Efficacy of florfenicol and intravenous fluid therapy for treatment of experimental salmonellosis in newborn calves

[*Eficácia do florfenicol e da fluidoterapia parenteral no tratamento
da salmonelose experimental em bezerros neonatos*]

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ABSTRACT

The efficacy of florfenicol associated or not to intravenous fluid therapy for treatment of *Salmonella* Dublin-infected calves was determined. Twenty-four healthy 10 to 15-day-old Holstein calves were randomly allotted into four groups, with six animals each: control (group 1); infected with 10^8CFU *Salmonella* Dublin and not treated (group 2); infected with 10^8CFU *Salmonella* Dublin and treated with florfenicol (group 3); and infected with 10^8CFU *Salmonella* Dublin and treated with florfenicol associated to fluid therapy (group 4). All animals were submitted to physical examination just before inoculation and every 24 hours, during seven days after experimental infection. Rectal swabs and blood samples were collected for *Salmonella* Dublin isolation and pH and blood electrolytes determination. The experimental infection with *Salmonella* Dublin induced clinical signs of salmonellosis, such as diarrhea and fever, and caused reduction in blood concentrations of pH, sodium, potassium and chlorides. The treated calves showed good clinical recovery, and the group treated with antibiotic in combination to fluid therapy presented a faster and more efficient correction of the hydro-electrolyte balance.

Keywords: calf, *Salmonella* Dublin, florfenicol, lactated Ringer's solution

RESUMO

Avaliou-se a eficácia terapêutica do florfenicol associado ou não à fluidoterapia intravenosa no tratamento de bezerros infectados experimentalmente com Salmonella *Dublin. Foram utilizados 24 bezerros sadios da raça Holandesa com 10 a 15 dias de idade, distribuídos aleatoriamente em quatro grupos experimentais, constituídos por seis animais cada: controle (grupo 1); infectado com 10^8UFC de* Salmonella *Dublin e não tratado (grupo 2); infectado com 10^8UFC de* Salmonella *Dublin e tratado com florfenicol (grupo 3); e infectado com 10^8UFC de* Salmonella *Dublin (grupo 4) e tratado com florfenicol associado à fluidoterapia. Todos os animais foram submetidos ao exame físico logo antes da inoculação e a cada 24 horas, durante sete dias após a infecção experimental. Foram colhidas amostras de suabes retais para o isolamento de* Salmonella *Dublin e amostras de sangue para determinação dos valores de pH e dosagem de eletrólitos sanguíneos. A infecção experimental com* Salmonella *Dublin induziu sinais clínicos de salmonelose, como diarreia e febre, e provocou redução do valor do pH e das concentrações sanguíneas de sódio, potássio e cloreto. Os bezerros submetidos aos tratamentos mostraram boa recuperação clínica, sendo que o grupo tratado com antibiótico combinado à fluidoterapia apresentou correção mais rápida e eficiente do equilíbrio hidroeletrolítico.*

Palavras-chave: *bezerro,* Salmonella *Dublin, florfenicol, Ringer com lactato de sódio*

INTRODUCTION

Bovine salmonellosis is a disease of great relevance due to its occurrence, economical importance, and implications in public health (Santos et al., 2002; Veling et al., 2002). The serovars *Salmonella* Dublin and *Salmonella* Typhimurium are the most isolated in cattle herds, and *S*. Dublin is the main cattle-adapted serovar (Smith et al., 1989; Pereira et al., 2004; Silva et al., 2008a).

The most common clinical signs reported in newborn calves with salmonellosis include dehydration, diarrhea, and metabolic acidosis (Fecteau et al., 2003; Silva et al., 2008b). Thus, practical and economical treatments for the control of the bacteremia, rehydration, and correction of electrolyte and acid-base imbalance are fundamental for the reduction of the incidence, mortality, and losses associated to this disease (House and Smith, 1998).

The aim of this study was to determine the efficacy of florfenicol associated or not to parenteral fluid therapy for treatment of *Salmonella* Dublin-infected calves.

MATERIAL AND METHODS

Twenty-four 10 to 15-day-old Holstein calves averaging 41kg body weight were evaluated and equally distributed into four groups: control (group 1); orally infected with 10^8CFU *Salmonella* Dublin and not treated (group 2); orally infected with 10^8CFU *Salmonella* Dublin and treated with 20mg of florfenicol/kg at the onset of the clinical signs of infection, in two doses via IM, every 48 hours (group 3); and orally infected with 10^8CFU *Salmonella* Dublin and treated with 20mg of florfenicol/kg at the onset of the clinical signs, in two IM doses, every 48 hours, associated with intravenous fluid therapy with lactated Ringer's solution at dose of 60mL/kg/day (group 4). During the experimental period, the calves were housed in individual stalls and received four liters of pasteurized milk, twice a day, and also were fed ration and water *ad libitum*. The Institutional Ethics and Animal Welfare Commission from the FCAV/UNESP/Campus de Jaboticabal approved this study.

The inoculum used in the experimental infection was prepared from *Salmonella* Dublin sample (register IOC 3101/03), naturally resistant to nalidixic acid, donated by Fundação Oswaldo Cruz (Manguinhos, RJ).

Blood samples and rectal swabs were collected minutes before the inoculation (0 hour) and also 24, 48, 72, 96, 120, 144, and 168 hours after experimental infection.

Blood samples were taken from the jugular vein using a 25x7mm needle attached to a 1mL syringe containing sodium heparin, according to the recommendations of Lisbôa et al. (2001), for determining values of pH, and concentrations of sodium, potassium, and chloride using an automatic analyzer (Omni C, Roche – São Paulo, Brazil).

The calves were submitted to a daily physical evaluation (Dirksen et al., 1993), and body weight was measured at the beginning and at the end of the study.

The detection of *Salmonella* Dublin in fecal samples was made by incubation of rectal swabs in two selective enrichment broth (selenite cystine and Muller-Kauffmann tetrathionate), followed by transfer into a semi-solid medium (modified brilliant-green agar containing 50µg nalidixic acid/mL), biochemical tests (triplice sugar iron agar and lysine-iron agar), and serological confirmation (*Salmonella* polyvalent somatic and flagellar antisera).

Repeated measures analysis of variance was the statistical method used to evaluate the parametric responses. Pairwise comparisons of means were made using Tukey's procedure. A P value <0.05 was considered significant. The fecal consistency scores were analyzed using non-parametric Kruskal-Wallis test and pairwise comparisons of means were made using Dunn's test. A P value <0.05 was considered significant (ZAR, 1999).

RESULTS AND DISCUSSION

Before inoculation with *Salmonella* Dublin, the clinical parameters of the evaluated groups were within the normal range for cattle (Dirksen et al., 1993). Twenty-four to 48 hours after the experimental infection with *Salmonella* Dublin, all calves from groups 2, 3, and 4 presented a

mild to severe diarrhea, with detection of the bacterium in at least one of the selective enrichment broths (Table 1 and Figure 1a). In the animals from groups 3 and 4 it was observed that the feces consistency improved immediately after the beginning of antibiotic therapy, with or without simultaneous fluid therapy, and most of the animals no longer shed the bacteria in feces (Table 1 and Figure 2a).

Table 1. Mean (±SD) of fecal consistency scores (0= normal; 1= mild diarrhea; 2= severe to moderate diarrhea); rectal temperature (ºC); pH, sodium (mMol/L), potassium (mMol/L), and chloride concentrations (mMol/L) in calves from control group (group 1); calves experimentally infected with 10^8CFU *Salmonella* Dublin (group 2); calves infected and treated with florfenicol (group 3); and calves infected and treated with florfenicol and fluid therapy (group 4), before inoculation (0 hour) and 24, 48, 72, 96, 120, 144, and 168 hours after experimental infection

Variables	Time after Salmonella Dublin inoculation (hours)							
	0	24	48	72	96	120	144	168
Fecal consistency scores*								
G1	0.00±0.00Aa	0.00±0.00Aa	0.00±0.00Aa	0.00±0.00Aa	0.00±0.00Aa	0.00±0.00Aa	0.00±0.00Aa	0.00±0.00Aa
G2	0.00±0.00Aa	0.80±0.80ABa	1.20±0.80ABa	1.30±0.50Ba	1.40±0.50Ba	1.40±0.50Ba	1.40±0.50Ba	1.00±1.20Aa
G3	0.00±0.00Aa	1.80±0.40Bb	1.70±0.50Bb	1.30±0.50Bab	1.00±0.00ABab	0.30±0.80ABab	0.30±0.50ABab	0.70±0.50Aab
G4	0.00±0.00Aa	1.50±0.50Ba	1.50±0.50Ba	1.20±0.80Ba	1.00±0.60Ba	0.80±0.80ABa	0.70±0.50ABa	0.50±0.50Aa
Rectal temperature (ºC)								
G1	38.5±0.50Aa	38.6±0.29Aa	38.3±0.22Aa	38.3±0.29Aa	38.3±0.46Aa	38.4±0.31Aa	38.4±0.42Aa	38.4±0.48Aa
G2	38.9±0.50Aa	39.0±0.16Aa	39.0±0.52ABa	39.4±0.79Aa	39.9±0.75Ba	39.7±0.77Ba	39.4±2.03Aa	40.4±0.97Bb
G3	39.1±0.65Aa	39.3±0.58Aa	39.5±0.75Ba	39.1±0.63Aa	38.7±0.41Aa	38.6±0.15ABa	38.6±0.17Aa	38.5±0.21Aa
G4	38.8±0.63Aa	38.9±0.38Aa	39.2±0.64ABa	38.6±0.20Aa	38.8±0.34ABa	38.5±0.21Aa	38.6±0.50Aa	38.7±0.42Aa
pH								
G1	7.35±0.03Aa	7.34±0.03Aa	7.34±0.01Aa	7.34±0.01Aa	7.36±0.04Aa	7.36±0.02Aa	7.37±0.02Aa	7.38±0.03Aa
G2	7.33±0.05Aa	7.35±0.05Aa	7.36±0.02Aa	7.32±0.07Aa	7.32±0.11Aa	7.31±0.11Aa	7.28±0.16Aa	7.35±0.03Aa
G3	7.31±0.05Aa	7.30±0.05Aa	7.29±0.06Aa	7.31±0.03Aa	7.31±0.04Aa	7.33±0.02Aa	7.32±0.04Aa	7.35±0.02Aa
G4	7.31±0.03Aa	7.30±0.11Aa	7.29±0.10Aa	7.33±0.07Aa	7.35±0.04Aa	7.36±0.02Aa	7.36±0.02Aa	7.35±0.04Aa
Na (mMol/L)								
G1	143±2.05Aa	143±1.22Aa	142±1.46Aa	141±0.70Aa	143±1.89Aa	143±1.23Aa	143±0.67Aa	143±1.33Aa
G2	142±3.26Aa	141±2.23Aa	140±2.24Aa	140±3.63Aa	140±4.62Aa	138±5.03Ba	139±5.55Aa	141±2.92Aa
G3	139±4.16Aa	139±4.97Aa	140±5.84Aa	140±5.00Aa	141±4.42Aa	141±4.00ABa	141±5.50Aa	142±2.64Aa
G4	141±3.35Aa	142±4.18Aa	142±5.06Aa	142±4.04Aa	143±1.98Aa	142±2.34Ba	142±2.46Aa	143±1.65Aa
K (mMol/L)								
G1	4.11±0.35Aa	3.98±0.36Aa	4.01±0.39Aa	3.97±0.19Aa	3.82±0.18Aa	3.87±0.19Aa	3.96±0.31Aa	4.00±0.22Aa
G2	3.76±0.15Aa	3.75±0.15Aa	3.76±0.05Aa	3.65±0.21Aa	3.57±0.16Aa	3.68±0.18Aa	3.78±0.12Aa	3.94±0.27Aa
G3	3.98±0.24Aa	3.80±0.12Aa	3.99±0.23Aa	4.04±0.27Aa	3.97±0.31Aa	3.83±0.17Aa	4.18±0.41Aa	4.00±0.26Aa
G4	3.99±0.21Aa	4.03±0.78Aa	3.79±0.31Aa	3.78±0.24Aa	3.63±0.21Aa	3.95±0.34Aa	3.81±0.24Aa	3.72±0.30Aa
Cl (mMol/L)								
G1	107±0.80Aa	105±1.52Aa	104±1.12Aa	103±0.60Aa	105±2.09Aa	104±0.83Aa	103±1.17Aa	105±0.93Aa
G2	105±4.08Aa	104±4.06Aa	103±2.62Aa	103±2.62Aa	104±1.53Aa	102±2.49Aa	103±1.75Aa	102±2.37Aa
G3	104±2.56Aa	103±2.66Aa	103±3.76Aa	105±4.31Aa	104±4.09Aa	106±4.17Aa	103±6.20Aa	104±4.17Aa
G4	106±2.89Aa	104±2.97Aa	105±1.96Aa	103±3.13Aa	104±2.24Aa	102±3.07Aa	103±2.13Aa	105±1.15Aa

Means followed by different capital letters in the same column and lower case letters in the same line differ statistically by Dunn's(*) test or Tukey's test (P<0.05).

It was observed a gradual increase of the rectal temperature in calves from group 2 along the experimental period, while the groups that were inoculated and treated presented a short period of temperature increase (group 3) or did not present significant alterations in rectal temperature (group 4) (Table 1 and Figure 1b). In a similar way, Osborne et al. (1978) and Fecteau et al. (2003) verified that calves infected with *Salmonella* and treated with antibiotic presented less days of fever and diarrhea. The mean values of blood pH presented mild variations during the experimental period, except for animals from group 2, whose pH values decreased markedly 48 hours after the inoculation, reaching the lowest value at 144 hours (Table 1 and Figure 1c). According to Naylor (1987) and Leal et al. (2007), one of the main biochemical alterations in animals with diarrhea is metabolic acidosis, characterized by decrease of the blood pH, due to fecal loss of bicarbonate. In groups 3 and 4 there was an increase of the pH, which was higher in calves that received fluid therapy (group 4). There were also reduction in sodium, potassium, and chloride concentrations 24 hours after the experimental infection in animals from groups 2, 3, and 4, due to fecal losses (Gonçalves et al., 1991), and increase in the concentration of these electrolytes in animals from groups 3 and 4 after the beginning of the treatment (Table 1 and Figure 1d to 1f).

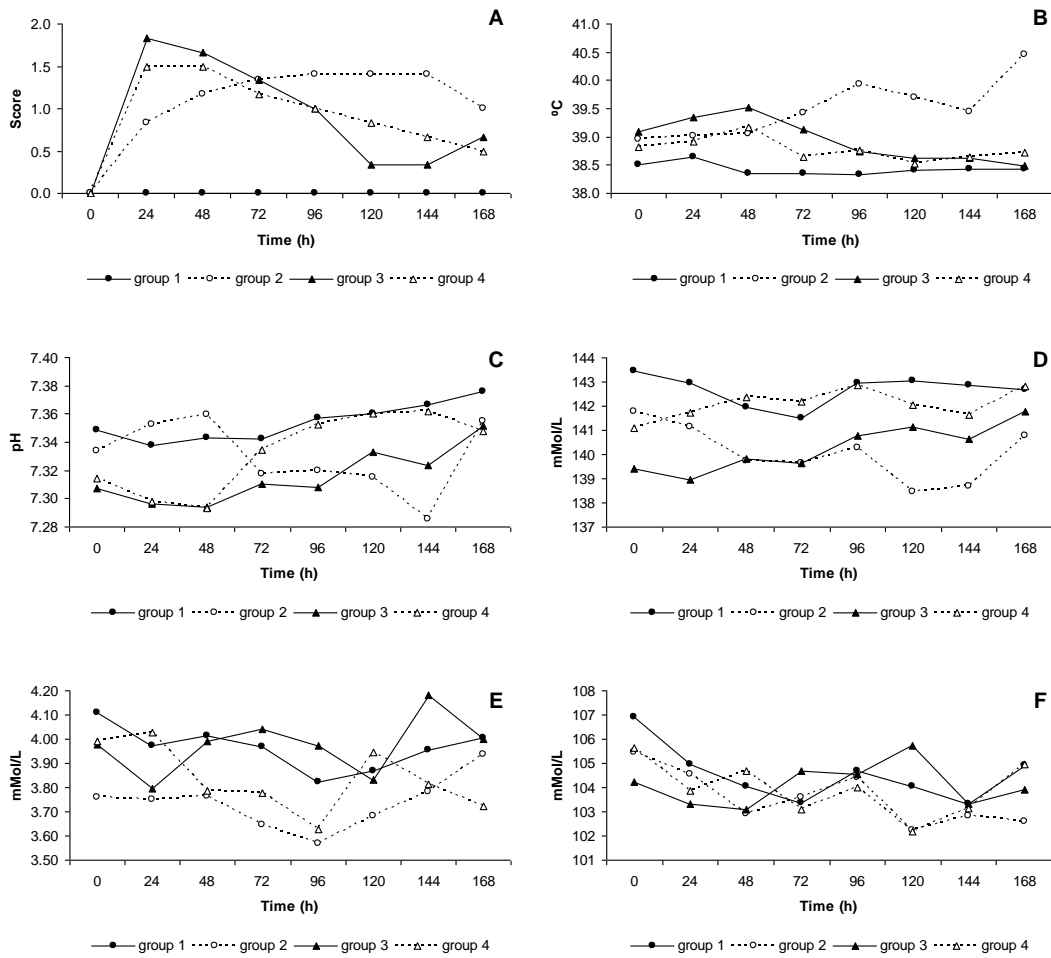*Arq. Bras. Med. Vet. Zootec.*, v.62, n.3, p.499-503, 2010

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Figure 1. **A**: fecal consistency scores (0= normal; 1= mild diarrhea; 2= severe to moderate diarrhea); **B**: rectal temperature (°C); **C**: blood pH; **D**: sodium (mMol/L); **E**: potassium (mMol/L); **F**: chloride concentrations (mMol/L). Group 1: control; group 2: calves experimentally infected with 10^8CFU *Salmonella* Dublin; group 3: calves infected and treated with florfenicol; group 4: calves infected and treated with florfenicol and fluid therapy, before inoculation (0 hour) and 24, 48, 72, 96, 120, 144, and 168 hours after experimental infection.
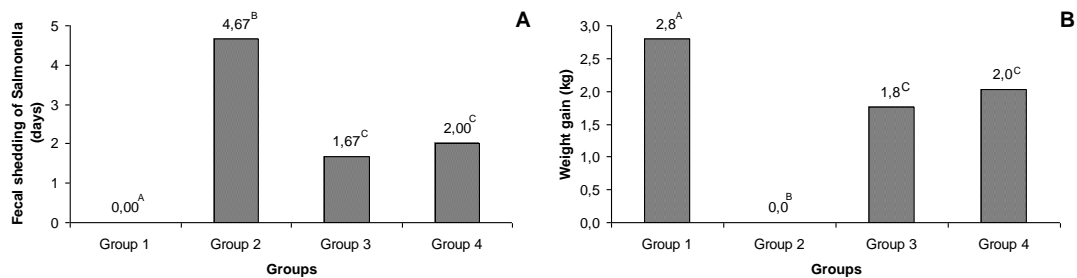

Figure 2. **A**: representation of the average period of *Samonella* fecal shedding and **B**: body weight gain, during the experimental period of calves from control (group 1), calves experimentally infected with 10^8CFU *Salmonella* Dublin (group 2), calves infected and treated with florfenicol (group 3), and calves infected and treated with florfenicol associated to fluid therapy (group 4). Means followed by the same letters do not differ statistically by Tukey's test (P>0.05).

Concerning live weight of the animals seven days after the inoculation, it was observed average gain or loss (kg/animal) of 2.8, -0.1, 1.8, and 2.0 for groups 1, 2, 3, and 4, respectively, indicating that the experimental infection interfered significantly with the weight gain, mainly in animals from group 2 (Figure 2b). According to Rebhun et al. (2000), in calves with salmonellosis, the anorexia usually accompanies the onset of diarrhea, which can be transitory or prolonged. The mortality rates were 83.3% and 16.7% in groups 2 and 3, respectively. In groups 1 and 4, there were no deaths.

CONCLUSIONS

The treatment of the experimental salmonellosis in calves with florfenicol was capable to reduce the fecal shedding of *Salmonella* and to improve the clinical recovery. However, the association of the antibiotic and fluid therapy provided a faster and more efficient control of the hydro-electrolyte imbalance and avoided the occurrence of deaths.

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