Outbreak of multidrug resistant Salmonella Typhimurium in calves at a veterinary hospital in Brazil

Carolina Pantuzza Ramos¹, Leticia Camêlo Vespasiano¹, Isabela Oliveira Melo¹, Rafael Gariglio Clark Xavier¹, Carlos Augusto Gomes Leal¹, Elias Jorge Facury Filho¹, Antônio Ultimo de Carvalho¹, Francisco Carlos Faria Lobato¹, Rodrigo Otávio Silveira Silva*¹

¹Universidade Federal de Minas Gerais (UFMG), Escola de Veterinária, 31270-901, Belo Horizonte, MG, Brazil. E-mail: rodrigo.otaviosilva@gmail.com.

*Corresponding author.

ABSTRACT: The present study aimed to describe and characterize a nosocomial outbreak caused by a multidrug resistant Salmonella Typhimurium in hospitalized calves at a veterinary medical teaching hospital from Brazil. Sixty-three (96.9%) calves showed lethargy, hyperthermia and profuse diarrhea and despite treatment, 26 (41.2%) animals died. Five animals were necropsied and stool samples of six calves were collected. The isolated strains were subjected to antimicrobial susceptibility test by disc-diffusion method and were fingerprinted by ERIC-PCR. Macroscopic lesions suggestive of salmonellosis, such as fibrinonecrotic enteritis and hepatosplenomegaly were observed. Salmonellosis was confirmed by isolation of S. Typhimurium from stool samples and organs from seven affected animals. Six out of seven isolates of S. Typhimurium, exhibited 100% of similarity at ERIC-PCR, suggesting occurrence of nosocomial transmission of S. Typhimurium among the hospitalized calves. All but one S. Typhimurium isolated were resistant to marbofloxacin, enrofloxacin, florfenicol, oxytetracycline and trimethoprim/sulfamethoxazole, antimicrobial agents largely used for humans and animal treatment. This is the first study of a nosocomial outbreak of multidrug resistant Salmonella Typhimurium in a veterinary hospital in Brazil and highlighted the need for preventive measures to reduce the risks for inpatients and humans in contact with animals.

Key words: salmonellosis, bovine, nosocomial, zoonosis, multiresistant.

INTRODUCTION

Salmonellosis is a zoonotic disease of worldwide distribution and responsible for great economic losses in animal production (SANCHEZ et al., 2002), being also reported as a nosocomial pathogen at veterinary hospitals (CUMMINGS et al., 2010; HOLSCBACH, PEEK, 2017). Among the several Salmonella enterica serovars associated with salmonellosis in cattle, S. Typhimurium stands out for its broad host range and its zoonotic transmission (MOHLER et al., 2009). In calves, it is commonly associated with enteric disease, which less frequently, could spread to other organs depending on the strain’ virulence factors and the host immunity (MOHLER et al., 2009; HOLSCBACH, PEEK, 2017).

Outbreaks of salmonellosis in hospitalized animals are frequently associated with nosocomial transmission.
transmission (CUMMINGS et al., 2010). It is known that some animals can shed *S. Typhimurium* intermittently for a variable period, even in the absence of clinical signs (HOLSCHBACH, PEEK, 2017). This fecal shedding can increase during hospitalization due to the presence of some risk factors, including antimicrobial therapy (CUMMINGS et al., 2009). In addition, *Salmonella* spp. can easily disseminate in the hospital environment, infecting other susceptible individuals and then eventually leading to nosocomial outbreaks (BURGESS et al., 2004; DUNOWSKA et al., 2007; CUMMINGS et al., 2010). There are few studies on *Salmonella* infections in hospitalized calves and, so far, all reports are restricted to American and Canadian veterinary hospitals (IKEDA et al., 1986; RA V ARY et al., 1998; CUMMINGS et al., 2009; CUMMINGS et al., 2010). The present study aimed to describe and characterize, for the first time, a nosocomial outbreak of multidrug resistant isolates of *S. Typhimurium* in hospitalized calves at a veterinary medical teaching hospital in Brazil. The isolated strains were evaluated for antimicrobial susceptibility by disc-diffusion method and were fingerprinted by ERIC-PCR.

**MATERIALS AND METHODS**

The outbreak

From November 2016 to January 2017, 65 calves with 1-to-3 days old were admitted to the Veterinary Hospital of the Universidade Federal de Minas Gerais. These animals were all acquired from the same dairy farm located in metropolitan area of Belo Horizonte (Minas Gerais – Brazil) and would be raised until 30 days old then would be part of a clinical experiment with *Anaplasma marginale* infection. Confined in a cattle shed, the animals were fed with hay, concentrate and milk replacer and would be daily examined until the clinical experiment.

The first group of calves (n=18) arrived in November 2016. Three to four days after admission, the animals exhibited lethargy, hyperthermia and profuse diarrhea. These animals received oral fluid therapy and antimicrobial therapy with florfenicol, followed by cefotiofur, penicillin, marbofloxacin or trimethoprim/sulfamethoxazole. Despite the treatment, four animals (22.2%) died. Similar to this first group, calves that arrived in December 2016 (n=26) and January 2017 (n=21) developed lethargy, hyperthermia and profuse diarrhea three to four days after admission. The same protocol of treatment was prescribed but, once no improvement of their clinical status was seen, antimicrobial therapy was changed to enrofloxacin. Eleven animals died in the months of December (42.3%) and also in January (52.3%). During the outbreak, a total of 63 (96.9%) animals were clinically affected, of which 26 (41.2%) died despite the treatment.

Calves that died during the outbreak were submitted to post mortem examination. Fragments of the liver, spleen, lungs and mesenteric lymph nodes from five animals were collected for histopathological analysis and bacterial culture. Biliary and intestinal contents were also collected from these animals. In addition, stool samples from other live animals were collected from six calves during the outbreak. All samples were stored at 4°C until processing in the Bacteriology and Research Laboratory at the School of Veterinary, Universidade Federal de Minas Gerais.

Isolation and characterization of *Salmonella* spp.

Specimens collected during post mortem examination were submitted to isolation of *Salmonella* spp. as previously described (RAMOS et al., 2018). Identification of *Salmonella* genus were performed by PCR (KWANG et al., 1996) and *Salmonella* species and subspecies were identified according to LE MINOR & POPPOFF (1987). The identification of the specific serovar was performed according to antigenic characterization reported by GRIMONT & WEILL (2007) at the Brazilian National Reference Laboratory of Enterobacteriaceae of Oswaldo Cruz Foundation (FIOCRUZ), Brazilian Ministry of Health. In addition, stool samples and intestinal content were tested for the most common pathogens in neonatal calves (HELLER & CHIGERWE, 2018) with previously described methods, including isolation and genotyping of *Clostridium perfringens* (DINIZ et al., 2018) and detection of rotavirus and coronavirus by RT-PCR (ASANO et al., 2010). Flotation method with Sheather’s sugar solution followed by light microscopy was also used for routine parasitological detection. Additionally, detection of diarrheagenic *Escherichia coli* was performed according to FRANCK et al (1998) based on identification of virulence genes of ETEC (f41, k99, sta), EPEC (eae) and EHEC/STEC (stx1, stx2).

The isolated strains were subjected to the antimicrobial susceptibility test by disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) manual VET01-A4 (CLSI, 2013). The following antimicrobials were tested: oxytetracycline (30µg), marbofloxacin (5µg), florfenicol (30µg), amoxicillin/clavulanic acid (30µg), trimethoprim/sulfamethoxazole (25µg), ceftriaxone (30µg) and enrofloxacin (5µg) (DME, Brazil). Additionally, to evaluate the genetic similarity
Outbreak of multidrug resistant *Salmonella Typhimurium* in calves at a veterinary hospital in Brazil.

RESULTS AND DISCUSSION

All necropsied animals showed the same pattern of macroscopic lesions suggestive for salmonellosis, such as fibrinonecrotic enteritis (Figure 1B), oedematous mesenteric lymph nodes and hepatosplenomegaly. Some calves have shown additional macroscopic finds such as congested meninges and pneumonia (Figure 1A).
Clinical signs and post mortem alterations seen during the outbreak are commonly described in salmonellosis in cattle (HOLSCHBACH, PEEK, 2017). Despite that, laboratorial diagnostic is still need to confirm the diagnosis of infection by *Salmonella* spp. and also to evaluate the possible association with other enteropathogens (BLANCHARD, 2012). *Salmonella Typhimurium* was recovered from the intestinal content, spleen, liver and mesenteric lymph nodes from all five necropsied calves, additionally from the lungs of one calf. This serovar was also isolated from two out of six tested stool samples from live calves, thus reinforcing the hypothesis of nosocomial transmission (Table 1).

The laboratory diagnosis also ruled out the presence of other common enteropathogens of young calves, including enterotoxigenic *E. coli*, rotavirus and *C. perfringens* type C (HELLER, CHIGERWE, 2018). *E. coli* was isolated in fecal material from all tested animals, but no virulence factor was detected in these strains. *C. perfringens* type A, which is commonly part of the intestinal microbiota of calves, was recovered from the stool samples of all sampled animals. *C. perfringens* type C, which is associated with necrotic enteritis in neonatal calves (UZAL et al., 2015), were not detected in the present report.

To better understand the transmission patterns during the outbreak, one *S. Typhimurium* isolate from each animal (n=7) (Figure 2) were fingerprinted by ERIC-PCR (VERSALOVIC et al., 1991). This molecular tool have been shown to efficiently fingerprinted *Salmonella* strains in outbreak investigations with a high discriminatory power (AMMARI et al., 2009; SMITH et al., 2011; HASHEMI & BAGHBANI-ARANI, 2015; PURIGHALLA et al., 2017). In the present report, the majority of the isolates showed an identical pattern in the ERIC-PCR (Figure 2), suggesting all emerged from the same clone. The only exception was the first isolate obtained during the outbreak, which was recovered from a calve in November 2016. Anyway, using 90% similarity as a cutoff point (HASHEMI & BAGHBANI-ARANI, 2015; RAMOS et al., 2018), this isolate also showed a high similarity with all other isolates, suggesting a closely relationship among all sampled strains in the outbreak and confirming the hypothesis of nosocomial transmission among the hospitalized calves.

*S. Typhimurium* is one of the most common *Salmonella* serovars responsible for infection in animals worldwide, being also known as an important zoonotic agent (HOLSCHBACH, PEEK, 2017). This serovar was also previously described as most common associated with nosocomial outbreaks at large-animal veterinary hospitals (RAVARY et al., 1998; WARD et al., 2005, CUMMINGS et al., 2010, LEON et al., 2018). In fact, it is known that cattle kept in a hospital settings are at risk of salmonellosis due to the presence of several predisposing factors, such as affected gut microbiota by antimicrobial and proton bomb inhibitors administration, concurrent diseases and status of immunosuppression (EWART et al., 2001; CUMMINGS et al., 2009). Additionally to these risk factors, previous reports have also shown a higher fecal shedding of *Salmonella* spp among hospitalized and dairy cattle in the summer and fall (PANGLOLI et al., 2008; CUMMINGS et al., 2009). Interestingly, the outbreak of *Salmonella Typhimurium* described in the present study occurred during the summer months in Brazil, corroborating with previous researches.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Month/year</th>
<th>Isolation</th>
<th>Resistance profile*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B7</td>
<td>Liver</td>
<td>November/2016</td>
<td>1, 2, 3, 5, 7</td>
<td></td>
</tr>
<tr>
<td>B38</td>
<td>Feces</td>
<td>December/2016</td>
<td>1, 2, 3, 5, 7</td>
<td></td>
</tr>
<tr>
<td>B47</td>
<td>Lungs</td>
<td>January/2017</td>
<td>1, 2, 3, 5, 7</td>
<td></td>
</tr>
<tr>
<td>B57</td>
<td>Spleen</td>
<td>January/2017</td>
<td>1, 2, 3, 5, 7</td>
<td></td>
</tr>
<tr>
<td>B58</td>
<td>Intestinal content</td>
<td>January/2017</td>
<td>1, 2, 3, 5, 7</td>
<td></td>
</tr>
<tr>
<td>B59</td>
<td>Feces</td>
<td>January/2017</td>
<td>Susceptible to all</td>
<td></td>
</tr>
<tr>
<td>B60</td>
<td>Mesenteric lymph nodes</td>
<td>January/2017</td>
<td>1, 2, 3, 5, 7</td>
<td></td>
</tr>
</tbody>
</table>

Outbreak of multidrug resistant *Salmonella Typhimurium* in calves at a veterinary hospital in Brazil.

Seven clinical isolates of *S. Typhimurium* from calves were also subjected to the antimicrobial susceptibility test by disc diffusion method (CLSI, 2013). Antimicrobials commonly used in animals, including for treatment of salmonellosis in humans and animals, were selected (HOHMANN, 2001; PARVATHI et al., 2011; CLSI, 2015; OIE, 2015). All *Salmonella* isolates were susceptible to amoxicillin/clavulanic acid and ceftriaxone. Of the seven sampled animals, just one *Salmonella* strain from a calf was susceptible to all antimicrobial agents tested, including the drugs used for their treatment (florfenicol, trimethoprim/sulfamethoxazole and enrofloxacin), contrasting with results of other isolates. It is important to note that this infected calf did not die, demonstrating, in addition to other factors, their clinical improvement due to response to the antimicrobial administration. Conversely, another animal, treated with ceftiofur, trimethoprim/sulfamethoxazole and enrofloxacin also survived to the infection; although, the resistance of their *Salmonella* isolates to trimethoprim/sulfamethoxazole and enrofloxacin also survived to the infection; although, the resistance of their *Salmonella* isolates to trimethoprim/sulfamethoxazole and enrofloxacin also survived to the infection; although, the resistance of their *Salmonella* isolates to trimethoprim/sulfamethoxazole and enrofloxacin also survived to the infection; although, the resistance of their *Salmonella* isolates to trimethoprim/sulfamethoxazole and enrofloxacin. Clinical improvement could be attributed, among other factors, to the administration of ceftiofur, a third-generation cephalosporin recommended for salmonellosis treatment in animals (OIE, 2015).

Multidrug-resistant strains of zoonotic *Salmonella* spp. are commonly associated with outbreaks in calves (MOHLER et al., 2009; HOLSCHBACH, PEEK, 2017). In fact, isolates in the present study were simultaneously resistant to more than three classes of antimicrobials, corresponding to marbofloxacin, enrofloxacin, florfenicol, oxytetracycline and trimethoprim/sulfamethoxazole. It is widely known that dissemination of antimicrobial resistance among *Salmonella serovars* has become a public health concern (WHO, 2017; PARISI et al., 2018). As described, six out of seven tested isolates were resistant to marbofloxacin and enrofloxacin, agents considered critically important for treating human and animals (OIE, 2015, WHO, 2017). Additionally, *Salmonella* fluoroquinolone-resistant is one of 12 bacteria for which new antibiotics are urgently needed, according to World Health Organization (WHO, 2017). It is also important to note that only one calf from which this isolates were recovered was previously treated with enrofloxacin and none had direct contact with marbofloxacin. Interestingly, both antimicrobial agents were used for treatment of others calves, of which could justify the emergence and spread of resistance for these drugs (ALLEN et al., 2010). *Salmonella* isolates from these six calves were also resistant to florfenicol, oxytetracycline and trimethoprim/sulfamethoxazole, antimicrobial agents commonly used in veterinary medicine as well as for treatment of diarrhea in calves (PEZZELLA et al., 2004; KUANG et al., 2015). It is noteworthy that three sampled calves were treated with florfenicol while four received trimethoprim/sulfamethoxazole. Although, none animal has been treated with oxytetracycline in the present report,

the resistance could be explained by the widespread increasing of tetracycline resistance, mainly attributed to the largely and indiscriminate use of this class of antimicrobial agent (CHOPRA & ROBERTS, 2001; SHIN et al., 2015).

Salmonella spp. isolates from outbreaks are commonly phenotypically similar, with shared antimicrobial susceptibility patterns and serotypes (BURGESS & MORLEY, 2014). In the present work, most isolates shared the same serotypes, antimicrobial resistance profile as well as genotypic patterns at ERIC-PCR, confirming its nosocomial transmission (BURGESS, MORLEY, 2014; CUMMINGS et al., 2014).

To reduce the consequences associated with Salmonella spp. into hospital settings, including the emergence of antimicrobial resistance and zoonotic transmission, active surveillance of this agent in patients and environments is necessary to identify and control the outbreaks early in their course. Control measures such as isolation of symptomatic calves and asymptomatic shedders, disinfection of shared equipment between patients and cleaning and disinfection of floors at least twice daily should be taken to reduce both nosocomial and zoonotic transmission of salmonellosis (STENERODEN et al., 2010).

CONCLUSION

This is the first description of a nosocomial outbreak of multidrug resistant Salmonella Typhimurium in a veterinary hospital in Brazil. The present research highlighted the need for preventive measures to reduce the risks for inpatients and humans in contact with animals.

ACKNOWLEDGMENTS

This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Pró Reitoria de Pesquisa da Universidade Federal de Minas Gerais (PRPq/UFMG). Francisco Carlos Faria Lobato have a research fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

BIOETHICS AND BIOSecurity COMMITTEE APPROVAL

We authors of the article entitled “Outbreak of multidrug resistant Salmonella Typhimurium in calves at a veterinary hospital in Brazil” declared, for all due purposes, the project that gave rise to the present data has not been submitted for evaluation to the Ethics Committee of the Federal University of Minas Gerais, but we are aware of the content of the Brazilian resolutions of the National Council for Control of Animal Experimentation - CONCEA “http://www.mct.gov.br/index.php/content/view/310553.html” if it involves animals. Thus, the authors assume full responsibility for the presented data and are available for possible questions, if they be required by the competent authorities.

DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS’ CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

REFERENCES


Outbreak of multidrug resistant Salmonella Typhimurium in calves at a veterinary hospital in Brazil.
