for resistance to new environments and

pathogenicity became possible, due to its ability to transfer, obtain and eliminate genetic elements between *E. coli* strains and other microorganisms (FRAZÃO et al., 2019; KAPER; NATARO; MOBLEY, 2004). Therefore, strains of *E. coli* can be pathogenic and capable of causing various types of infections in the intestinal tract (diarrheogenic *E. coli*) and extraintestinal infections, such as urinary tract infection, sepsis and meningitis (MENG et al., 2013; FOCACCIA, 2005).

Diarrheogenic *E. coli* (DEC) are associated with outbreaks related to the consumption of contaminated food, resulting in diarrhea, gastroenteritis, and nutrient malabsorption. DEC can be subdivided into six subgroups, also called pathotypes, they are enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), Shiga toxin-producing *E. coli* (STEC/ EHEC), diffusely adherent *E. coli* (DAEC), and enteropathogenic *E. coli* (EPEC) (NATARO & KAPER, 1998). STEC/EHEC in particular is important for public health as they are responsible for causing severe diseases in humans, from diarrhea to hemolytic uremic syndrome. Their main reservoir is the intestinal tract of livestock, mainly ruminants, and therefore can eliminate the pathogen through feces (OLIVEIRA, 2008).

Therefore, animals can be important sources of contamination in the environment of slaughterhouses (FONTOURA et al., 2020), and, consequently, microorganisms can spread reaching the final product (ISLAM et al., 2008; XIA et al., 2010). And, due to the consumption of meat contaminated by pathogens harmful to public health, meat can then be an important vehicle of food infections reflecting serious consequences for human health (NASCIMENTO et al., 2014).

The cattle rearing system can be considered a determining factor in the contamination of meat products with DEC. In the intensive production system, animals are raised in feedlots, and due to the high concentration and proximity of cattle, a greater accumulation of waste and consequently of microorganisms can occur, both in the environment and on the animals' skin. On the other hand, cattle raised in the extensive system are raised loosely in pastures, with greater space for movement. Therefore, animals raised in the intensive production system may be responsible for carrying a greater load of microorganisms to slaughter, increasing the risk of contamination of the carcass and processing environment when compared to those raised in an extensive system (GOMES et al., 2015; JARDIM et al., 2006).

The present study aimed to evaluate the influence of different beef production systems (intensive and extensive rearing) on the presence of pathogenic strains of *E. coli* at different points in the production line of a slaughterhouse located in Minas Gerais, Brazil, contemplating a One Health approach.

Material and methods

Ethical aspects

This research project was approved by the Ethics Committee for Research on Human Beings with Certificate of Presentation for Ethical Assessment (CAAE) 04536218.6.3006.5411 and opinion 3,489,847. Those involved were submitted to the research adherence through the Free and Informed Consent Term (FICT), collaborating in an autonomous, conscious, free, and informed manner.

Sample collection

The samples were collected in a cattle slaughterhouse, located in Triângulo Mineiro (Minas Gerais), supervised by the Federal Inspection Service of the Ministry of Agriculture, Livestock and Supply. Samples were collected from 50 animals raised in extensive production systems and 50 from animals from the intensive system. The animals were selected by lots according to their origin (10 animals in each lot), totaling 10 lots and 100 animals.

Samples for *E. coli* identification were obtained from the surface of carcasses after bleeding (A), after evisceration (B), after final washing (C), final meat cuts (D), handling surface (S), residual water (AR), animal feces (F) and handlers' feces (H). Carcass surfaces were collected using a swab, using two pre-moistened sterile sponges (10 ml of 0.85% w/v saline water, per

sponge), in four areas of the carcass (limb pelvic, hock/buttocks, external/Xifoidin, neck/dewlap) (ISO, 2015).

The meat cuts (D) were also sampled through a superficial smear of the chuck, shoulder, rump and rump. Surface samples (S) were obtained by smearing the surface of mats and tables that transported the meat cuts. For all these points mentioned above, the delimitation of the area occurred through sterile molds of 10x10 cm (100 cm²), obtaining a sampled area of 400 cm² per point (Table 1).

To collect the feces of the animals (F), the rectal ampulla of each carcass was swabbed immediately before the occlusion of the rectum, and the samples were stored in Cary Blair transport medium. Regarding the water samples (AR), they were collected with the aid of a sterile flask, at the establishment's water treatment station, where residual water from the entire slaughterhouse accumulates, before undergoing any type of treatment. The collection of human feces (H) was performed with the consent of the employees. Sterile containers, a swab in Cary Blair transport medium, and a collection manual prepared by the research team with collection guidelines and recommendations were provided. The stool samples were then deposited initially in the sterile bottle and later placed in the tube containing the transport medium with the aid of the swab. All samples were stored in isothermal boxes and sent to the laboratory.

Table 1 - Description of points, samples and collection methods for the detection of *E. coli* carried out at the cattle slaughterhouse.

Sample Type	Collect point	Method	Area/volume	n/lot	n total
Animal	Carcasses after bleeding	Swabe	400 cm ²	10	100
Animal	Carcasses post-evisceration	Swabe	400 cm ²	10	100
Animal	Carcass after final wash	Swabe	400 cm ²	10	100
Animal	Stools in pre-occlusion of the rectum	Swabe	-	10	100
Ambience	Treated and in-process water	Bottle	100 MI	-	5
Ambience	Equipment surface	Swabe	400 cm ²	-	20
Human	Employee feces	Swabe	-	1	10
Final product	Rump, chuck steak, hard top, shoulder and sirloin	Swabe	400 cm ²	-	50
Total					485

Isolation and characterization of *E. coli*

The isolation and characterization of *Escherichia coli* were performed following the methodology described in ISO17603 and ISO13136 for the STEC pathotype and the traditional microbiology for diarrheagenic *E. coli* (DEC).

The swabs of animal and human feces were initially tested for DEC, being seeded on MacConkey Agar plates, incubated at 37°C for 24 hours. Then, each swab was immersed in 20 mL of 1% Buffered Peptone Water (APT) for 24 h at 37°C.

Subsequently, through the Polymerase Chain Reaction - PCR test, the product was tested for STEC, and the samples positive for the *stx* gene were seeded on MacConkey Sorbitol Agar (SMAC) and incubated at 37°C for 24 hours as proposed by March & Ratnam (1986).

The other samples collected were initially pre-enriched. For that, the sponges used in the smears of carcass surface, meat cuts and the surface of mats and deboning tables were stored in a sterile bag and added 90 mL of 1% APT, homogenized and incubated at 37°C for 24 h. For water analysis, 25 mL of the collected sample was used, plus 225 mL of 1% APT, incubated at 37°C for 24 h. After incubation, the pre-enrichment products were seeded on MacConkey agar (Oxoid) for the isolation of DEC, and another part of the product was tested for the *stx* gene as per ISO recommendation. The *stx* gene-positive samples were then seeded on SMAC agar and incubated at 37°C for 24 h. The suspected colonies of *E. coli* present in the MacConkey Agar plates were selected, being four colonies fermenting lactose, and one non-fermenting lactose. Regarding the SMAC plates, five sorbitol positive and five sorbitol negative colonies were selected. Each colony was biochemically analyzed by culture in EPM (Escola Paulista de Medicina) (TOLEDO, FONTES & TRABULSI, 1982a), Simmons Citrate (TOLEDO, FONTES & TRABULSI, 1982b) and MILi (motility, indole and lysine). Samples biochemically detected as *E. coli* were then preserved on agar for further testing.

Identification of diarrheogenic *E. coli* pathotypes through Polymerase Chain Reaction – multiplex PCR

Samples biochemically identified as *E. coli* were investigated for virulence genes associated with different pathotypes (Table 2) through the Polymerase Chain Reaction.

Table 2 - *E. coli* pathotypes and their virulence markers used for identification

<i>E. coli</i> pathotypes	Virulence markers
Enteropathogenic <i>E. coli</i> (EPEC)	LEE Region (<i>eae</i>) and/or EAF Plasmid (<i>bfp</i>)
Shiga toxin producing <i>E. coli</i> (STEC)	<i>stx</i> genes (<i>stx1/stx2</i>)
Enterohemorrhagic <i>E. coli</i> (EHEC)	LEE region (<i>eae</i>) and <i>stx</i> gene
Enterotoxigenic <i>E. coli</i> (ETEC)	LT toxin and/or ST toxin
Enteroinvasive <i>E. coli</i> (EIEC)	Plasmid Inv (<i>ipaH</i>)
Enteraggregative <i>E. coli</i> (EAEC)	Plasmid EAEC (<i>aggR</i>)

DNA extraction was performed using the boiling technique (DE MÉDICI et al., 2003). Amplification was performed using the GoTaq® Green Master Mix (Promega, Madison, WI, USA) according to the manufacturer's recommendations and primers according to Toma et al., 2003 and Aranda et al., 2007, presented in Table 3.

Table 3 - List of primers for identifying *E. coli* pathotypes.

Primers	Sequence (5'-3')	Virulence genes	Amplicom (bp)
SK1	CCCGAATTCGGCACAAGCATAAGC	<i>Eae</i>	881
SK2	CCCGGATCCGTCTCGCCAGTATTCG		
VTcom-u	GAGCGAAATAATTTATATGTG	<i>Stx</i>	518
VTcom-d	TGATGATGGCAATTCAGTAT		
AL65	TTAATAGCACCCGGTACAAGCAGG	<i>Est</i>	147
AL125	CCTGACTCTTCAAAAGAGAAAATTAC		
LTL	TCTCTATGTGCATACGGAGC	<i>Elt</i>	322
LTR	CCATACTGATTGCCGCAAT		

IpaIII	G TTCCTTGACCGCCTTTCCGATACCGTC		
		<i>IpaH</i>	619
IpaIV	G CCGGTCAGCCACCCTCTGAGAGTAC		
AggRks1	G TATACACAAAAGAAGGAAGC		
		<i>AggR</i>	254
AggRks2	A CAGAATCGTCAGCATCAGC		

The program used to carry out the reaction consisted of: initial denaturation at 95°C for 5 minutes; 30 cycles of denaturation at 95°C for 20 seconds, annealing at 52°C for 20 seconds and extension at 72°C for 30 seconds and final extension at 72°C for 7 minutes. The products of the PCR reactions were submitted to electrophoresis in 1.5% agarose (w/v; Promega), stained with Unisafe (UniScience, Osasco, Brazil) according to Aranda et al. 2007. The DNA fragments were then analyzed comparatively with 100bp DNA markers (Promega), visualized and photographed in an image analyzer (L PIX – Loccu Biotechnology), and the classification of the isolates was performed according to Table 02. In all reactions A negative control was used, by replacing the sample with nuclease-free water, and strains E2348 / 69, 042, H10407, EDL1284 and O157: H7 EDL933 were used as a positive control for EPEC, EAEC, ETEC, EIEC and STEC respectively.

Identification of *hlyA*, *stx1* and *stx2*

The isolates identified as STEC were tested for enterohemolysin according to the primers presented in Table 3 (XIA et al., 2010) and Shiga toxin types 1 and 2 according to the primers presented in Table 4 (BAI, SHI & NAGARAJA, 2010). Both DNA extraction and amplification and electrophoresis were performed as described above.

Table 4- List of primers for identification of *hlyA*, *stx1* / *stx2* genes in STEC positive samples.

Primers	Sequence (5'-3')	Virulence genes	Amplicom (bp)
<i>stx1F</i>	TGTCGCATAGTGGAACCTCA		
<i>stx1R</i>	TGCGCACTGAGAAGAAGAGA	<i>stx1</i>	655
<i>stx2F</i>	CCATGACAACGGACAGCAGTT		
<i>stx2R</i>	TGTCGCCAGTTATCTGACATTC	<i>stx2</i>	477
<i>hlyAF</i>	AGCCGGAACAGTTCTCTCAG		
<i>hlyAR</i>	CCAGCATAACAGCCGATGT	<i>hlyA</i>	527

Determination of phylogroups according to the Clermont classification

The isolates classified as diarrheogenic *E. coli* were tested by quadriplex PCR for characterization in phylogenetic groups A, B1, B2, D and F according to Clermont et al. (2013). They were also tested by means of two simplex PCR, in order to classify them into phylogenetic groups E and C, respectively, as shown in Table 5 (CLERMONT et al., 2013).

Table 5- Primers according to Clermont et al. (2013) for the determination of phylogenetic groups.de DEC.

PCR Reaction	Primers	Sequence (5'-3')	Virulence genes	Amplicon (bp)
Quadruplex	chuA.1b	ATGGTACCGGACGAACCAAC	<i>chuA</i>	288
	chuA.2	TGCCGCCAGTACCAAAGACA		
	yjaA.1b	CAAACGTGAAGTGTCTCAGGAG	<i>yjaA</i>	211
	yjaA.2b	AATGCGTTCCTCAACCTGTG		
	TspE4C2.1b	CACTATTCGTAAGGTCATCC	<i>TspE4C2</i>	152
	TspE4C2.2b	AGTTTATCGCTGCGGGTCGC		
Group E	AceK.f	AACGCTATTCGCCAGCTTGC	<i>arpA</i>	400
	ArpA1.r	TCTCCCCATACCGTACGCTA		
	ArpAgpE.f	GATTCCATCTTGTCAAAATATGCC	<i>arpA</i>	301
Group C	ArpAgpE.r	GAAAAGAAAAAGAATTCCCAAGAG		
	trpAgpC.1	AGTTTTATGCCCAGTGCGAG	<i>trpA</i>	219
	trpAgpC.2	TCTGCGCCGGTCACGCCC		

For this, the DNA was extracted through the boiling technique (DE MÉDICI et al., 2003). For amplification, the GoTaq® Green Master Mix (Promega®) mix was used according to the manufacturer's recommendations and the primers according to Clermont et al. (2013) and presented in Table 3. The protocol used to carry out the reaction consisted of: initial denaturation of 94°C for 4 minutes, 30 cycles of 94°C for 5 seconds, 57°C (Group E) or 59°C (quadruplex and group C) for 20 seconds and final extension at 72°C for 5 minutes.

The products of the PCR reactions were submitted to electrophoresis in 1.5% agarose (w/v; Promega), stained with Unisafe (UniScience, Osasco, Brazil) according to Aranda et al. 2007. The DNA fragments were then comparatively analyzed with 100bp DNA markers (Promega), visualized and photographed in an image analyzer (L PIX – Loccu Biotechnology). In all reactions, a negative control was used, by replacing the sample with nuclease-free water and a positive control for each gene incorporated in the identification of pathotypes. The results achieved through PCR were classified according to the decision tree of Clermont et al. (2013).

Determination of serogroups of diarrheagenic *E. coli* isolates

The isolates confirmed as DEC were tested for serogroups O157, O104, O113 and the “big six” O26, O103, O111, O45, O121 and O145, based on the protocols established by Toro et al. (2013), Bugarel et al. (2010) and Chen et al. (2013). The extraction was performed using the boiling technique (DE MÉDICI et al., 2003). For amplification, the GoTaq® Green Master Mix (Promega®) mix was used according to the manufacturer's recommendations and the primers according to Toro et al. (2003) and Bugarel et al. (2010) and presented in Table 5.

The protocol used to carry out the reaction for the “big six” and O157 consisted of: initial denaturation at 95°C for 5 minutes, 30 cycles of 95°C for 30 seconds, 56°C for 30 seconds, 72°C for 40 seconds and final extension of 72°C for 10 minutes (TORO et al., 2013). For serogroup O104, the protocol followed was following Bugarel et al. (2010), consisted of: initial denaturation of 95°C for 3 minutes, 30 cycles of 95°C for 30 seconds, 64°C for 60 seconds, 72°C for 60 seconds and final extension of 72°C for 5 minutes. For serogroup O113, the protocol followed was according to Chen et al. (2013), consisted of: initial denaturation of 95°C for 15 minutes, 30 cycles of 94°C for 30 seconds, 57°C for 90 seconds, 72°C for 90 seconds and final extension of 72°C for 10 minutes.

Table 6- List of primers for the identification of DEC serogroups

Primers	Sequence(5'-3')	Virulence genes	Amplicom (bp)
O26F	GTGTGTCTGGTTCGTATTTTTTATCTG	O26	438
O26R	CCTTATATCCCAATATAGTACCCACCC		
O45F	GGTCGATAACTGGTATGCAATATG	O45	341
O45R	CTAGGCAGAAAGCTATCAACCAC		
O103F	TTATACAAATGGCGTGGATTGGAG	O103	385
O103R	TGCAGACACATGAAAAGTTGATGC		
O111F	TTCGATGTTGCGAGGAATAATTC	O111	361
O111R	GTGAGAGCCCACCAGTTAATTGAAG		
O121F	AGTGGGGAAGGGCGTTACTTATC	O121	366
O121R	CAATGAGTGCAGGCAAAATGGAG		
O145F	CCTGTCTGTTGCTTCAGCCCTTT	O145	392
O145R	CTGTGCGCGAACCCTGCTAAT		
O157F	TCGTTCTGAATTGGTGTGCTCA	O157	278
O157R	CTGGTGTGCGAAAGAAATCGTTC		
O104F	TGTCGCGCAAAGAATTTCAAC	O104	100
O104R	AAAATCCTTTAACTATACGCCC		
O113F	TGCCATAATTCAGAGGGTGAC	O113	514
O113R	AACAAAGCTAATTGTGGCCG		

Statistical Analysis

All samples with typical results in biochemical tests were considered positive for *E. coli*. The chi-square test ($p < 0.05$) was used to compare the frequency of occurrence of generic *E. coli* and DEC between intensive and extensive rearing systems using the GraphPad program. The same was done for the frequency of DEC pathotypes, phylogroups and serogroups, among the rearing systems.

Results

Of a total of 485 samples analyzed, 126 were positive for generic *E. coli* (Table 7). The feces of the animals were the point where the microorganism was most detected (100%), both in the intensive and extensive systems.

Regarding the other sampled points, animals from the intensive system showed greater positivity for generic *E. coli* at point A than animals from the extensive system, with 16% and 2% positivity respectively ($P = 0.0309$). At point B, it was also possible to detect *E. coli* in both systems, 2% for intensive and 4% for extensive and at point C, it was only found in one carcass (2%) from extensive production. At both points, there were no statistical differences between the systems ($p > 0.05$). Regarding the points of residual water, meat cuts, handling surface and feces of the employees, it was also possible to isolate *E. coli*, as described in table 7.

Table 7- Frequency of generic *Escherichia coli* according to the sample collected in a cattle slaughterhouse in the state of Minas Gerais.

Ponit*	Intensive		Extensive		Mist		P value
	Samples positives	%	Samples positives	%	Samples positives	%	
A	8/50 ^b	16,0	1/50 ^b	2,0	-	-	0,0309
B	1/50 ^c	2,0	2/50 ^b	4,0	-	-	>0,9999
C	0/50 ^c	0,0	1/50 ^b	2,0	-	-	>0,9999
F	50/50 ^a	100,0	50/50 ^a	100,0	-	-	>0,9999
AR	-	-	-	-	1/5 ^{a,b}	20	
D	-	-	-	-	2/50 ^b	4	
S	-	-	-	-	3/20 ^b	15	
H	-	-	-	-	8/10 ^a	80	
P value **	<0,0001		<0,0001		<0,0001		

*A – Post-Bleeding; B – Post-Evisceration; C – Post – Final Wash; F – Animal feces; AR – Wastewater; D- Meat cuts; S- Surfaces of tables and mats that had contact with the meat cuts; H – Human feces. **Different lowercase letters indicate difference between frequencies in the same column.

Regarding diarrheagenic *E. coli* (DEC), only in the feces of the animals (F) it was possible to detect them through the screening performed on the samples, as described in table 8. Of the animals analyzed, 35 were positive for some DEC, being 28 % of the animals from the intensive system and 42% of the animals from the extensive system, with no statistical difference between the rearing systems (P=0.2081).

Table 8 - Frequency of pathogenic *Escherichia coli* according to the type of sample collected at a cattle slaughterhouse in Minas Gerais.

Point*	Intensive		Extensive		Mist		P value
	Samples positives	%	Samples positives	%	Samples positives	%	
A	0/50	0	0/50	0	-	-	-
B	0/50	0	0/50	0	-	-	-
C	0/50	0	0/50	0	-	-	-
F	14/50	28%	21/50	42%	-	-	0,2081
AR	-	-	-	-	0/5	0	
D	-	-	-	-	0/50	0	
S	-	-	-	-	0/20	0	
H	-	-	-	-	0/10	0	

*A – Post-Bleeding; B – Post-Evisceration; C – Post – Final Wash; F – Animal feces; AR – Wastewater; D- Meat cuts; S- Surfaces of tables and mats that had contact with the meat cuts; H

– Human feces. **Different lowercase letters indicate difference between frequencies in the same column.

Subsequently, according to the biochemical confirmation and classification of the isolates using the virulence markers of each DEC, a total of 33 samples of feces of the animals were positive for STEC, being 14 obtained from the intensive system and 19 from the extensive system. In addition, 2 animals (4%) from pasture farming were positive for EIEC (Table 9). In view of these results, no statistical evidence was found that the frequency of STEC ($p = 0.3952$) and EIEC ($p = 0.4949$) in animals are associated with production systems.

Of all samples confirmed as STEC, the *stx* gene was classified, where it was possible to identify that one animal (2%) from the extensive system carried the *stx1* gene, 22 animals, 9 (18%) from the intensive system and 13 (26%) of the extensive animals carried the *stx2* gene and a total of 10 animals carried both the *stx1* and *stx2* genes, with 5 (10%) animals being reared in the intensive system and 5 (10%) in the extensive system, as shown in Table 9.

Table 9 - Frequency of diarrheagenic *Escherichia coli* pathotypes from animal feces collected at a cattle slaughterhouse in the state of Minas Gerais.

Cattle slaughterhouse in the state of Minas Gerais.					
Pathotype	Origin of samples and frequency of positivity				P value
	Intensive		Extensive		
	Samples positives	%	Samples positives	%	
STEC	14/50	28	19/50	38	0,3952
<i>stx1</i>	0/50	0	1/50	2	>0,9999
<i>stx2</i>	9/50	18	13/50	26	>0,9999
<i>stx1e2</i>	5/50	10	5/50	10	>0,9999

* EHEC, EPEC, EAEC and ETEC pathotypes were not detected in any of the samples.

Data related to phylogenetic groups were determined according to Clermont et al. (2013), and are shown in Table 10. The phylogroup most found was B1, with 24% of the animals originating from the intensive system and 68% of the animals from the extensive system. Followed by phylogroup E, in which 2% of the animals of the intensive system and 8% of the extensive system were identified. For phylogroup A, C and F, only animals from the extensive system showed corresponding genes, with 4%, 2% and 2% positivity, respectively. In the E. clades group, only one (2%) animal from the intensive system was positive. Regarding phylogroups B2 and D, none of the samples were positive.

Table 10 - Frequency of diarrheagenic *Escherichia coli* phylogroups from animal feces collected at a cattle slaughterhouse in the state of Minas Gerais.

Phylogroups	Origin of samples and frequency of positivity				P value
	Intensive		Extensive		
	Samples positives	%	Samples positives	%	
A	0/50	0	2/50	4	0,4949
B1	12/50	24	17/50	34	0,3783
B2	0/50	0	0/50	0	>0,9999
C	0/50	0	1/50	2	>0,9999
D	0/50	0	0/50	0	>0,9999
E	1/50	2	4/50	8	0,3622
F	0/50	0	1/50	2	>0,9999

E. clades	1/50	2	0/50	0	>0,9999
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With regard to serogroups, O157 and non-O157 (O113, O104, O26, O45, O103, O111, O121, O145) were analyzed. As shown in Table 11, serogroup O113 was identified, in which 2 (4%) of animals raised in an intensive system and 3 (6%) in the extensive system were positive, and also serogroup O104, in which 2 (4%) were positive. % of animals raised in an intensive system and 3 (6%) in an extensive system. The other serogroups were not identified. Also, the flagellar antigen H7 was investigated, and it was detected in 16 animals, being 5 (10%) of the intensive system and 11 (22%) of the extensive system, and of these, 3 were positive for O104:H7, 2 (4%) from the extensive system and 1 (2%) from the intensive.

Table 11 - Frequency of pathogenic *Escherichia coli* serogroups from animal feces collected at a cattle slaughterhouse in the state of Minas Gerais.

Serogroups	Origin of samples and frequency of positivity				P value
	Intensive		Extensive		
	Samples positives	%	Samples positives	%	
O113	2/50	4	3/50	6	>0,9999
O104	2/50	4	3/50	6	>0,9999
H7	5/50	10	11/50	22	0,1714

Serogroups O26, O45, O103, O111, O121, O145 and O157 were not identified in any of the samples analyzed.

Discussion

In the present study, the main source of generic *E. coli* were stool samples from animals and handlers, an expected result, since it is a microorganism that is part of the intestinal microbiota of mammals and is eliminated in the feces (KAPER & NATARO, 1998). The second point with the highest positivity for *E. coli* was point A, collected from the skin of the animals, where animals from the intensive system showed a higher frequency of *E. coli*.

The fact that the skin is the second point with the highest frequency of *E. coli*, and that animals from the intensive system showed greater positivity, can be explained by the closer proximity of animals during confinement, favoring a greater accumulation of fecal material. and, consequently, a greater number of microorganisms, when compared to animals submitted to the extensive rearing system (JARDIM et al., 2006). This factor may still be responsible for increasing the load of contamination that will be carried into the refrigerator environment (ISLAM et al., 2008; XIA et al., 2010).

Bovine feces are the main source of contamination of the skin and carcasses of animals in the refrigerated environment. Since, during the skinning or evisceration stages, cross-contamination can occur, spreading the microorganism from the skin or gastrointestinal tract to the carcass and consequently to meat products (RHOADES et al., 2009).

Similar results were also found in the study by Aslam et al, 2003, in which of the *E. coli* isolates from the collected samples, 61% were of fecal origin from cattle, 23% from skin smears, 10% from carcasses and 5% of ground meat, presenting, therefore, a greater number of *E. coli* in the feces of the animals, followed by the skin, carcass and meat cuts.

In relation to DEC, it was only possible to detect them in the feces samples of the animals, and there were no statistical differences between the production systems. The fact that DEC was detected only in animal feces can be justified by the effective control at the slaughterhouse, through adherence to good hygienic practices at slaughter. Since the quality of the final product and consumer safety are undoubted factors for the food industries (ARTILHA-MESQUITA et al., 2021).

Therefore, a well-designed and planned quality management system is indispensable in industries so that it can be adhered to throughout the product processing. There are several tools

used for quality management, designed to meet requirements such as the production of safe food, reducing waste, process improvements, meet national and international requirements. Among the available tools, Good Manufacturing Practices (GMP), Standard Operational Hygiene Procedure (PPHO) and Hazard Analysis and Critical Control Points (HACCP) can be mentioned (GOBIS & CAMPANATTI, 2012; SANTOS et al., 2020; HUBNER & KLAIC, 2019).

The pathotype most frequently found in both rearing systems was STEC (*E. coli* producing Shiga Toxin), however, there was no statistical difference between the systems. The same result was found in studies, in which the detection of STEC is not related to production systems (FEGAN et al., 2004; RENTER, SARGEANT AND HUNGERFOR, 2004).

However, in some studies, authors found that animals from the intensive system have a higher frequency of STEC. As in the work by Venegas-Vargas et al., 2016, carried out in the state of Michigan, in the United States, where they found STEC positivity in 21% of confined animals and 13% in animals raised on pasture. Hussein and Bollinger (2005) also found similar results, and reported that the prevalence of STEC ranged from 4.6 to 55.9% in feedlot cattle, from 4.7 to 44.8% in pasture cattle.

The different results between the studies can be explained, due to the significant impact of environmental factors on the prevalence of STEC (VENEGAS-VARGAS et al., 2016). For example, management practices, food diets, season, age of the animals, level of animal-to-animal contact, stress, among others (VENEGAS VARGAS et al., 2016; COBBOLD et al., 2004).

The fact that the pathotype most frequently found in the stool samples of the animals in this study was STEC can be explained by the fact that cattle and other ruminants are their primary reservoirs. The microorganism colonizes the rectum-anal junction of cattle (NAYLOR et al., 2003), and consequently the elimination occurs mainly through feces (RICE et al., 2003).

Other Brazilian studies also found high frequencies of STEC from bovine feces, such as Pigatto (2008) who found a frequency of 37% in samples analyzed in Paraná. Studies carried out in the states of Paraná, Rio de Janeiro, Rio Grande do Sul and São Paulo also showed results with a high frequency of STEC, being 57%, 53%, 49% and 25% respectively (FARAH et al., 2007; CERQUEIRA et al. al., 1999; MOREIRA et al., 2003; IRINO et al., 2005).

Furthermore, samples identified as STEC were classified as stx1, stx2 or stx1/stx2. It was found that 22% of the analyzed samples are stx2, 1% stx1 and 10% stx1/stx2. According to studies, the stx2 type appears to be more pathogenic than stx1 and is more often associated with severe forms of human morbidity (GONZALEZ & CERQUEIRA, 2020).

Another pathotype found was enteroinvasive *E.coli* (EIEC), which is not frequently detected in animals, since infected humans are considered the main reservoirs of this pathogen (MENG et al., 2013). In the present study, two animals from the extensive system were positive for EIEC. However, there are not many studies that address the presence of this pathogen in cattle feces. Kagambèga et al. (2012) carried out a survey in Africa on the prevalence of pathogenic *E. coli* in feces of poultry, swine and cattle, and only 1% of the feces samples from birds was detected EIEC. In view of this, there is a need for further research investigating this pathotype in cattle, since EIEC is responsible for causing invasive inflammatory colitis, and occasionally dysentery in humans (MORENO et al., 2012).

Both EIEC and STEC are important pathogens related to foodborne diseases and consequently a problem for public health. Mainly STEC which was identified as the third most related agent to food outbreaks between the years 2016 to 2019 in the United States (TACK, 2020). In 2010, the FERG - Foodborne Disease Burden Epidemiology Reference Group, estimated about 2.5 million cases due to STEC worldwide, resulting in more than 3,000 cases of HUS and 269 deaths (KIRK et al., 2015).

All samples identified as DEC were classified according to phylogroups according to Clermont et al, 2013. Phylogroup B1 was the most identified in both intensive and extensive animals. The same result is shown in the works of Madoshi et al (2016) and Mercat et al, 2016, and can be clarified due to the fact that phylogroup B1 is predominant in non-human mammals while B2 is more associated with humans and swine (ESCOBAR -PARAMO et al., 2006; CARLOS et al., 2010). According to studies, there is an evolutionary relationship of ancestors between

groups A and B1, as they have a smaller genome and fewer virulence factors when compared to B2 and D (ESCOBAR-PARAMO et al., 2006; CARLOS et al., 2010).

The second most frequently identified phylogroup was E, and A, C, F and E.clades were also detected, but at a lower frequency, while B2 and D were not identified. As in the work by Mercat et al., (2016), carried out in South Africa, which tested buffalo and cattle feces samples and detected 44.5% of the B1 samples, followed by phylogroup E with 29%, and phylogroups A, B2 and C were rarely detected.

With regard to serogroups, the present study was positive for two non-O157, which are O113 and O104. Cattle are the main reservoirs of STEC O157 and not O157, as they harbor such microorganisms in the gastrointestinal tract and eliminate them through feces (SHRIDAR et al., 2018).

Serogroup O113 is one of the most commonly found in beef products, such as ground beef, for example, in the United States, however its virulence potential is still unknown (FENG et al., 2017). However, some authors have reported that *E.coli* O113 strains are able to colonize the gastrointestinal tract of humans and consequently cause HUS (PATON et al., 1999). In the work by Vicente et al (2005) carried out in the region of Jaboticabal, state of São Paulo, Brazil, they also detected the presence of O113 in samples of bovine feces.

Bando and collaborators (2017) detected that some STEC O113:H7 strains from animal sources in Brazil belong to the same clonal group of STEC strains isolated from Argentine HUS patients. In view of this, the presence of this pathogen during meat processing shows a direct risk to the consumer, which may increase the risk of cases of foodborne diseases or outbreaks (DOS SANTOS et al., 2018).

Food contamination by STEC O104 is very common, even in developed countries (YANG et al.2017). In the study by Sridhar et al (2016), after analyzing fecal samples from cattle from feedlots, they detected a prevalence of 45.1% of *E.coli* O104, while Paddock et al (2013) detected a prevalence of 20.6%.

In the present study, the investigation of the H7 antigen was carried out, and three samples from bovine feces were identified as O104:H7. Sridhar et al (2016) through the analysis of PFGE, detected that O104:H7 strains from bovine feces were about 67.9% to 77.5% similar to strains from human samples, and they also address that this serogroup has a large potential to be a foodborne diarrheal pathogen in humans.

It is worth mentioning that even though it was not researched in this study, the H4 antigen is relevant to public health. The best-known serogroup is O104:H4, involved in one of the largest foodborne outbreaks in 2011 in Germany, however, they were not detected in cattle feces. However, cattle harbor other serotypes of O104, such as O104:H7, which has been associated with sporadic cases of diarrhea in humans (SRIDHAR et al., 2018).

Conclusion

In view of this, only in the stool samples it was possible to detect DEC, and STEC was the most found pathotype, followed by EIEC. Regarding the phylogroups, B1 was the most detected among the samples, followed by E, A, F and E.clade. And, among all the serogroups analyzed, positive isolates were detected for O113 and O104, which are considered important in terms of public health and need further research related to positivity in bovine feces.

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Remote Hosting and Large Data Sets

Data may be deposited with specialized service providers or institutional/subject repositories, preferably those that use the DataCite mechanism. Large data sets and files greater than 60 MB must be deposited in this way. For a list of other repositories specialized in scientific and experimental data, please consult [databib.org](#) or [re3data.org](#). The data repository name, link to the data set (URL) and accession number, doi or handle number of the data set must be provided in the paper. The journal [Data](#) also accepts submissions of data set papers.

Deposition of Sequences and of Expression Data

New sequence information must be deposited to the appropriate database prior to submission of the manuscript. Accession numbers provided by the database should be included in the submitted manuscript. Manuscripts will not be published until the accession number is provided.

- *New nucleic acid sequences* must be deposited in one of the following databases: [GenBank](#), [EMBL](#), or [DDBJ](#). Sequences should be submitted to only one database.
- *New high throughput sequencing (HTS) datasets* (RNA-seq, ChIP-Seq, degradome analysis, ...) must be deposited either in the [GEO database](#) or in the NCBI's [Sequence Read Archive \(SRA\)](#).
- *New microarray data* must be deposited either in the [GEO](#) or the [ArrayExpress](#) databases. The "Minimal Information About a Microarray Experiment" (MIAME) guidelines published by the Microarray Gene Expression Data Society must be followed.
- *New protein sequences* obtained by protein sequencing must be submitted to UniProt (submission tool [SPIN](#)). Annotated protein structure and its reference sequence must be submitted to [RCSB of Protein Data Bank](#).

All sequence names and the accession numbers provided by the databases must be provided in the Materials and Methods section of the article.

Deposition of Proteomics Data

Methods used to generate the proteomics data should be described in detail and we encourage authors to adhere to the "[Minimum Information About a Proteomics Experiment](#)". All generated mass spectrometry raw data must be deposited in the appropriate public database such as [ProteomeXchange](#), [PRIDE](#) or [iPOST](#). At the time of submission, please include all relevant information in the materials and methods section, such as repository where the data was submitted and link, data set identifier, username and password needed to access the data.

[\[Return to top\]](#)

Research and Publication Ethics

Research Ethics

Research Involving Human Subjects

When reporting on research that involves human subjects, human material, human tissues, or human data, authors must declare that the investigations were carried out following the rules of the Declaration of Helsinki of 1975 (<https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/>), revised in 2013. According to point 23 of this declaration, an approval from the local institutional review board (IRB) or other appropriate ethics committee must be obtained before undertaking the research to confirm the study meets national and international guidelines. As a minimum, a statement including the project identification code, date of approval, and name of the ethics committee or institutional review board must be stated in Section 'Institutional Review Board Statement' of the article.

Example of an ethical statement: "All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of XXX (Project identification code)."

For non-interventional studies (e.g. surveys, questionnaires, social media research), all participants must be fully informed if the anonymity is assured, why the research is being conducted, how their data will be used and if there are any risks associated. As with all research involving humans, ethical approval from an appropriate ethics committee must be obtained prior to conducting the study. If ethical approval is not required, authors must either provide an exemption from the ethics committee or are encouraged to cite the local or national legislation that indicates ethics approval is not required for this type of study. Where a study has been granted exemption, the name of the ethics committee which provided this should be stated in Section 'Institutional Review Board Statement' with a full explanation regarding why ethical approval was not required.

A written informed consent for publication must be obtained from participating patients. Data relating to individual participants must be described in detail, but private information identifying participants need not be included unless the identifiable materials are of relevance to the research (for example, photographs of participants' faces that show a particular symptom). Patients' initials or other personal identifiers must not appear in any images. For manuscripts that include any case details, personal information, and/or images of patients, authors must obtain signed informed consent for publication from patients (or their relatives/guardians) before submitting to an MDPI journal. Patient details must be anonymized as far as possible, e.g., do not mention specific age, ethnicity, or occupation where they are not relevant to the conclusions. A [template permission form](#) is available to download. A blank version of the form used to obtain permission (without the patient names or signature) must be uploaded with your submission. Editors reserve the right to reject any submission that does not meet these requirements.

You may refer to our sample form and provide an appropriate form after consulting with your affiliated institution. For the purposes of publishing in MDPI journals, a consent, permission, or release form should include unlimited permission for publication in all formats (including print, electronic, and online), in sublicensed and reprinted versions (including translations and derived works), and in other works and products under open access license. To respect patients' and any other individual's privacy, please do not send signed forms. The journal reserves the right to ask authors to provide signed forms if necessary.

If the study reports research involving vulnerable groups, an additional check may be performed. The submitted manuscript will be scrutinized by the editorial office and upon request, documentary evidence (blank consent forms and any related discussion documents from the ethics board) must be supplied. Additionally, when studies describe groups by race, ethnicity, gender, disability, disease, etc., explanation regarding why such categorization was needed must be clearly stated in the article.

Ethical Guidelines for the Use of Animals in Research

The editors will require that the benefits potentially derived from any research causing harm to animals are significant in relation to any cost endured by animals, and that procedures followed are unlikely to cause offense to the majority of readers. Authors should particularly ensure that their research complies with the commonly-accepted '3Rs [1]':

- Replacement of animals by alternatives wherever possible,
- Reduction in number of animals used, and
- Refinement of experimental conditions and procedures to minimize the harm to animals.

Authors must include details on housing, husbandry and pain management in their manuscript.

For further guidance authors should refer to the Code of Practice for the Housing and Care of Animals Used in Scientific Procedures [2], American Association for Laboratory Animal Science [3] or European Animal Research Association [4].

If national legislation requires it, studies involving vertebrates or higher invertebrates must only be carried out after obtaining approval from the appropriate ethics committee. As a minimum, the project identification code, date of approval and name of the ethics committee or institutional review board should be stated in Section 'Institutional Review Board Statement'. Research procedures must be carried out in accordance with national and institutional regulations. Statements on animal welfare should confirm that the study complied with all relevant legislation. Clinical studies involving animals and interventions outside of routine care require ethics committee oversight as per the American Veterinary Medical Association. If the study involved client-owned animals, informed client consent must be obtained and certified in the manuscript report of the research. Owners must be fully informed if there are any risks associated with the procedures and that the research will be published. If available, a high standard of veterinary care must be provided. Authors are responsible for correctness of the statements provided in the manuscript.

If ethical approval is not required by national laws, authors must provide an exemption from the ethics committee, if one is available. Where a study has been granted exemption, the name of the ethics committee that provided this should be stated in Section 'Institutional Review Board Statement' with a full explanation on why the ethical approval was not required.

If no animal ethics committee is available to review applications, authors should be aware that the ethics of their research will be evaluated by reviewers and editors. Authors should provide a statement justifying the work from an ethical perspective, using the same utilitarian framework that is used by ethics committees. Authors may be asked to provide this even if they have received ethical approval.

MDPI endorses the ARRIVE guidelines (arriveguidelines.org/) for reporting experiments using live animals. Authors and reviewers must use the ARRIVE guidelines as a checklist, which can be found at <https://arriveguidelines.org/sites/arrive/files/documents/ARRIVE%20Compliance%20Questionnaire.pdf>. Editors reserve the right to ask for the checklist and to reject submissions that do not adhere to these guidelines, to reject submissions based on ethical or animal welfare concerns or if the procedure described does not appear to be justified by the value of the work presented.

1. NSW Department of Primary Industries and Animal Research Review Panel. Three Rs. Available online: <https://www.animaethics.org.au/three-rs>
2. Home Office. Animals (Scientific Procedures) Act 1986. Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes. Available online: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/388535/CoPanimalsWeb.pdf
3. American Association for Laboratory Animal Science. The Scientific Basis for Regulation of Animal Care and Use. Available online: <https://www.aalas.org/about-aalas/position-papers/scientific-basis-for-regulation-of-animal-care-and-use>
4. European Animal Research Association. EU regulations on animal research. Available online: <https://www.eara.eu/animal-research-law>

Research Involving Cell Lines

Methods sections for submissions reporting on research with cell lines should state the origin of any cell lines. For established cell lines the provenance should be stated and references must also be given to either a published paper or to a commercial source. If previously unpublished *de novo* cell lines were used, including those gifted from another laboratory, details of institutional review board or ethics committee approval must be given, and confirmation of written informed consent must be provided if the line is of human origin.

An example of Ethical Statements:

The HCT116 cell line was obtained from XXXX. The MLH1⁺ cell line was provided by XXXXX, Ltd. The DLD-1 cell line was obtained from Dr. XXXX. The DR-GFP and SA-GFP reporter plasmids were obtained from Dr. XXX and the Rad51K133A expression vector was obtained from Dr. XXXX.

Research Involving Plants

Experimental research on plants (either cultivated or wild) including collection of plant material, must comply with institutional, national, or international guidelines. We recommend that authors comply with the [Convention on Biological Diversity](#) and the [Convention on the Trade in Endangered Species of Wild Fauna and Flora](#).

For each submitted manuscript supporting genetic information and origin must be provided. For research manuscripts involving rare and non-model plants (other than, e.g., *Arabidopsis thaliana*, *Nicotiana benthamiana*, *Oryza sativa*, or many other typical model plants), voucher specimens must be deposited in an accessible herbarium or museum. Vouchers may be requested for review by future investigators to verify

the identity of the material used in the study (especially if taxonomic rearrangements occur in the future). They should include details of the populations sampled on the site of collection (GPS coordinates), date of collection, and document the part(s) used in the study where appropriate. For rare, threatened or endangered species this can be waived but it is necessary for the author to describe this in the cover letter.

Editors reserve the rights to reject any submission that does not meet these requirements.

An example of Ethical Statements:

Torenia fournieri plants were used in this study. White-flowered Crown White (CrW) and violet-flowered Crown Violet (CrV) cultivars selected from 'Crown Mix' (XXX Company, City, Country) were kindly provided by Dr. XXX (XXX Institute, City, Country).

Arabidopsis mutant lines (SALKxxxx, SAILxxxx,...) were kindly provided by Dr. XXX, institute, city, country).

Clinical Trials Registration

Registration

MDPI follows the International Committee of Medical Journal Editors (ICMJE) [guidelines](#) which require and recommend registration of clinical trials in a public trials registry at or before the time of first patient enrollment as a condition of consideration for publication.

Purely observational studies do not require registration. A clinical trial not only refers to studies that take place in a hospital or involve pharmaceuticals, but also refer to all studies which involve participant randomization and group classification in the context of the intervention under assessment.

Authors are strongly encouraged to pre-register clinical trials with an international clinical trials register and cite a reference to the registration in the Methods section. Suitable databases include [clinicaltrials.gov](#), [the EU Clinical Trials Register](#) and those listed by the World Health Organisation [International Clinical Trials Registry Platform](#).

Approval to conduct a study from an independent local, regional, or national review body is not equivalent to prospective clinical trial registration. MDPI reserves the right to decline any paper without trial registration for further peer-review. However, if the study protocol has been published before the enrolment, the registration can be waived with correct citation of the published protocol.

CONSORT Statement

MDPI requires a completed CONSORT 2010 [checklist](#) and [flow diagram](#) as a condition of submission when reporting the results of a randomized trial. Templates for these can be found here or on the CONSORT website (<http://www.consort-statement.org>) which also describes several CONSORT checklist extensions for different designs and types of data beyond two group parallel trials. At minimum, your article should report the content addressed by each item of the checklist.

[\[Return to top\]](#)

Sex and Gender in Research

We encourage our authors to follow the '[Sex and Gender Equity in Research – SAGER – guidelines](#)' and to include sex and gender considerations where relevant. Authors should use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Article titles and/or abstracts should indicate clearly what sex(es) the study applies to. Authors should also describe in the background, whether sex and/or gender differences may be expected; report how sex and/or gender were accounted for in the design of the study; provide disaggregated data by sex and/or gender, where appropriate; and discuss respective results. If a sex and/or gender analysis was not conducted, the rationale should be given in the Discussion. We suggest that our authors consult the full [guidelines](#) before submission.

[\[Return to top\]](#)

Borders and Territories

Potential disputes over borders and territories may have particular relevance for authors in describing their research or in an author or editor correspondence address, and should be respected. Content decisions are an editorial matter and where there is a potential or perceived dispute or complaint, the editorial team will attempt to find a resolution that satisfies parties involved.

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Publication Ethics Statement

Veterinary Sciences is a member of the Committee on Publication Ethics ([COPE](#)). We fully adhere to its [Code of Conduct](#) and to its [Best Practice Guidelines](#).

The editors of this journal enforce a rigorous peer-review process together with strict ethical policies and standards to ensure to add high quality scientific works to the field of scholarly publication. Unfortunately, cases of plagiarism, data falsification, image manipulation, inappropriate authorship credit, and the like, do arise. The editors of *Veterinary Sciences* take such publishing ethics issues very seriously and are trained to proceed in such cases with a zero tolerance policy.

Authors wishing to publish their papers in *Veterinary Sciences* must abide to the following:

- Any facts that might be perceived as a possible conflict of interest of the author(s) must be disclosed in the paper prior to submission.
- Authors should accurately present their research findings and include an objective discussion of the significance of their findings.
- Data and methods used in the research need to be presented in sufficient detail in the paper, so that other researchers can replicate the work.
- Raw data should preferably be publicly deposited by the authors before submission of their manuscript. Authors need to at least have the raw data readily available for presentation to the referees and the editors of the journal, if requested. Authors need to ensure appropriate measures are taken so that raw data is retained in full for a reasonable time after publication.
- Simultaneous submission of manuscripts to more than one journal is not tolerated.
- The journal accepts exact translations of previously published work. All submissions of translations must conform with our [policies on translations](#).
- If errors and inaccuracies are found by the authors after publication of their paper, they need to be promptly communicated to the editors of this journal so that appropriate actions can be taken. Please refer to our [policy regarding Updating Published Papers](#).
- Your manuscript should not contain any information that has already been published. If you include already published figures or images, please obtain the necessary permission from the copyright holder to publish under the CC-BY license. For further information, see the [Rights and Permissions](#) page.
- Plagiarism, data fabrication and image manipulation are not tolerated.
 - **Plagiarism is not acceptable** in *Veterinary Sciences* submissions.
 Plagiarism includes copying text, ideas, images, or data from another source, even from your own publications, without giving any credit to the original source.

 Reuse of text that is copied from another source must be between quotes and the original source must be cited. If a study's design or the manuscript's structure or language has been inspired by previous works, these works must be explicitly cited.

 If plagiarism is detected during the peer review process, the manuscript may be rejected. If plagiarism is detected after publication, we may publish a correction or retract the paper.
 - **Image files must not be manipulated or adjusted in any way** that could lead to misinterpretation of the information provided by the original image.

 Irregular manipulation includes: 1) introduction, enhancement, moving, or removing features from the original image; 2) grouping of images that should obviously be presented separately (e.g., from different parts of the same gel, or from different gels); or 3) modifying the contrast, brightness or color balance to obscure, eliminate or enhance some information.

 If irregular image manipulation is identified and confirmed during the peer review process, we may reject the manuscript. If irregular image manipulation is identified and confirmed after publication, we may correct or retract the paper.

Our in-house editors will investigate any allegations of publication misconduct and may contact the authors' institutions or funders if necessary. If evidence of misconduct is found, appropriate action will

be taken to correct or retract the publication. Authors are expected to comply with the best ethical publication practices when publishing with MDPI.

Citation Policy

Authors should ensure that where material is taken from other sources (including their own published writing) the source is clearly cited and that where appropriate permission is obtained.

Authors should not engage in excessive self-citation of their own work.

Authors should not copy references from other publications if they have not read the cited work.

Authors should not preferentially cite their own or their friends', peers', or institution's publications.

Authors should not cite advertisements or advertorial material.

In accordance with COPE guidelines, we expect that "original wording taken directly from publications by other researchers should appear in quotation marks with the appropriate citations." This condition also applies to an author's own work. COPE have produced a discussion document on [citation manipulation](#) with recommendations for best practice.

[\[Return to top\]](#)

Reviewer Suggestions

During the submission process, please suggest three potential reviewers with the appropriate expertise to review the manuscript. The editors will not necessarily approach these referees. Please provide detailed contact information (address, homepage, phone, e-mail address). The proposed referees should neither be current collaborators of the co-authors nor have published with any of the co-authors of the manuscript within the last five years. Proposed reviewers should be from different institutions to the authors. You may identify appropriate Editorial Board members of the journal as potential reviewers. You may suggest reviewers from among the authors that you frequently cite in your paper.

[\[Return to top\]](#)

English Corrections

To facilitate proper peer-reviewing of your manuscript, it is essential that it is submitted in grammatically correct English. Advice on some specific language points can be found [here](#).

If you are not a native English speaker, we recommend that you have your manuscript professionally edited before submission or read by a native English-speaking colleague. This can be carried out by MDPI's [English editing service](#). Professional editing will enable reviewers and future readers to more easily read and assess the content of submitted manuscripts. All accepted manuscripts undergo language editing, however **an additional fee will be charged** to authors if very extensive English corrections must be made by the Editorial Office: pricing is according to the service [here](#).

[\[Return to top\]](#)

Preprints and Conference Papers

Veterinary Sciences accepts submissions that have previously been made available as preprints provided that they have not undergone peer review. A preprint is a draft version of a paper made available online before submission to a journal.

MDPI operates [Preprints](#), a preprint server to which submitted papers can be uploaded directly after completing journal submission. Note that *Preprints* operates independently of the journal and posting a preprint does not affect the peer review process. Check the *Preprints* [instructions for authors](#) for further information.

Expanded and high-quality conference papers can be considered as articles if they fulfill the following requirements: (1) the paper should be expanded to the size of a research article; (2) the conference paper should be cited and noted on the first page of the paper; (3) if the authors do not hold the copyright of the published conference paper, authors should seek the appropriate permission from the copyright holder; (4) authors are asked to disclose that it is conference paper in their cover letter and include a statement on what

has been changed compared to the original conference paper. *Veterinary Sciences* does not publish pilot studies or studies with inadequate statistical power.

Unpublished conference papers that do not meet the above conditions are recommended to be submitted to the [Proceedings Series journals](#).

[\[Return to top\]](#)

Authorship

MDPI follows the International Committee of Medical Journal Editors ([ICMJE](#)) guidelines which state that, in order to qualify for authorship of a manuscript, the following criteria should be observed:

- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
- Drafting the work or revising it critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Those who contributed to the work but do not qualify for authorship should be listed in the acknowledgments. More detailed guidance on authorship is given by the [International Council of Medical Journal Editors \(ICMJE\)](#).

Any change to the author list should be approved by all authors including any who have been removed from the list. The corresponding author should act as a point of contact between the editor and the other authors and should keep co-authors informed and involve them in major decisions about the publication. We reserve the right to request confirmation that all authors meet the authorship conditions.

For more details about authorship please check [MDPI ethics website](#).

Reviewers Recommendation

Authors can recommend potential reviewers. Journal editors will check to make sure there are no conflicts of interest before contacting those reviewers, and will not consider those with competing interests. Reviewers are asked to declare any conflicts of interest. Authors can also enter the names of potential peer reviewers they wish to exclude from consideration in the peer review of their manuscript, during the initial submission progress. The editorial team will respect these requests so long as this does not interfere with the objective and thorough assessment of the submission.

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Lack of Interference With Editorial Decisions

Editorial independence is of utmost importance and MDPI does not interfere with editorial decisions. All articles published by MDPI are peer reviewed and assessed by our independent editorial boards, and MDPI staff are not involved in decisions to accept manuscripts. When making an editorial decision, we expect the academic editor to make their decision based only upon:

- The suitability of selected reviewers;
- Adequacy of reviewer comments and author response;
- Overall scientific quality of the paper.

In all of our journals, in every aspect of operation, MDPI policies are informed by the mission to make science and research findings open and accessible as widely and rapidly as possible.

Editors and Editorial Staff as Authors

Editorial staff or editors shall not be involved in processing their own academic work. Submissions authored by editorial staff/editors will be assigned to at least two independent outside reviewers. Decisions will be

made by other Editorial Board Members who do not have a conflict of interest with the author. Journal staff are not involved in the processing of their own work submitted to any MDPI journals.

Conflict of Interests

According to The International Committee of Medical Journal Editors, “Authors should avoid entering into agreements with study sponsors, both for-profit and non-profit, that interfere with authors’ access to all of the study’s data or that interfere with their ability to analyze and interpret the data and to prepare and publish manuscripts independently when and where they choose.”

All authors must disclose all relationships or interests that could inappropriately influence or bias their work. Examples of potential conflicts of interest include but are not limited to financial interests (such as membership, employment, consultancies, stocks/shares ownership, honoraria, grants or other funding, paid expert testimonies and patent-licensing arrangements) and non-financial interests (such as personal or professional relationships, affiliations, personal beliefs).

Authors can disclose potential conflicts of interest via the online submission system during the submission process. Declarations regarding conflicts of interest can also be collected via the [MDPI disclosure form](#). The corresponding author must include a summary statement in the manuscript in a separate section “Conflicts of Interest” placed just before the reference list. The statement should reflect all the collected potential conflict of interest disclosures in the form.

See below for examples of disclosures:

Conflicts of Interest: Author A has received research grants from Company A. Author B has received a speaker honorarium from Company X and owns stocks in Company Y. Author C has been involved as a consultant and expert witness in Company Z. Author D is the inventor of patent X.

If no conflicts exist, the authors should state:

Conflicts of Interest: The authors declare no conflicts of interest.

[\[Return to top\]](#)

Editorial Procedures and Peer-Review

Initial Checks

All submitted manuscripts received by the Editorial Office will be checked by a professional in-house *Managing Editor* to determine whether they are properly prepared and whether they follow the ethical policies of the journal, including those for human and animal experimentation. Manuscripts that do not fit the journal’s ethics policy or do not meet the standards of the journal will be rejected before peer-review. Manuscripts that are not properly prepared will be returned to the authors for revision and resubmission. After these checks, the *Managing Editor* will consult the journals’ *Editor-in-Chief* or *Associate Editors* to determine whether the manuscript fits the scope of the journal and whether it is scientifically sound. No judgment on the potential impact of the work will be made at this stage. Reject decisions at this stage will be verified by the *Editor-in-Chief*.

Peer-Review

Once a manuscript passes the initial checks, it will be assigned to at least two independent experts for peer-review. A single-blind review is applied, where authors’ identities are known to reviewers. Peer review comments are confidential and will only be disclosed with the express agreement of the reviewer.

In the case of regular submissions, in-house assistant editors will invite experts, including recommendations by an academic editor. These experts may also include *Editorial Board Members* and Guest Editors of the journal. Potential reviewers suggested by the authors may also be considered. Reviewers should not have published with any of the co-authors during the past five years and should not currently work or collaborate with any of the institutions of the co-authors of the submitted manuscript.

Optional Open Peer-Review

The journal operates optional open peer-review: *Authors are given the option for all review reports and editorial decisions to be published alongside their manuscript. In addition, reviewers can sign their review, i.e., identify themselves in the published review reports.* Authors can alter their choice for open review at any time before publication, but once the paper has been published changes will only be made at the discretion of the *Publisher* and *Editor-in-Chief*. We encourage authors to take advantage of this opportunity as proof of the

rigorous process employed in publishing their research. To guarantee impartial refereeing, the names of referees will be revealed only if the referees agree to do so, and after a paper has been accepted for publication.

Editorial Decision and Revision

All the articles, reviews and communications published in MDPI journals go through the peer-review process and receive at least two reviews. The in-house editor will communicate the decision of the academic editor, which will be one of the following:

- *Accept* *after* *Minor* *Revisions:*
The paper is in principle accepted after revision based on the reviewer's comments. Authors are given five days for minor revisions.
- *Reconsider* *after* *Major* *Revisions:*
The acceptance of the manuscript would depend on the revisions. The author needs to provide a point by point response or provide a rebuttal if some of the reviewer's comments cannot be revised. Usually, only one round of major revisions is allowed. Authors will be asked to resubmit the revised paper within a suitable time frame, and the revised version will be returned to the reviewer for further comments.
- *Reject* *and* *Encourage* *Resubmission:*
If additional experiments are needed to support the conclusions, the manuscript will be rejected and the authors will be encouraged to re-submit the paper once further experiments have been conducted.
- *Reject:*
The article has serious flaws, and/or makes no original significant contribution. No offer of resubmission to the journal is provided.

All reviewer comments should be responded to in a point-by-point fashion. Where the authors disagree with a reviewer, they must provide a clear response.

Author Appeals

Authors may appeal a rejection by sending an e-mail to the Editorial Office of the journal. The appeal must provide a detailed justification, including point-by-point responses to the reviewers' and/or Editor's comments. The *Managing Editor* of the journal will forward the manuscript and related information (including the identities of the referees) to the Editor-in-Chief, Associate Editor, or Editorial Board member. The academic Editor being consulted will be asked to give an advisory recommendation on the manuscript and may recommend acceptance, further peer-review, or uphold the original rejection decision. A reject decision at this stage is final and cannot be reversed.

In the case of a special issue, the *Managing Editor* of the journal will forward the manuscript and related information (including the identities of the referees) to the *Editor-in-Chief* who will be asked to give an advisory recommendation on the manuscript and may recommend acceptance, further peer-review, or uphold the original rejection decision. A reject decision at this stage will be final and cannot be reversed.

Production and Publication

Once accepted, the manuscript will undergo professional copy-editing, English editing, proofreading by the authors, final corrections, pagination, and, publication on the www.mdpi.com website.

[\[Return to top\]](#)

Promoting Equity, Diversity and Inclusiveness Within MDPI Journals

Our Managing Editors encourage the Editors-in-Chief and Associate Editors to appoint diverse expert Editorial Boards. This is also reflective in our multi-national and inclusive workplace. We are proud to create equal opportunities without regard to gender, ethnicity, sexual orientation, age, religion, or socio-economic status. There is no place for discrimination in our workplace and editors of MDPI journals are to uphold these principles in high regard.

[\[Return to top\]](#)

Resource Identification Initiative

To improve the reproducibility of scientific research, the [Resource Identification Initiative](#) aims to provide unique persistent identifiers for key biological resources, including antibodies, cell lines, model organisms and tools.

We encourage authors to include unique identifiers - RRIDs- provided by the [Resource Identification Portal](#) in the dedicated section of the manuscript.

To help authors quickly find the correct identifiers for their materials, there is a single [website](#) where all resource types can be found and a 'cite this' button next to each resource, that contains a proper citation text that should be included in the methods section of the manuscript.