Rev. Bras. Saúde Prod. Anim., Salvador, v.19, n.4, p.371-380 out./dez., 2018 **ISSN 1519 9940**
http://dx.doi.org/10.1590/S1519-99402018000400002

Colimetric index and virulence genes *iss* and *iut*A in *Escherichia coli* isolates in cellulitis of poultry carcasses under sanitary inspection

Índice colimétrico e genes de virulência iss e iutA em isolados de "Escherichia coli" em celulites de carcaças de frangos sob Inspeção Sanitária

SILVA, Ricardo Mendes da[1]*; JESUS, Maykson Costa de[2]; MACÊDO, Kelly Menezes[2]; CARDOSO, Vaneza Leal[2]; SANTANA, Jerusa Mota[3]; SILVA, Isabella de Matos Mendes da[1]; BALIZA, Marcílio[1]; EVÊNCIO-NETO, Joaquim[4]

[1]Universidade Federal do Recôncavo da Bahia, Centro de Ciências da Saúde, Complexo Multidisciplinar de Estudos e Pesquisas em Saúde, Santo Antônio de Jesus, Bahia, Brasil.
[2]Universidade Federal do Recôncavo da Bahia, Centro de Ciências Agrárias, Ambientais e Biológicas, Cruz das Almas, Bahia, Brasil.
[3]Universidade Federal da Bahia, Escola de Medicina Vaterinária e Zootecnia, Salvador, Bahia, Brasil.
[4]Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brasil.
*Endereço para correspondência: ricardomendes@ufrb.edu.br

SUMMARY

Current study determines the population of total coliforms and *Escherichia coli* and identifies *iss* and *iut*A virulence genes in *Escherichia coli* strains isolated from cellulitis in poultry carcasses retrieved from a slaughterhouse. One hundred cellulitis lesions were collected between August 2013 and January 2014. The population of total coliforms and *Escherichia coli* was verified by Petrifilm™ rapid counting method (AOAC 998.8). *Escherichia coli* samples were analyzed for *iss* and *iut*A genes by Polymerase Chain Reaction (PCR) technique. Total coliforms were present in 96.0% (96/100) of the analyzed samples, with a population between 3.4 and 9.5 log CFU/g. *Escherichia coli* was present in 82.0% (82/100) of cellulitis samples and the population ranged between <1.0 and 9.0 log CFU/g. The *iss* gene was found in 89.0% of isolates and the *iut*A gene in 97.6%. High populations of total coliforms and *Escherichiacoli* in cellulitis samples indicate that hygienic-sanitary failures may have occurred in the production of broilers. When high prevalence of virulence genes under analysis, characteristic of Avian Pathogenic *Escherichia coli* (APEC) and possible zoonotic character of the pathotype are taken into account, it is important to highlight the need to adopt Good Manufacturing Practices, Standard Procedures of Operational Hygiene and Hazard Analysis and Critical Control Points in poultry slaughterhouses to ensure the safety of the final product.

Keywords: APEC, colibacillosis, zoonotic potential

RESUMO

Objetivou-se determinar a população de coliformes totais e de Escherichia coli e identificar os genes de virulência iss e iutA nas cepas de Escherichia coli isoladas de celulites em carcaças de frangos em um abatedouro. No periodo de agosto de 2013 a janeiro de 2014 foram coletadas 100 lesões de celulite, sendo verificada a população de coliformes totais e Escherichia coli, pelo método rápido de contagem Petrifilm™ (AOAC 998.8). As amostras de Escherichia coli foram analisadas para verificar a presença dos genes iss e iutA, utilizando a técnica de Reação em Cadeia da Polimerase (PCR). Houve a presença de coliformes totais em 96,0 % (96/100) das amostras analisadas, com a população entre 3,4 a 9,5 log UFC/g. Constatou-se também a presença de Escherichia coli em 82,0 % (82/100) das amostras de celulite, com população entre <1,0 e 9,0 log UFC/g. O gene iss foi encontrado em 89,0 % dos isolados e o gene iutA em 97,6 %. As populações elevadas

de coliformes totais e de Escherichia coli nas amostras de celulite indicam que falhas higiênico-sanitárias podem ter ocorrido na produção dos frangos de corte. Considerando a prevalência elevada dos genes de virulência pesquisados, característicos de Escherichia coli Patogênica para Aves (APEC) e o possível caráter zoonótico deste patotipo, é importante ressaltar a necessidade da adoção das Boas Práticas de Fabricação, Procedimentos Padrão de Higiene Operacional e da Análise de Perigos e Pontos Críticos de Controle nos abatedouros, para garantir a inocuidade do produto final.

Palavras-chave: APEC, colibacilose, potencial zoonótico

INTRODUCTION

Colibacillosis is one great challenge in broiler industry. This is due to health risks and economic losses. Is a localized or systemic infection caused by Avian Pathogenic *Escherichia coli* (APEC), (BARNES et al., 2008; FERREIRA et al., 2011).

Avian cellulitis, one of the colibacillosis presentations, is a subcutaneous inflammation, particularly in the thigh and abdomen (ANDRADE et al., 2006); requiring partial or whole carcass condemnation. Almeida et al. (2017) ranked cellulitis, as a second (with 19.68%) for whole condemnation of carcasses in slaughterhouses under Federal Inspection in northeastern Brazil.

Federal Inspection in 2016 reported there were 63,008,095 condemnations of broiler carcasses in slaughterhouses under federal inspection, specifically, 7,000,000 and 6,800,000 due to cellulitis and colibacillosis, respectively (MAPA, 2016).

Cost per kilo of broiler live weight in Paraná state, in June 2018 was R$ 2.91, and the mean weight of each broiler was 2.5 kg, one may estimate a liability of R$49,470,000.00 per year due to colibacillosis condemnations. If the retail consumer is calculated, losses may be reached close to R$ 150,000,000.00 (EMBRAPA, 2018).

Colibacillosis is caused by the invasion of bacteria in the tissues, with skin surface lesions due to contact with other broilers or to the bad quality poultry litter. It is caused by APEC pathotype which belong of extra-intestinal pathogenic *E. coli* (ExPEC) (HUJA et al., 2015).

APECs provide the *iss* (*Increased Serum Survival*) gene. The latter expresses cell wall protein which resists the host´s lithic serum effects and the. virulence factor which may often be found (ROCHA et al., 2008). Strains may express iron acquisition systems, siderophore compounds of aerobactin and their external membrane receptors (gene *iut*A) (CHOUIKHA et al., 2008).

E. coli may cause intestinal infections and extra intestinal and its classification is based on virulence genes (KAPER et al., 2004). Studies insist on similarity between human and avian ExPEC strains which imply a zoonotic potential of APEC (PITOUT, 2012).

Since the zoonotic potential of APEC caused by cellulitis lesions in broilers and the economic liability this inflammation should be taken into account, current assay quantifies the population of total coliforms and *E. coli* and identifies virulence genes *iss* and *iut*A in *E. coli* strains isolated from cellulitis of broiler carcasses retrieved from a slaughterhouse in the region of the Recôncavo of Bahia, Brazil.

MATERIALS AND METHODS

One hundred cellulitis samples from broiler carcasses were retrieved between August 2013 and January 2014 in a broiler slaughterhouse under federal sanitary inspection in the Recôncavo

Sul da Bahia, Brazil. Animal carcasses hailed from nine aviaries. Carcasses were removed from the inspection line; samples were collected aseptically by a sterile scalpel and conditioned in sterile collectors and identified; they were then transported in an isothermal box with recycled ice; microbiological analyses were done immediately. All broiler carcasses were examined for lesions associated with cellulitis, and weighed. The following parameters were employed to evaluate cellulitis lesions: weight, color, conformation and consistency. The samples were photographed with an Olympus E330 camera.

One gram of cellulitis was weighed and added to 9 mL of saline solution 0.9%, forming a dilution 10^{-1}, with successive dilutions, till dilution 10^{-8} for the later addition of 1 mL of dilutions in plates. Populations of total coliforms and *Escherichia coli* were determined by fast count method in Petrifilm™ (3M Company), by plate Petrifilm EC, following manufacturer´s instructions (AOAC 998.8). Characteristic colonies were counted with colony counter CP600 Plus (Phoenix®) by calculating log CFU/g (SILVA et al., 2007).

Up to three *Escherichia coli*-type colonies from the Petrifilm EC™ plate (3M Company) were isolated for research on virulence genes. Each isolated colony was inoculated in microtube with Brain Heart Infusion (BHI) broth, with a platinum inoculating loops, and incubated at 35±1°C for 24±2 h. Further, 2 mL glycerol (final concentration 15%) were added and samples were frozen at minus 20°C for DNA extraction. DNA extraction comprised the reactivation of samples in BHI broth and incubated at 35±1°C for 24±2h. A pool with strains retrieved from the same cellulitis sample was prepared. Microtubes were centrifuged for 5 minute at 13,500 rpm. Supernatant was then removed and 800 µL of de-ionized water were added. Homogenization and centrifugation were performed as described above. The supernatant was removed once more and 80 µL of de-ionized water were added. Samples were then homogenized and heated at 96°C for 10 minutes in a warm bath. The samples were then centrifuged for 20 seconds at 13,500 rpm, and 2 µL of the supernatant were removed and placed in microtubes with 18 µL of de-ionized water.

DNA extracted from samples was quantified by spectrophotometer BioPhotometer D30 (Eppendorf™), UV wavelength 260 nm. Samples were diluted in ultrapure water (Hexapur™) to standardize final concentration at 50 ng/10µL. DNA samples were stored at minus 20°C untill analysis. Virulence genes from *E. coli* strains isolated from the cellulitis of broiler carcasses, associated with APEC pathotype (*iss* and *iut*A), were screened by Polymerase Chain reaction (PCR), by starters shown in Table 1.

Table 1. Sequences of PCR primers in broiler cellulites retrieved from a slaughterhouse in the Recôncavo of Bahia, Brazil

Gene	Sequence of oligonucleotides	Amplicon (MW)	Function
*iss**	5`GTGGCGAAAACTAGTAAAACAGC3` 5`CGCCTCGGGGTGGATAA 3`	760	Resistant to serum
*iut*A**	5`GGCTGGACATCATGGGAACTGG3` 5`CGTCGGGAACGGGTAGAATCG3`	302	Iron uptake

* Source : Knöbl et al. (2012) ** Source : Johnson et al. (2008). MW-molecular weight.

Rev. Bras. Saúde Prod. Anim., Salvador, v.19, n.4, p.371-380 out./dez., 2018 ISSN 1519 9940
http://dx.doi.org/10.1590/S1519-99402018000400002

Table 2 provides components employed in PCRs and their respective volumes and/or concentrations.

Reactions were prepared in aseptic chambers where 24 µL of the mixture were distributed in 0.2 mL polypropylene tubes, to which 1 µL of each sample was added to each tube. Ultrapure water (Hexapur™) was employed for negative control, whereas standard strain APEC (ATCC 25922), donated by the Oswaldo Cruz Foundation (Fiocruz) of Rio de Janeiro, Brazil, was used for positive control. Amplification reactions were performed in thermocycler Mastercycler (Amplitherm™).

Table 2. PCR components for the amplification of genes iss and iutA in samples of broiler cellulitis retrieved from a slaughterhouse in the Recôncavo of Bahia, Brazil

Components	Volume (µL)	Concentration
De-ionized water	14.7	-
PCR buffer	5	2x
PCR buffer	5	2x
MgCl2	1.5	1.5 µMol
MgCl2	1.5	1.5 µMol
Starter I	1.0	-
Starter II	1.0	-
DNA-mold	1	50 ng/µL
Total	25	-

Further, 10 µL of the amplified product, positive and negative controls, and 2 µL of molecular weight 50 pb DNA ladder were added to each well of agar gel 2%, stained with ethidium bromide (10 mg/mL). Separation occurred by electrophorese, using GSR® 1000STD at 100 minutes, 60V, 37mA and 2W. Results were then observed with UV transilluminator (Loccus®). Analyses were performed at the Research Laboratories of Food and Nutrition Safety Nucleus (SANUTRI) of the Center of Health Sciences of the Universidade Federal do Recôncavo da Bahia (CCS/UFRB), Brazil.

Statistical analysis was performed with SPSS 17.0. Descriptive analysis was made, with mean and standard deviation for quantitative variables and percentage for qualitative variables. Analysis of Variance (ANOVA) compared means of bacteria according to poultry categories, whilst Tukey´s test was employed for multiple comparisons. Statistically significant results occurred when p value was lower than or equal to 0.05.

RESULTS AND DISCUSSION

Broiler carcasses from the nine different aviaries were analyzed for cellulitis and other macroscopic alterations. Carcass weight ranged between 1.1 and 4.0 kg; five carcasses (5.0%) were diagnosed with ascites and four (4.0%) with cachexia. One carcass presented cachexia plus petechia. Samples of cellulitis had a yellowish (71.0%) or whitish (29.0%) hue, weighing between 2.0 and 18.0 g, with firm cartilaginous or adipose lesions. Macroscopic alterations plus avian cellulitis should be taken seriously since they may foreground carcass disposal. A similar study by Barros et al. (2013) on avian cellulitis in the same region

Rev. Bras. Saúde Prod. Anim., Salvador, v.19, n.4, p.371-380 out./dez., 2018 ISSN 1519 9940
http://dx.doi.org/10.1590/S1519-99402018000400002

revealed two carcasses with cachexia and one with pericarditis associated with carcasses with cellulitis. They weighed between 0.9 and 1.7 g, or rather, lower than cellulitis lesions in current study.

According to Instruction 210/1998 by the Ministry of Agriculture, Livestock and Food Supply (BRASIL, 1998), inflammatory processes, such as cellulitis, demand the condemnation of the diseased area. Moreover, carcass and viscera should be disposed of if the process is systemic. It may be suggested that cellulitis lesions should not be taken into account in the case of discussing the disposal or not of carcasses. The colisepticemia process exists even in small lesions and, therefore, a risk to public health (VIEIRA et al., 2014). The importance of health inspection should be underscored to identify and determine the disposal of carcasses.

The number of total coliforms and *E. coli* isolated from cellulitis was also determined. In fact, 96.0% of samples featured total coliforms, with a population ranging between 3.4 and 9.5 log CFU/g. The above is very high and is a concern for public health.

Several research works on total coliform counts in broilers have been undertaken. Hemmat et al. (2015) determined the population of total coliforms in broiler carcasses from markets in the town of Beni Suef, Egypt. The authors registered total coliform counts between <1 and 4.9 log CFU/g, lower than those in current study. This fact may have occurred due to samples retrieved from carcasses at the purchase end. They may have had a lower microbial load when compared to that in samples from subcutaneous tissues with cellulitis lesions.

Table 3 gives results on the frequency of *E. coli* and virulence genes *iss* and *iut*A in broiler cellulitis samples in nine aviaries from the Recôncavo of Bahia. *E. coli* was identified in 82.0% of samples, with a population between <1.0 and 9.0 log CFU/g. ANOVA test revealed no statistically significant difference between aviaries for *E. coli* populations (F = 1.44; Sig. = 0.189).

Table 3. Frequency of Escherichia coli and virulence genes iss and iutA in samples of broiler cellulitis in aviaries in the Recôncavo da Bahia, Brazil

Origin	Population*	Mean E. coli	*E. coli*	*iss*	*iut*A	n**
Aviary A	2.9-6.3 (±1.4)[a]	5.3	5/5	2/5	5/5	5
Aviary B	3.4-7.3 (±1.7)[a]	6.0	3/5	3/3	2/3	5
Aviary C	<1.0-9.0 (±2.9)[a]	4.8	30/39	26/30	30/30	39
Aviary D	<1.0-7.6 (±2.3)[a]	5.2	14/16	12/14	13/14	16
Aviary E	<1.0-8.1 (±3.6)[a]	4.6	4/6	4/4	4/4	6
Aviary F	<1.0-7.0 (±2.4)[a]	4.3	9/11	9/9	9/9	11
Aviary G	<1.0-7.4 (±2.3)[a]	5.5	7/8	7/7	7/7	8
Aviary H	7.1-7.6 (±0.2)[a]	7.4	4/4	4/4	4/4	4
Aviary I	7.0-9.0 (±0.4)[a]	7.7	6/6	6/6	6/6	6

*E. coli population in log CFU/g **n – number of cellulitis samples.
Mean – mean counts of Escherichia coli.
***Similar letters in the column Population do not show any statistically significant difference between results with ANOVA.

According to Mohamed et al. (2014), *E.coli* in conventional poultry breeding system is well-known and its high rates were detected by several authors, such as Barros et al. (2013) for Bahia and Vieira et al. (2014) for Rio de Janeiro. These authors isolated *E. coli* respectively in 82.5% and 96.0% of cellulitis lesions. This fact brings deep concern for the poultry industry. Microorganisms in cellulitis lesions is a serious matter since certain *E. coli* strains have a greater capacity to adhere to the tissue´s deep layers, which explains the high prevalence rates of the bacterium in cellulitis-affected stocks (LECLERC et al., 2003).

Further, broilers from contaminated aviaries may have their carcass infected by *E. coli*, including eatable viscera, such as the liver, the organ responsible for biotransformation, according to results by Silva et al. (2012). These researchers isolated thirty *Escherichia coli* strains in 62 samples of broiler liver retrieved from two abattoirs in the Recôncavo da Bahia, under the State Health Inspection Service. Coupled to the isolation of *E. coli*, research on virulence genes associated with the bacterium´s pathogenicity was undertaken especially APEC. Genes *iss* and *iut*A were identified in 89.0% (73/82) and 97.6% (80/82) of isolates, respectively.

Several authors, such as Jeong et al. (2012), have reported the high prevalence rate of gene *iss* amplification. The authors amplified the gene in 78.2% (79/101) of isolated colibacillosis strains from Korea. Moreover, Barbieri et al. (2013) detected *iss* gene in 78.5% (113/144) of isolates from serious cellulitis lesions in broilers from abattoirs of the northern region of the state of Paraná, Brazil.

Huja et al. (2015) underscored that gene *iss* is crucial for the survival of the microorganism in the host´s serum. Ewers et al. (2009) pointed out that serum resistance is an attribute that determines the strain´s virulence. Vandekerchove et al. (2005) insisted that, combined to several pathogenic genes, the gene *iss* prevalent for the development of colibacillosis, especially in outbreaks with high mortality rates.

Virulence gene *iss* may be associated with *E. coli* which is pathogenic in humans, as a study by Frömmel et al. (2013), developed in Germany, showed. The researchers investigated 20 isolates of uropathogenic *E. coli* (UPEC) and 19 isolates of intestinal *E. coli* of healthy people and detected gene *iss* in 45.0% and 26.0% of samples, respectively. Aerobactin Gene *iut*A was also detected in assays with pathogenic strains of *Escherichia coli*. Schouler et al. (2012) identified gene *iut*A in 82.7% (291/352) of pathogen strains retrieved from *Escherichia coli* isolates in France, Spain and Belgium.

Iron is also crucial for the survival of *Escherichia coli*, since it facilitates numberless cell activities, such as peroxide reduction, transport of electrons and biosynthesis of nucleotides. Since iron exists at low concentrations in extra-intestinal places of the infection, ExPEC strains developed several strategies for iron acquisition as from the host, among which aerobactin may be mentioned (HEINEMANN et al., 2008).

Genes *iss* and *iutA* were simultaneously amplified from *E. coli* isolates of cellulitis samples in 87.8 % (72/82) of *Escherichia coli* strains. The amplification of a single gene occurred in 1.2% (1/82) for gene *iss* and in 9.8 % (8/82) for gene *iut*A.

Results revealed that gene *iut*A was more prevalent than gene *iss* in the samples under analysis. Similar genotypic characteristics were registered in a research by Brito et al. (2003) who amplified genes *iut*A and *iss* in 92% (48/52) and 83% (43/52), respectively, of *E. coli* strains retrieved from broiler

carcasses with cellulitis in south Brazil. Contrasting results were obtained by Corrêa et al. (2013), who amplified genes *iut*A and *iss* respectively in 71.4% (20/28) and 92.8% (26/28) of samples collected in birds in Brazil. However, the authors researched psittacine birds rather than broilers.

The occurrence of genes *iss* and *iut*A in *E. coli* isolates from cellulitis samples is a serious risk to public health due to APEC´s pathogenic potential (BARROS et al. 2013). Further, Ewers et al. (2009) registered that APEC strains have a zoonotic potential risk due to their similarity with ExPEC strains that cause diseases in humans.

High population rates of total coliforms and *E. coli* in cellulitis samples pinpoint hygiene and sanitary faults in the production of broilers due to inadequate management in aviaries (JAENISCH et al., 2004). Intensification of hygiene in aviaries, including cleanness of toilettes, adequate change or management of feather bed between lots, care in storing and distribution of water and diet to broilers, the choice of suppliers of chickens and a good working vaccine program are highly recommended (MENDES & KOMIYAMA, 2011).

Cellulitis samples under analysis revealed high counts in total coliforms and *E. coli*, whilst virulence genes *iss* and *iut*A, APEC characteristics, occurred in all *E. coli* isolates in most samples. Consequently, due to the zoonotic feature of the pathotype, the introduction of Good Manufacturing Practices, Standard Procedures of Operational Hygiene and Hazard Analysis and Critical Control Points in poultry slaughterhouses should be a must to ensure the safety of the final product and prevent crossed contamination.

ACKNOWLEDGEMENTS

The authors would like to thank the Coordenação de Aperfeiçoamento de Nível Superior for the scholarship awarded. Thanks are also due to the abattoir which gave the authors permission to collect the samples, and to the Fundação Oswaldo Cruz (Fiocruz) of Rio de Janeiro for donating standard strains as positive control.

REFERENCES

ALMEIDA, T.J.O.; ASSIS, A.S.; MENDONÇA, M.; ROLIM, M.B.Q., Causas de condenação de carcaças de Gallus gallus domesticus em abatedouros frigoríficos sob Inspeção Federal no Nordeste do Brasil. **Medicina Veterinária (UFRPE)**, v.11, n.4, p.285-291, 2017.

ANDRADE, C.L.; FERREIRA, G.B.; FRANCO, R.M.; NASCIMENTO, E.R.; TORTELLY, R. Alterações patológicas e identificação da *Escherichia coli* como agente causal da celulite aviária em frangos de corte inspecionados em um matadouro de São Paulo. **Revista Brasileira de Ciências Veterinárias**, v.13, n.3, p.139-143, 2006.

BARBIERI, N.L.; OLIVEIRA, A.L.; TEJKOWSKI, T.M.; PAVANELO, D.A.; ROCHA, D.A.; MATTER, L.B.; CALLEGARI-JACQUES, S.M.; BRITO, B.G.; HORN, F. Genotypes and pathogenicity of cellulitis isolates reveal traits that modulate APEC virulence. **Plos One**, 2013. Available in: <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0072322>. Access in: 24 nov. 2016.

BARNES, H.J.; NOLAN, L.K.; VAILLANCOUT, J. Colibacillosis. In: Saif, Y.M. (Ed.). **Disease of Poultry**. Ames: Blackwell Publishing, 2008. p.691-737.

BARROS, L.S.S.; SILVA, R.M.; SILVA, I.M.; BALIZA, M.D.; VIRGÍLIO, F.F. *Escherichiacoli* from cellulitis lesions in broilers. **Food Measure**, v.7, p.40-45, 2013.

BRASIL. Portaria nº 210, de 26 de novembro de 1998. Aprova o Regulamento Técnico da Inspeção Tecnológica e Higiênico Sanitária da Carne de Aves. **Diário Oficial da República Federativa do Brasil**, Brasília, DF, 1998.

BRITO, B.G.; GAZIRI, L.C.J.; VIDOTTO, M.C. Virulence Factors and Clonal Relationships among Escherichia coli Strains Isolated from Broiler Chickens with Cellulitis. **Infection and Immunity**, v.71, n.7, p.4175–4177, 2003.

CHOUIKHA, I.; BREE, A.; MOULIN-SCHOULEUR, M.; GILOT, P.; GERMON, P. Differential survey of aerobic bacteria and fungi in the feces of healthy psittacine birds. **Avian Disease**, v.32, p.46-52, 2008.

CORRÊA, I.M.O.; FLORES, F.; SCHNEIDERS, G.H.; PEREIRA, L.Q.; BRITO, B.G.; LOVATO, M. Detecção de fatores de virulência de *Escherichia coli* e análise de *Salmonella* spp. em psitacídeos. **Pesquisa Veterinária Brasileira**, v.33, n.2, p.241-246, 2013.

EMBRAPA. **Custos/Frangos de Corte Junho 2018**. Available in: <https://www.embrapa.br/suinos-e-aves/cias/custos/frango-uf>. Access in: 1 ago. 2018.

EWERS, C.; ANTÃO, E.M.; DIEHL, I.; PHILIPP, H.C.; WIELER, L.H. Intestine and environment of the chicken as reservoirs for extraintestinal pathogenic *Escherichia coli* strains with zoonotic potential. **Applied and Environmental Microbiology**, v.75, n.1, p.184-192, 2009.

FERREIRA, T.Z.F.; SESTERHENN, R.; KINDLEIN, L. Principais causas de condenação em matadouro-frigoríficos de frangos de corte sob Inspeção Estadual e Federal no Estado do Rio Grande do Sul. In: CONGRESSO BRASILEIRO DE AVICULTURA, 22., 2011, São Paulo. **Anais...**(São Paulo, 2011.

FRÖMMEL, U.; LEHMANN,W.; RÖDIGER, S.; BÖHM,A.; NITSCHKE, J.; WEINREICH, J.; GROB, J.; ROGGENBUCK, D.; OLAF ZINKE, O.; ANSORGE, H.; VOGEL, S.; KLEMM, P.; WEX, T.; SCHRÖDER, C.; WIELER, L.H.; SCHIERACKA, P. Adhesion of Human and Animal *Escherichia coli* Strains in Association with Their Virulence-Associated Genes and Phylogenetic Origins. **Applied and Environmental Microbiology**, v.79, n.19, p.5814-5829, 2013.

HEINEMANN, I.U.; JAHN, M.; JAHN, D. The biochemistry of heme biosynthesis. **Archives Biochemistry Biophysics**, v.474, n.2, p.238–251, 2008.

HEMMAT, M.I.; REHAM, A.A.; MOHAMMED, A.E-S.; SALWA, M.H. Bacteriological evaluation of freshly slaughtered chicken carcasses. **Benha Veterinary Medical Journal**, v.28, n.2, p.74-82, 2015.

HUJA, S.; OREN, Y.;TROST, E.; BRZUSZKIEWICZ, E.; BIRAN, D.; BLOM, J.; GOESMANN, A.; GOTTSCHALK, G.; HACKER, J.; RON, E.Z.; DOBRINDT, U.Genomic Avenue to Avian Colisepticemia. **Mbio**, v.6, p.1-13, 2015.

JAENISCH, F.R.F.;COLDEBELLA, A.; MACHADO, H.G.P.; ABREU, P.G.; ABREU, V.M.N.; SANTIAGO, V. **Importância da higienização na produção avícola**. Concórdia, SC: Embrapa Suínos e Aves, 2004. 5p. (Comunicado Técnico, 363). Available in: <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/85826/1/DCOT-363.pdf>. Access in: 31 ago. 2018.

JEONG, Y.W.; KIM, T.E.; KIM, J.H.; KWON, H.J. Pathotyping avian pathogenic *Escherichia* coli strains in Korea. **Journal of Veterinary Science**, v.13, n.2, p.145-152, 2012.

JOHNSON, T.J.; WANNEMUEHLER, Y.; DOETKOTT, C.; JOHNSON, S.J.; ROSENBERGER, S.C.; NOLAN, L.K. Identification of Minimal Predictors of Avian Pathogenic Escherichia coli Virulence for Use as a Rapid Diagnostic Tool. **Journal of Clinical Microbiology**, v.46, n.12, p.3987–3996, 2008.

KAPER, J.B.; NATARO, J.P.; MOBLEY, H.L.T. Pathogenic *Escherichia coli*. **Nature**, v.2, p.123-140, 2004.

KNÖBL, T.; MORENO, A.M.; PAIXÃO, R.; GOMES, T.A.T.; APARECIDA, M.; VIEIRA, M.; LEITE, D.S.; BLANCO, J.E.; FERREIRA, A.J.P. Prevalence of Avian Pathogenic *Escherichia coli* (APEC) Clone Harboring *sfa* Gene in Brazil. **The Scientific World Journal**, v.2012, p.1-7, 2012.

LECLERC, B.; FAIRBROTHER, J.M.; BOULIANNE, M.; MESSIER, S. Evaluation of the adhesive capacity of *Escherichia coli* isolates associated with avian cellulitis. **Avian Diseases**, v.47, p.21-31, 2003.

MAPA. Ministério da Agricultura, Pecuária e Abastecimento. Serviço de Inspeção Federal (SIF): **Relatório Anual 2016**.11p. Available in: <file:///C:/Users/Cliente/Downloads/RELAT%C3%93RIO%20DE%20GEST%C3%83O%20SIF%202016.pdf>. Access in: 1 ago.2018.

MENDES, A.A.; KOMIYAMA, C.M. Estratégias de manejo de frangos de corte visando qualidade de carcaça e carne. **Revista Brasileira de Zootecnia**, v.40, p.352-357, 2011.

MOHAMED, M.A.; SHEHATA, M.A.; RAFEEK, E. Virulence Genes Content and Antimicrobial Resistance in *Escherichia coli* from Broiler Chickens. **Veterinary Medicine International**, v.2014, p.1-6, 2014.

PITOUT, J.D.D. Extraintestinal pathogenic *Escherichia coli*: a combination of virulence with antibiotic resistance. **Frontiers in Microbiology**, v.3, p.1-7, 2012.

ROCHA, A.C.G.P.; ROCHA, S.L.S.; LIMA-ROSA, C.A.V.; SOUZA, G.F.; MORAES, H.L.S.; SALLE, F.O.; MORAES, L.B.; SALLE, C.T.P. Genes associated with pathogenicity of avian *Escherichia coli* (APEC) isolated from respiratory cases of poultry. **Pesquisa Veterinária Brasileira**, v.28, n.3, p.183-186, mar, 2008.

SCHOULER, C.A.; SCHAEFFER, B.; BRÉE, A.; MORA, A.; DAHBI, G.; BIET, F.; OSWALD, E.; MAINIL, J.; BLANCO, J.; MOULIN-SCHOULEUR, M. Diagnostic Strategy for Identifying Avian Pathogenic *Escherichia coli* Based on Four Patterns of Virulence Genes. **Journal of Clinical Microbiology**, v. 50, n. 5, p.1673–1678, fev, 2012.

SILVA, N.; JUNQUEIRA, V.C.A.; SILVEIRA, N.F.A.; TANIWAKI, M.H.; SANTOS, F.R.S. dos; GOMES, R.A.R.; OKAZAKI, M.M. **Manual de métodos de análise microbiológica de alimentos**, 3. ed. São Paulo: Livraria Varela, 2007.

SILVA, I.M.M.; BALIZA, M.;SANTOS, M.P.; REBOUÇAS, L.T.; ROCHA, É.V.S;SANTOS,.V.A.; SILVA, R.M.; EVÊNCIO-NETO, J., Presença de *Escherichia coli* em fígados de frangos provenientes de matadouros avícolas. **Revista Brasileira Saúde Produção Animal,** Salvador, v.13, n.3, p.694-700 jul/set, 2012.

VANDEKERCHOVE, D.; VANDEMAELE, F.; ADRIAENSES, C.; ZALESKA, M.; HERNALSTEENS, J.P.; BAETS, L. De; BUTAYE, P.; IMMERSEEL. F.V.; WATTIAU, P.; LAEVENS, H.; MAST, J.; GODDEERIS, B.; PASMANS, F. Virulence-associated traits in avian *Escherichia coli*:Comparison between isolates from colibacillosis-affectedand clinically healthy layer flocks. **Veterinary Microbiology**, Amsterdam, v.108, n.1-2, p.75-87, 2005.

VIEIRA, T.B., PEREIRA, V.L.A.; ROBSON MAIA FRANCO, R.M.; NASCIMENTO, E.R. do; SILVA, R.C.F.; TORTELLY, R. Potencial patogênico e caráter séptico de *Escherichia coli* pela identificação dos fatores de virulência *iss* e *fel*A em celulite e miúdos de frangos sob Inspeção Sanitária. **Revista Brasileira de Medicina Veterinária**, v.36, n.2, p.144-152, abr-jun, 2014.

Receipt date: 22/06/2018
Approval date: 16/09/2018