DIARRHEAGENIC Escherichia coli IN RAW MILK, WATER, AND CATTLE FECES IN NON-TECHNIFIED DAIRY FARMS

Escherichia coli DIARREIOGÊNICA EM LEITE CRU, ÁGUA E FEZES BOVINAS DE PROPRIEDADES LEITEIRAS NÃO TECNIFICADAS

Laryssa F. Ribeiro¹ ORCID – http://orcid.org/0000-0003-1943-7897
Mayhara M. C. Barbosa² ORCID – http://orcid.org/0000-0002-1970-8266
Fernanda R. Pinto³ ORCID – http://orcid.org/0000-0002-0794-1984
Leticia F. Lavezzo⁴ ORCID – http://orcid.org/0000-0002-1461-6362
Gabriel A. M. Rossi⁵ ORCID – http://orcid.org/0000-0001-7967-7628
Henrique M. S. Almeida⁴ ORCID – http://orcid.org/0000-0002-7631-4271
Luiz A. Amaral⁶ ORCID – http://orcid.org/0000-0002-5297-4961

¹Centro Universitário Mário Palmério (UNIFUCAMP), Monte Carmelo, MG, Brazil
²Instituto Federal de Educação, Ciência e Tecnologia do Ceará (IFCE), Quixadá, CE, Brazil.
³Universidade Federal de Pelotas (UFPEL), Pelotas, RS, Brazil.
⁴Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, Brazil.
⁵Centro Universitário Central Paulista (UNICEP), São Carlos, SP, Brazil.
⁶Correspondent author - laryssaribeiro84@gmail.com

Abstract
This study focused on detecting diarrheagenic Escherichia coli, enteropathogenic E. coli (EPEC), Shiga-toxin-producing E. coli (STEC), enterohemorrhagic E. coli (EHEC or STEC:EPEC), enterotoxigenic E. coli (ETEC), and enteraggregative E. coli (EAEC) in raw milk, water, and cattle feces sampled from non-technified dairy farms located in the northeastern São Paulo State, Brazil. Thirty-six water samples were collected at different points, namely, water wells (8 samples), water intended for human consumption (8 samples), water from milking parlor (8 samples), and water intended for animal consumption (7 samples), headwaters (1 sample), rivers (3 samples), and reservoirs (1 sample). Three raw milk samples were taken directly from bulk tanks in each farm, totaling 24 samples. Feces samples were collected using rectal swabs from 160 bovines (20 animals per farm). E. coli was detected in 128 feces samples (80%), 16 raw milk samples (66.67%), and 20 water samples (55.56%). STEC (26 samples, 16.25%), EPEC (10 samples, 6.25%), STEC: EPEC (5 samples, 3.13%), and STEC: ETEC (1 sample, 0.63%) were the most prevalent strains detected in samples from cattle feces. EPEC, STEC, and STEC: EPEC strains were detected in 4.17% (1 sample), 16.67% (4 samples), and 4.17% (1 sample) of raw milk samples, respectively. STEC strains were detected in water used in the milking parlor, while no EAEC strain was detected. As a conclusion, cattle feces are important contamination sources of pathogenic E. coli in non-technified dairy farms and, consequently, cross-contamination among feces, water, and/or raw milk can occur. The use of quality water and hygienic practices during milking are recommended to avoid contamination since pathogens can be transmitted to humans via raw milk or raw milk cheese ingestion.

Keywords: EAEC, ETEC, EPEC, STEC, public health

Resumo
Este estudo teve como objetivo realizar a detecção de Escherichia coli diarreiogênicas (EPEC, STEC,
ETEC e EAEC) em leite, água e fezes bovinas em pequenas propriedades leiteiras localizadas na Região Nordeste do Estado de São Paulo, Brasil. *E. coli* foi detectada em amostras obtidas de fezes (80%), leite cru (66,67%) e água (55,56%). STEC, EPEC, STEC:EPEC e STEC:ETEC foram as cepas mais prevalentes em amostras de fezes bovinas, respectivamente. Em relação ao leite cru, cepas de EPEC, STEC e STEC:EPEC foram detectadas em 4,17%, 16,67% e 4,17% das amostras, respectivamente. Ainda, detectou-se STEC na amostra de água utilizada na sala de ordenha, enquanto EAEC não foi detectada em nenhuma amostra. Conclui-se que fezes de bovinos é uma importante fonte de contaminação de *E. coli* patogênicas em propriedades leiteiras e podem consequentemente contaminar o leite cru e água. A importância da qualidade da água e da adoção efetiva de práticas higiénicas durante a obtenção do leite para evitar a contaminação são recomendadas devido à possibilidade de transmissão de microorganismos patogênicos a seres humanos devido a ingestão de leite cru ou queijos produzidos a partir de leite não pasteurizado.

**Palavras-chave:** EAEC, ETEC, EPEC, STEC, saúde pública

Received on: June, 17th, 2017.
Accepted on: June, 10th, 2019.

**Introduction**

Waterborne disease outbreaks are major challenges for public health worldwide\(^{(1)}\). Developing countries are more susceptible to such epidemics due to their poorer sanitation control and water quality than developed countries, which are most affected by emerging water-treatment resistant pathogens, technical failures, or even inadequate control measures\(^{(2)}\).

The presence of pathogenic *Escherichia coli* strains is an important indicator of public health hazards. These bacteria are divided into six diarrheagenic groups: enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC)\(^{(3)}\). EPEC is a common cause of childhood diarrhea in developing countries due to its ability to adhere to the intestinal mucosa, resulting in nutrient malabsorption\(^{(4)}\). The formation of A/E lesions causes a decrease in the absorption capacity of the intestinal mucosa, which leads to a rupture of the electrolyte balance and hence to diarrhea\(^{(5)}\).Diarrhea caused by EPEC probably results from multiple mechanisms, including ion secretion, increased intestinal permeability, intestinal inflammation, and loss of absorption surface\(^{(6)}\). STEC can produce toxins that cause illnesses if ingested, such as hemorrhagic colitis and hemolytic uremic syndrome (HUS)\(^{(4)}\). These toxins, also known as verotoxins, produce a distinct and irreversible cytotoxic effect on Vero cells\(^{(7)}\).

ETEC toxins induce fluid secretion, causing childhood diarrhea. Their outbreaks are frequently associated with poor handling or poor water quality and hence contaminated food consumption\(^{(4)}\). These microorganisms colonize intestinal mucosa surface and release LT (thermolabile) and ST (thermostable) enterotoxins, causing diarrhea\(^{(8)}\). In addition, they express surface adhesins which allow the colonization of intestinal epithelial cells\(^{(8)}\). EAEC is also a cause of persistent diarrhea in children and can lead to chronic inflammation. EAEC pathogenesis is determined by the organism’s ability to adhere to intestinal cells, produce enterotoxins and cytotoxins, and induce inflammation. Dispersin, which is antigenic antiaggregatory protein encoded by the aap gene in the pAA plasmid and regulated by aggR, modulates fimbrial adhesion and facilitates penetration of the microorganism through intestinal mucus by binding to lipopolysaccharide and altering the electrostatic properties of
the EAEC outer membrane surface (10).

Some pathogenic E. coli strains are adapted to survive in aquatic environments and associated with high-mortality in waterborne outbreaks. Diarrheagenic strains were detected in some water sources such as raw water, surface water, animal/human wastewater, irrigation water, swimming water, and municipal water contaminated with feces. Thus, preventing the contamination of public water supplies and proper disinfection methods such as chlorine and ultraviolet light treatments are recommended (11).

Raw milk and dairy products are usually associated with diarrheagenic E. coli contamination (12). Some diarrheagenic E. coli outbreaks due to the consumption of unpasteurized milk are described in the literature (13, 14). Furthermore, high prevalence of potentially pathogenic E. coli strains in raw milk and raw milk cheeses is reported in several countries worldwide (15).

Contamination sources of diarrheagenic E. coli in dairy products are poorly treated water or even direct contact with cattle feces. Vicente et al. (9) detected a prevalence of 100% on dairy herds and detected at least one animal containing STEC strains in feces. These authors highlighted bovines as important sources of environmental contamination, mainly during the rainy season. Furthermore, bovines are considered as the most important reservoirs of STEC and EPEC for humans, and transmission occurs via ingestion of contaminated food or water (16).

The detection of virulence factors such as stx1 (stx1A), stx2 (stx2A), intimin (eae), transcriptional activator AggR (aggR), heat-labile toxin type 1 (eltB), heat-stable enterotoxin (estA, estB), and plasmid pCVD432 (aatA) in E. coli isolates has been recommended to evaluate potential risk for public health (17 - 22). Overall, this study focused on detecting the presence of diarrheagenic E. coli (EPEC, STEC, ETEC, and EAEC) in samples of milk, water, and cattle feces from dairy farms in the northeastern São Paulo State, Brazil.

**Materials and methods**

Sampling was performed in eight non-technified dairy farms located in Jaboticabal municipality, São Paulo State (Brazil), between 2010 and 2011. And by non-technified dairy farms, we mean properties with a maximum cattle population of 20 cross-breed animals, maximum milk production of 300 liters, no attending veterinarian, no sanitary control, and family production with family employees. Seven farms used hand milking and one mechanical milking. These farms have a daily production of about 250-260 liters of raw milk. Milking is performed twice a day (in the morning and afternoon).

Thirty-six water samples were collected at different sampling points in the farms, according to their own rearing conditions, such as from wells (8 samples), milking parlor (8 samples), headwater (1 sample), river (3 samples), and reservoir (1 sample), and water intended for human (8 samples) and animal (7 samples) consumptions. Three raw milk samples were collected directly from bulk tanks in each farm (at the beginning, middle, and end of milking), totaling 24 samples. Water and milk samples were taken using sterilized glasses with a maximum capacity of 500 mL (23). Feces samples were collected using rectal swabs from 160 bovines (20 animals per farm) (24).

*E. coli* was isolated according to Vicente et al. (9). Three to five colonies were picked from each plate for biochemical identification (25). DNA from all isolates was extracted (26) and examined for quality and integrity by running on an agarose gel.

To detect diarrheagenic E. coli strains, *E. coli* isolates were screened for virulence genes, using three
conventional multiplex PCR with a set of specific primers for amplification. A multiplex PCR was performed to detect EPEC and STEC. For EPEC, it was detected eae gene, and for STEC genes stx1 and stx2. Another multiplex PCR was done for ETEC genes, and the genes eltB, estA, and estB were detected. Moreover, a third multiplex PCR was performed for EAEC, and the genes aggR and aatA were detected. Table 1 describes the genes, sequences, sizes (bp), annealing temperatures (°C), and strain positive controls.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Size (bp)</th>
<th>Annealing temperature (°C)</th>
<th>Positive control</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>stx1</td>
<td>AGAGCGATGTTACGGTTTG&lt;br&gt;TGCCCCCAGATGGATG&lt;br&gt;TGGGTTTCTTCGGTATC&lt;br&gt;GACATTCTGGTGAACCTCTCT&lt;br&gt;AGGCTTCGTCCACAGTT&lt;br&gt;CCACGTCAACCAGAGA</td>
<td>388</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>stx2</td>
<td>807&lt;br&gt;55</td>
<td>EDL933</td>
<td>(17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eae</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eltB</td>
<td>TTA CGG GTG TAC TAT CCT CTC TA&lt;br&gt;GTT CTC GTG CAG ATA TGT GAT TC&lt;br&gt;TCC CCT CTT TTA GTC AGT CAA CTG&lt;br&gt;GCA CAG GCA GGA TTA CAA CAA AGT</td>
<td>275</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>estA</td>
<td>163&lt;br&gt;55</td>
<td>EeL7805 (University of Montreal)</td>
<td>(20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>estB</td>
<td>368</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aggR</td>
<td>GCA ATA AGG TGT AGG TGA T&lt;br&gt;GCC TGC AGT GAG AAA TGG AC</td>
<td>457&lt;br&gt;57</td>
<td>O42 (São Paulo State Federal University)</td>
<td>(18)</td>
<td></td>
</tr>
<tr>
<td>aatA</td>
<td>378</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results and discussion

Out of the 220 samples collected, *E. coli* was isolated from 164 samples (74.55%), as shown in Table 2. This bacterium was detected with higher prevalence in samples of feces (80.00%), raw milk (66.67%), and water sources (55.56%). It was detected in samples of water for human consumption (62.50%), demonstrating a possible risk to public health due to the transmission of waterborne diseases. Furthermore, the presence of STEC in water from water milking parlor demonstrates a risk of raw milk contamination with major pathogens during milking if hygienic practices are not properly carried out (27).

Five hundred and thirty-one *E. coli* isolates were obtained: 67 from water, 392 from feces, and 72 from milk samples. All farms (100%) had at least one potentially pathogenic isolate (Table 3). An atypical pathotype containing stx1: sta genes was detected in one bovine feces sample.

Ingestion of raw milk or raw milk cheeses can pose risks to public health (15) since we detected EPEC (4.17%), STEC (16.67%), and EHEC (4.17%) strains in raw milk. Furthermore, pathogenic strains were detected in cattle feces, highlighting bovine as an important reservoir of such strains, thus being a potential source of contamination of milk and other dairy products (9, 28). There was a great prevalence of STEC, EPEC, EHEC, and STEC: ETEC in cattle feces.
Diarrheagenic *Escherichia coli* in raw milk, water, and cattle feces in non-technified dairy farms

Table 2. Number of samples collected, positive for *E. coli*, and positive for diarrheagenic *E. coli* (EPEC, STEC, EHEC, and STEC: ETEC pathotypes) from different sources in non-technified dairy farms located in the municipality of Jaboticabal, São Paulo State (Brazil), between 2010 and 2011

<table>
<thead>
<tr>
<th>Source</th>
<th>Total sample collected (n)</th>
<th><em>E. coli</em> positive (%)</th>
<th>EPEC positive (%)</th>
<th>STEC positive (%)</th>
<th>STEC: EPEC positive (%)</th>
<th>STEC: ETEC positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water wells</td>
<td>8</td>
<td>4 (50.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Water from milking parlor</td>
<td>8</td>
<td>3 (37.50)</td>
<td>0 (0.00)</td>
<td>1 (12.50)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Animal consumption</td>
<td>7</td>
<td>5 (71.43)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Headwater</td>
<td>1</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>River</td>
<td>3</td>
<td>3 (100.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Reservoir</td>
<td>1</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>36</td>
<td>20 (55.56)</td>
<td>0 (0.00)</td>
<td>1 (2.78)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Raw milk</td>
<td>24</td>
<td>16 (66.67)</td>
<td>1 (4.17)</td>
<td>4 (16.67)</td>
<td>1 (4.17)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Feces</td>
<td>160</td>
<td>128 (80.00)</td>
<td>10 (6.25)</td>
<td>25 (15.62)</td>
<td>5 (3.13)</td>
<td>1 (0.62)</td>
</tr>
<tr>
<td>Total</td>
<td>220</td>
<td>164 (74.55)</td>
<td>11 (5.00)</td>
<td>31 (14.10)</td>
<td>6 (2.73)</td>
<td>1 (0.45)</td>
</tr>
</tbody>
</table>

Table 3. Diarrheagenic *E. coli* isolated from different sources, profiles, pathotypes, and dairy farms located in the municipality of Jaboticabal, São Paulo State (Brazil), between 2010 and 2011

<table>
<thead>
<tr>
<th>Source</th>
<th>Profile</th>
<th>Pathotype</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water from milk room</td>
<td>str2</td>
<td>STEC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (0.98)</td>
</tr>
<tr>
<td>Milk</td>
<td>str2</td>
<td>STEC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (1.59)</td>
</tr>
<tr>
<td></td>
<td>str1:str2</td>
<td>STEC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11 (10.78)</td>
</tr>
<tr>
<td></td>
<td>str1:stx2</td>
<td>EHEC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (0.98)</td>
</tr>
<tr>
<td></td>
<td>stxl</td>
<td>STEC</td>
<td>2 (1.68)</td>
<td>1 (2.2)</td>
<td>1 (2.2)</td>
<td>3 (2.94)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>stx2</td>
<td>STEC</td>
<td>7 (5.88)</td>
<td>3 (7.88)</td>
<td>5 (6.17)</td>
<td>2 (4.26)</td>
<td>1 (1.59)</td>
<td>6 (5.88)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>eae</td>
<td>EPEC</td>
<td>1 (2.44)</td>
<td>2 (4.74)</td>
<td>6 (8.52)</td>
<td>4 (3.92)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>stx1:stx2</td>
<td>STEC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10 (9.80)</td>
</tr>
<tr>
<td></td>
<td>stx1:stx2:stx1</td>
<td>EHEC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5 (4.90)</td>
</tr>
<tr>
<td></td>
<td>stx1:stx2:stx1</td>
<td>EHEC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (1.23)</td>
</tr>
<tr>
<td></td>
<td>stx1:stx2:stx1</td>
<td>EHEC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (2.44)</td>
</tr>
<tr>
<td>Total number of pathogenic isolates / Total number of isolates in each farm (%)</td>
<td>9/119 (7.56)</td>
<td>4/41 (9.75)</td>
<td>1/40 (2.5)</td>
<td>3/38 (7.89)</td>
<td>9/81 (11.11)</td>
<td>2/47 (4.26)</td>
<td>8/63 (12.70)</td>
<td>41/102 (40.20)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*E. coli* strains belonging to EAEC group were isolated from food and stool samples of diarrheagenic patients in Colombia, demonstrating the importance of this foodborne pathogen in developing countries(29). No EAEC strains were detected in the samples evaluated in the present study, which could be explained by the fact that animals are not commonly reservoirs for EAEC strains(30).

Similar high frequencies of diarrheagenic strains have also been reported in other Brazilian dairy farms. Sandrini et al.(31) detected the presence of STEC strains in 95% of dairy farms evaluated in the municipality of Pelotas, Rio Grande do Sul State (Brazil). Moreover, Irino et al.(32) observed STEC isolation rates ranging from 3.8 to 84.6% in six dairy farms of São Paulo State (Brazil).

Farrokh et al.(28) stated that milk and other dairy products are important contamination sources of STEC strains causative of outbreaks, as demonstrated in our study. Pathogen surveillance is a risk management measure for public health protection(33), in which it is highlighted the need for long-term strategies to ensure safe dairy products(34). Also, there has been reported a regular consumption of raw milk and other dairy products in Brazil(35).

Cienc. anim. bras., Goiânia, v.20, 1-9, e-47449, 2019
In this study, the seven farms (1, 2, 3, 4, 5, 6, and 7) showed low percentages of pathogenic *E. coli* strains, but only farm 8 had 40.20% of the total isolates (41/102) (Table 3). This farm had a high occurrence of isolates containing the gene stx2 in milk and feces samples, and one isolate in water samples. In this property, there might have been cross-contamination among animal feces, milking parlor water, and raw milk due to high environmental contamination, demonstrating the need for hygienic practices\(^{36}\).

Cattle feces are a common source of pathogenic *E. coli* (STEC and EPEC) as demonstrated in the present study. Vicente et al.\(^9\) evaluated the frequency of stx1, stx2 and eae genes in dairy farms and observed high prevalence (72.16%) of the gene stx in isolates from feces samples of asymptomatic animals. Thus, teats and udders can be contaminated with infected feces and hence contaminate raw milk in case of unsatisfactory hygienic conditions during milking\(^{37}\) or by mastitic milk\(^{38}\).

Raw milk from bulk tanks can pose a risk of transmission of STEC strains to consumers\(^{39}\), just as other dairy products, which could threaten public health if contaminated with *E. coli*. Outbreaks can be caused by pathogenic *E. coli* when consuming cheese made of raw milk\(^{13, 14, 40, 41}\).

Paneto et al.\(^{42}\) evaluated 50 samples of raw cheeses from supermarkets in the Brazilian midwestern region and observed that 96% of them were contaminated with *E. coli*, posing a risk to the local population. Other authors also observed a higher risk of *E. coli* infection in areas where consumption of unpasteurized dairy products is common, such as the state of São Paulo\(^{43}\), highlighting the importance of pasteurizing milk to assure public health\(^{44}\).

**Conclusion**

Cattle feces were the most important sources of pathogenic *Escherichia coli* in the studied dairy farms and hence could contaminate raw milk and water. Therefore, water quality and effective hygienic practices during milking are crucial to avoid raw milk contamination. These conditions should be considered owing to the possibility of transmission of pathogenic microorganisms to humans via ingestion of raw milk or its derived cheeses.

**References**


24 Cerqueira AMF, Guth BEC, Joaquim RM, Andrade JRC. High occurrence of Shiga toxin-producing Escherichia coli (STEC) in healthy cattle in Rio de Janeiro State, Brazil. Veterinary Microbiology. 1999; 70, 111-121.


27 Martin A, Beutin L. Characteristics of Shiga toxin-producing Escherichia coli from meat and milk products of different origins and association with food producing animals as main contamination sources. International Journal of Food Microbiology. 2011; 146:99-104.


36 Ferreira MRA, Stella AE, Freitas-Filho EG, Silva TS, Nascimento KA, Pinto JFN, Dias M, Moreira CN. Distribution of the stx1 and stx2 genes in *Escherichia coli* isolated from milk cattle according to season, age and production scale in southwestern region of Goiás, Brazil. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2018; 70(6):1807-1813.


