

Brief Original Article

Outbreak investigation of septicemic salmonellosis in calves

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Abstract

Introduction: An early and accurate diagnosis of septicemic salmonellosis is critical for implementing timely and proper treatment, prevention, and control measures.

Methodology: Here, we report a study on three outbreaks of septicemic salmonellosis in calves from Midwestern Brazil.

Results: the morbidity, mortality and lethality rates were of 10.55%, 2.79%, and 26.4%, respectively. Higher susceptibility was detected in *Bos taurus* than in *Bos indicus* cattle. Clinical manifestations consisted of apathy, hyperthermia, difficulty breathing and panting, and pallor of the mucous membranes. Chronic cases had necrosis of the tail tip and ears. Gross findings included enlarged liver, non-collapsed edematous lungs and diphtheritic enteritis. Significant histopathological changes included paratyphoid nodules in the liver and acute interstitial pneumonia. *Salmonella enterica* subsp. *enterica* serotype Dublin was detected by culture and by PCR from the blood of live calves, and from the spleen, liver, bile, mesenteric lymph node and lung samples of necropsied calves.

Conclusions: We suggest that in clinical cases of septicemic salmonellosis, blood samples are better than fecal samples for detection of the agent, being a sound test to identify animal carriers in the herd.

Key words: *Salmonella* Dublin; *Bos Taurus*; carrier; blood; culture; PCR.

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Introduction

Bovine salmonellosis is responsible for causing severe economic losses due to high mortality, especially in cases of the septicemic form caused by *Salmonella enterica* serotype Dublin (for conciseness, hereafter referred to as *Salmonella* Dublin or *S. Dublin*). Early and accurate diagnosis of this type of salmonellosis presentation is critical to allow veterinary practitioners to implement proper treatment, prevention, and control measures [1]. One of the main characteristics of *Salmonella* Dublin is its ability to induce a carrier state in cattle, in which bacteria are then shed intermittently throughout life [2]. Carriers are one of the primary sources of infection, and their recognition and identification, although challenging to perform, are essential to control salmonellosis in a herd [2,3]. Usually, the detection of the bacterium in live animals is performed through fecal microbiological culture

[4,5]. However, due to the intermittent bacterial shedding in the feces, false negatives can occur, which might be a source of further outbreaks. A diagnostic method to detect carriers of *S. Dublin* is therefore desirable. Here, we report a study of three outbreaks of septicemic salmonellosis in calves and suggest a diagnostic protocol for the identification of carrier cattle.

Methodology

Three outbreaks occurred on three different farms. Data on the outbreaks were provided by the veterinary practitioners who supervised the farms. Additionally, over five months, the authors made five on-site visits to the farms to gather epidemiological data and to perform necropsy and sample collections from live and necropsied calves. Outbreaks were numbered 1-3 chronologically.

Of all outbreaks, among all calves that died spontaneously, five were necropsied, and fragments of several organs were sampled and stored in 10% formalin solution for further routine processing for histopathology. Samples of liver, intestine loops, intestinal contents, lung, and bile were stored separately for microbiological culture.

In two calves, spleen smears were performed and stained using DiffQuick® (Siemens Healthcare Limited, Camberley, UK) stain for hematozoa. A detailed clinical examination was carried out in 60 calves with clinical signs compatible with salmonellosis; blood samples were obtained by jugular puncture and stored in two types of Vacutainer® (Becton, Dickinson and Company – BD, New Jersey, USA), one containing EDTA. Feces were sampled using swabs from the rectal ampulla in a tube containing Stuart medium. All samples were refrigerated at 4°C until the bacteriological culture was performed using MacConkey agar (Prodimol Biotecnology, Belo Horizonte, Brazil, for fecal samples) and Hemoprov II pediatric® (Newprov, Pinhais, Brazil, for blood samples) media. Fecal samples were cultured on MacConkey and incubated at 37°C for 24 hours. Subsequently, colorless colonies were identified using Gram-stain and specific biochemical tests for enterobacteria (catalase, oxidase and urease activity, sugar fermentation, citrate utilization, motility, indole production). Samples, whose biochemical profile was compatible with the genus *Salmonella*, were submitted to the rapid slide agglutination test. Blood samples were cultured on Hemoprov II pediatric® at 37°C for 24 hours. After this time an aliquot from the culture was transferred for BHI (brain heart infusion) agar and placed at 37°C for 24 hours. Then, colonies were cultured on MacConkey and incubated at 37°C for 24 hours. Colorless colonies were identified as previously mentioned.

All cultures that yielded organisms with biochemical characteristics of the genus *Salmonella* were submitted to DNA extraction by thermolysis [6], followed by specific Polymerase Chain Reaction (PCR)

to *S. Dublin* using the primer set pDm10-F (5'-GGGCTGGTTCTGAAAGGTGTTA-3') and pDm10-R (5'-ACTGAAGTTGTATCGGGTGGTC-3'), which delimits a 463 bp fragment of a gene encoding a hypothetical protein as previously described [7]. The PCR products were observed under ultraviolet light after electrophoresis in 2% agarose gel stained with GelRed® (Biotium, Landing Parkway Fremont, USA) according to the manufacturer's instructions. A 100 bp DNA ladder was used to measure the amplified fragments.

The morbidity, mortality and lethality rates were calculated based on the number of calves at risk, which was defined as the total of calves that remained in the same environment as the affected calves [8].

Results

The three outbreaks occurred in Midwestern Brazil, in two counties in Mato Grosso do Sul (MS) state, namely Amambai (latitude 23°06'15" south, longitude 55°13'33" west) and Juti (latitude 22°51'38" south, longitude 54°36'10" west). The three farms adopted an extensive system with rotational grazing to raise cattle. The lots consisted of calves from Nelore (*Bos indicus*) and European (*Bos taurus*) mixed breeds between 30 and 90 days of age. Nelore calves did not become ill, although they were held in the same pastures as the European mixed-breed calves. The total number of calves in the three farms was 2860, 860 Nelore and 2000 European mixed breeds; 302 became ill, and 80 died, indicating morbidity, mortality and lethality rates of 10.55%, 2.79%, and 26.4%, respectively.

Clinical manifestations consisted of apathy, hyperthermia, difficulty breathing and panting, and pallor of the mucous membranes. A few calves developed mild yellow and fetid diarrhea. Several calves were found dead. Others had a fatal clinical course of 1-2 days. Affected calves were treated with single doses of ceftiofur (4 mL/calf) and dipyrone (5 mL/calf). Several calves recovered after treatment, and some recovered cases presented necrosis of the tail tip and ears.

Table 1. Results for bacterial culture and PCR from calves with Salmonellosis.

Calf	Blood culture	PCR	Comments
A	<i>Salmonella</i>	<i>S. Dublin</i>	Recovered after treatment
B	<i>Salmonella</i>	<i>S. Dublin</i>	NI
C	<i>Salmonella</i>	ND	Treated one day before sampling
D	<i>Salmonella</i>	<i>S. Dublin</i>	Treated 12 days before sampling
E	<i>Salmonella</i>	ND	Treated 12 days before sampling; necrosis of the tail tip and ear
F	<i>Salmonella</i>	ND	Clinical signs manifested one day before sampling
G	<i>Salmonella</i>	<i>S. Dublin</i>	Clinical signs manifested one day before sampling

ND = not done; NI = not informed.

Figure 1. Salmonellosis in calf. The lungs are non-collapsed and are diffusely red. The liver is enlarged, with multiple orange foci at the capsular surface.



Figure 2. Salmonellosis in calf. The cecal content is dark brown and has big clots of fibrin.



Bacteriological fecal and blood samples from 60 calves were negative. Twenty of those were healthy calves, and the remaining 40 were from calves that had developed clinical signs of salmonellosis. Blood culture from seven calves, of which one had recovered from the clinical disease and six were sick, yielded *Salmonella* in pure culture. Of these seven positive cases, *S. Dublin* was identified in four (Table 1). There was no growth of this agent in any of the 60 fecal samples.

Five calves (one from Farm 1, one from Farm 2 and three from Farm 3) were necropsied. Gross findings were similar in all necropsied cattle and consisted of an enlarged liver with rounded edges and multiple yellowish or hemorrhagic areas on the capsular surface (Figure 1); gelatinous edema in the gallbladder wall and non-collapsed lungs with smooth and bright pleura surfaces and gelatinous edema in the interlobular pulmonary septa. In the intestinal lumina, fibrin clots were adhered to the mucosa (diphtheritic enteritis)

(Figure 2). The histopathological lesions were also similar in all five necropsied calves. In the liver, there were multiple randomly distributed minute nodules consisting of necrosis associated with neutrophilic and macrophagic infiltrates (paratyphoid nodules) (Figure 3). Some small hepatic arteries were thrombosed. In the lungs, there was thickening of the lobular and interstitial pulmonary septa by dense fibrin bundles, moderate to severe infiltrate of macrophages and neutrophils, and alveolar edema (Figure 4). Foci of necrosis with fibrin, cellular debris and neutrophils

Figure 3. Salmonellosis in calf. In the liver, there are multiple random foci of necrosis with fibrin, necrotic debris, neutrophils and a few macrophages (arrows). Fibrin thrombi are seen in the vessels' lumen (asterisks). The sinusoids and the cytoplasm of hepatocytes are filled with bile pigment (arrowheads). H&E, 10X.

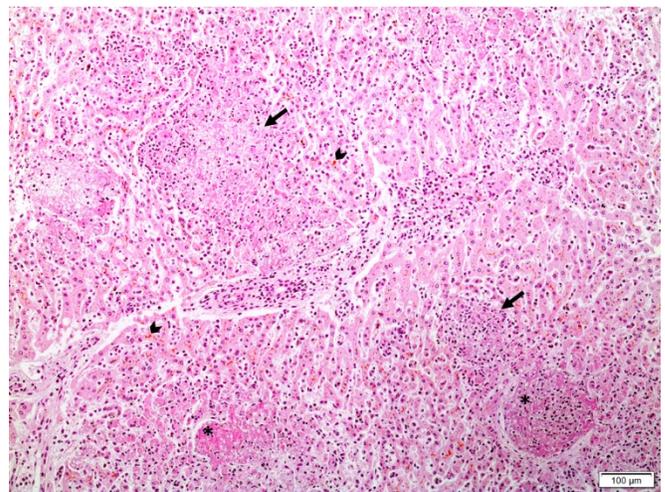
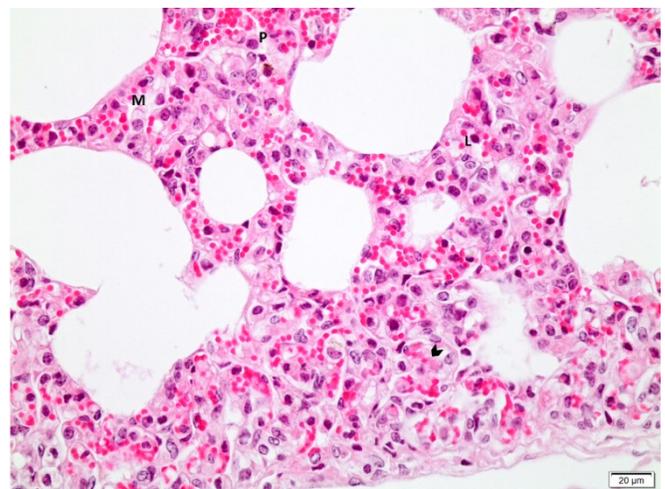


Figure 4. Salmonellosis in calf. In the lungs, alveolar septa are thickened due to infiltrate of macrophages (M), lymphocytes (L) and plasma cells (P). Fibrin clots are seen in some alveolar lumen (arrowhead). H&E, 40X.



were seen in spleen (Figure 5). Bacterial clusters were associated with the lesions in the lymph nodes, spleen and cecum. *Salmonella* was cultured from spleen, liver, bile, mesenteric lymph node and lung samples from the five necropsied calves and identified as *S. Dublin* by PCR.

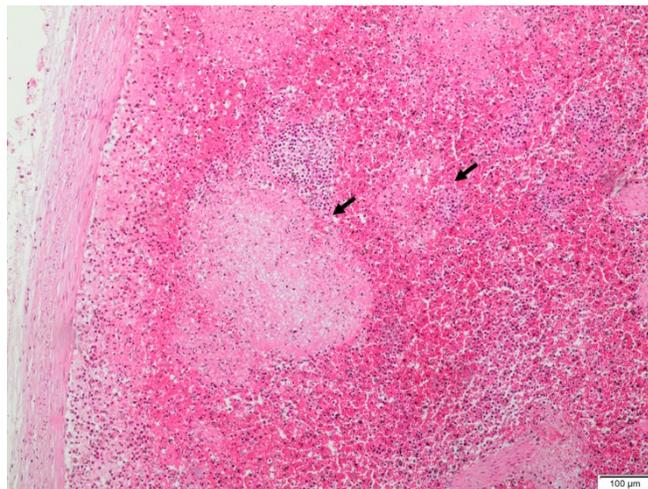
Discussion

The diagnosis of septicemic salmonellosis was based on clinical and pathological findings and confirmed through microbiological culture and identification of *S. Dublin* by the PCR technique. The diagnosis followed the criteria recommended by others [9-11].

Only calves aged between 30 and 90 days became ill, and PCR detected Dublin as the serotype involved in the outbreaks. The age group was one of the risk factors for the disease since *S. Dublin* is an established cause of diarrhea and septicemia in calves within this age range [12,13]. Considering that the farms' breeding systems are extensive, factors like clustering of calves with different ages and the stress caused by environment factors (e.g. high environment temperature) may be considered, in this study, as potential causes for the disease occurrence, since these contribute to the decreased immunity levels of the calves in age of risk [14,15]. The clinical signs manifested by the calves in the outbreaks reported here are characteristic of those produced by this serotype, which may induce a disease in which diarrhea is absent and signs of respiratory distress predominate, as observed in the calves of the outbreaks reported here [13,16,17]. The necrosis and slough of the tail tips and ear that were observed in some calves of this report are chronic changes of septicemic salmonellosis, possibly resulting from immunomediated host reactions [9]. The necropsy and histopathological findings were similar to those observed in outbreaks previously described [1,10,11,13].

The microbiological culture of mesenteric lymph nodes, spleen, liver, lung, and bile of the necropsied calves yielded bacteria of the genus *Salmonella*. These are considered samples of choice for postmortem *Salmonella* isolation [5]. In antemortem attempts at diagnosis (blood and fecal cultures) in our study, *Salmonella* was isolated and identified only in the blood samples from calves both in the acute phase of the disease and recovered from salmonellosis. Although the blood of live animals is also indicated as an adequate sample for *Salmonella* isolation [5], in several reports, this procedure has not been attempted [1,9,13]. In the current study, blood was a useful tool for the

Figure 5. Salmonellosis in calf. The parenchyma is disrupted by multiple extensive foci of necrosis with fibrin deposition. H&E, 10X.



antemortem diagnosis of septicemic salmonellosis. The negative result in fecal samples can be explained in three possible ways: (i) Most of the time, calves with septicemic salmonellosis without enteric clinical signs do not shed the bacterium in the feces since it does not colonize the intestines [17]. (ii) Calves that are carriers may not shed the bacterium in the feces or may shed it intermittently; the agent will then only be detected when it is shed in the feces [2]. (iii) Diarrhea may dilute out a particular load of bacteria in the feces to an insignificant load for microbiological culture to detect [10].

Identification of the bacteria in the blood of the calves recovered with signs of recurrence is an indication of the presence of carrier calves in the herd [2]. However, because the bacterium was not demonstrated in the feces, we were unable to identify the route of bacterial shedding during the outbreaks. Vaginal discharge, saliva and urine are also paths for *Salmonella* shedding. Flies may also be involved in transmission between animals [2,18]. Thus, we cannot rule out any of this transmission mode of the disease in the present outbreak.

Antimicrobial agents are usually not indicated as a treatment due to reports of induced resistant *Salmonella* strains [17,18]. However, treatment with antimicrobials resulted in a complete recovery of several calves in this study, although relapses occurred in some cases. In cases of large outbreaks, this may be a useful measure for reducing calf mortality.

The results of this study and previously cases [1,19] show that septicemic salmonellosis is endemic in Brazil and may cause high losses to the livestock. Due to its

zoonotic character, in our country, salmonellosis is a disease that has official surveillance guidelines only in aviculture [20], despite its also being a potential cause of economic losses in beef cattle.

Conclusion

In clinical cases of septicemic salmonellosis, blood samples are better than fecal samples for the detection of *Salmonella*, and blood culture is a good test to identify animal carriers in the herd.

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