Serum protein concentrations, including acute phase proteins, in calves experimentally infected with *Salmonella* Dublin[1]

Daniela Gomes da Silva[2], Péricles Ricardo Lacerda e Silva[2], Paulo César da Silva[2]
and José Jurandir Fagliari[2]

ABSTRACT.- Silva D.G., Silva P.R.L., Silva, P.C. & Fagliari J.J. 2011. [**Serum protein concentrations, including acute phase proteins, in calves experimentally infected with *Salmonella* Dublin.**] *Pesquisa Veterinária Brasileira 31(7):551-554*. Departamento de Clínica e Cirurgia Veterinária, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Via de Acesso Prof. Paulo Donato Castellane s/n, Jaboticabal, SP 14884-900, Brazil. E-mail: danielafcav@yahoo.com.br

The aim of this study was to evaluate serum protein concentrations in calves experimentally inoculated with *Salmonella* Dublin. Twelve healthy 10 to 15-day-old Holstein calves were randomly allotted into two groups, control and infected with 10^8 CFU of *Salmonella* Dublin orally. The calves were subjected to physical evaluation and blood samples were collected shortly before administration of the bacteria and also 24, 48, 72, 96, 120 and 168 hours post-infection. The concentration of serum proteins was determined through sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Thirty serum proteins ranging from molecular weight of 24,000 Da to molecular weight of 236,000 Da were detected. Serum concentrations of ceruloplasmin (125,000 Da), haptoglobin (45,000 Da), acid glycoprotein (40,000 Da) and a 34,000 Da protein were significantly increased in the experimentally infected calves, when compared with their concentrations in the control animals. Therefore, this study showed that *S.* Dublin infection could lead to the increase of certain serum proteins in calves.

INDEX TERMS: *Salmonella* Dublin, SDS-PAGE, serum proteins, calves.

RESUMO.- [**Teores séricos de proteínas, incluindo proteínas de fase aguda, em bezerros infectados experimentalmente com *Salmonella* Dublin.**] O objetivo do estudo foi avaliar as concentrações séricas de proteínas em bezerros inoculados experimentalmente com *Salmonella* Dublin. Foram examinados 12 bezerros sadios da raça Holandesa com 10 a 15 dias de idade, distribuídos aleatoriamente em dois grupos, controle e infectado experimentalmente com 10^8 UFC de *Salmonell*a Dublin. As amostras de sangue foram colhidas minutos antes e 12, 24, 48, 72, 96, 120 e 168 horas após a inoculação. As concentrações séricas das proteínas ceruloplasmina (125.000 Da), haptoglobina (45.000 Da), glicoproteína ácida (40.000 Da) e proteína de peso molecular de 34.000 Da foram significativamente maiores nos bezerros infectados do que nos do grupo controle. Portanto, o estudo mostrou que a infecção por *S.* Dublin pode elevar a concentração de certas proteínas séricas em bezerros.

TERMOS DE INDEXAÇÃO: *Salmonella* Dublin, SDS-PAGE, proteínas séricas, bezerros.

INTRODUCTION

Salmonellosis is one of the main causes of morbidity and mortality in calves (Smith et al. 1994). Although the cattle may be infected by several serovars of *Salmonella*, *Salmonella* Dublin and *Salmonella* Typhimurium are the most prevalent serovars isolated from calves with clinical salmonellosis (Smith et al. 1989, Santos et al. 2002). After oral infection, the bacteria colonize the digestive system causing cellular injury, mainly in the epithelium of the colon and ileum (Kirk et al. 2002). Once it reaches the lamina propria of the bowel wall, *Salmonella* induces a remarkable inflammatory reaction characterized by intense infiltrate of polimorphonuclear cells and fluid secretion into the intestinal lumen (Wallis et al. 1995). Calves with 1 to 6 weeks of age are the most susceptible to *S.* Dublin infection which is frequently characterized by fever, diarrhea, bacteremia and respiratory

signs (Wray & Davies 2000, Veling et al. 2002). Classically, these signs have been useful in monitoring the evolution of the disease; however, changes in these parameters are difficult to quantify (Deignan et al. 2000). Because of the inflammatory process, salmonellosis leads to changes in serum concentrations of acute phase proteins (Heinrich et al. 1990, Kent 1992) that could be identified by means of electrophoresis (Kaneko et al. 2008). Since acute phase proteins increase more rapidly after the onset of inflammation, in response to inflammatory cytokines, the identification of changes in serum concentrations of these proteins might be useful to detect the early stage of infection and to monitor the progression of salmonellosis. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) has been widely used to evaluate the acute phase proteins of the inflammatory response, contributing to the diagnosis and prognosis of several diseases, as equine laminits (Fagliari et al. 1998), equine acute abdomen (Fagliari & Silva 2002), pneumonia in calves (Fagliari et al. 2003) and mastitis in ewes (Costa et al. 2010). However, there is no previous report on the use of SDS-PAGE to measure serum proteins changes in calves with salmonellosis.

Therefore, the aim of the study was to determine, by means of SDS-PAGE, whether serum protein concentrations were altered in calves experimentally infected with *Salmonella* Dublin, with special regard to acute phase proteins.

MATERIALS AND METHODS

Blood samples from twelve healthy 10 to 15-day-old Holstein calves, obtained from a dairy farm that had no history of salmonella isolations, were evaluated. During the experimental period the calves were housed in individual stalls with fenestrated plastic floor (1.30m x 1.50m x 1.35m) suspended 0.4m above the ground and located in an isolated area with concrete floor of the Laboratório de Apoio à Pesquisa do Departamento de Clínica e Cirurgia Veterinária. The calves were bedded on rubber floor and received four liters of pasteurized milk, twice a day, and were fed ration and water *ad libitum*. The Institutional Ethics and Animal Welfare Commission from FCAV/UNESP (CEBEA) approved this study (Protocol 013597-06). During the experimental period, the cleaning and desinfection of bucktes and stalls was performed twice a day with detergent and 2.5% sodium hypochlorite solution.

Prior to experimental exposure, only animals that had normal blood counts[3], negative cultures for salmonella and no fever were randomly allocated in two groups: control and infected. Calves from control group (n=6) received orally 10mL of *Brain Heart Infusion* (BHI) broth[4] through a plastic syringe before the first milk intake, whereas calves from the infected group (n=6) were challenged orally with 10^8 colony-forming unit (CFU) of *Salmonella* Dublin suspended in 10mL of BHI broth (Fecteau et al. 2003). Infected calves were housed in a separated area from control calves to avoid cross contamination between groups.

Salmonella Dublin samples (register IOC 3101/03) used for the preparation of the inoculum (Miles & Misra 1938, Wray & Sojka 1981) were originally isolated from feces of infected calves during the natural outbreak of salmonellosis and were donated by Fundação Oswaldo Cruz (Manguinhos/RJ, Brazil).

Blood samples were collected by venopunction before inoculation of BHI with or without *Salmonella* Dublin, and 24, 48, 72, 96, 120 and 168 hours later. Blood samples were centrifuged, and serum was obtained and frozen until being analyzed. During each collection, a physical examination was performed (Dirksen et al. 1993). The isolation of *Salmonella* Dublin was made from samples collected with rectal swabs (Rice et al. 1997, Santos et al. 2002). The PCR assay was also used for detection of the bacteria (Itoh et al. 1997).

Total serum protein concentrations were determined by the Biuret Method with a commercial kit[5] and the changein optical density was read by spectrophotometry[6]. Serum proteins were fractionated by means of sodium dodecyl sulphate-polyacrylamide gel electrophoresis SDS-PAGE) (Weber & Osborn 1969). Concentration of protein fractions was determined by use of computer-assisted densitometry[7]. Proteins were identified by means of reference markers[8] with molecular weights of 24,000 Da, 29,000 Da, 36,000 Da, 45,000 Da, 55,000 Da, 66,000 Da, 97,000 Da, 116,000 Da and 205,000 Da, and by comparison with the electrophoretic mobility of purified albumin, transferrin, ceruloplasmin, α_1-antitrypsin, haptoglobin and immunoglobin G^7.

The data obtained were analyzed by use of ANOVA for repeated measures. Means were compared by Tukey's test. A value of P<0.05 was considered significant (ZAR 1999).

RESULTS

About 24 to 96 hours after experimental infection, the calves inoculated with 10^8 CFU of *Salmonella* Dublin showed clinical signs of salmonellosis, characterized by severe diarrhea, dehydration and fever (Veling et al. 2002), and the bacteria was isolated from rectal samples collected on swabs and detected by PCR. In animals from the control group, no clinical alteration was observed, and cultures and PCR for detection of *Salmonella* were negative. Control calves also had normal blood counts during the experiment.

Total serum protein concentration from control and infected group were similar (Table 1). Serum protein concentrations obtained by SDS-PAGE allowed to detect 30 proteins with molecular weights ranging from 24,000 Da to 236,000 Da. From these, 8 proteins were submitted to statistical analysis due to their importance for the diagnosis and evidence of difference between the control and infected groups. Twenty-four hours after *Salmonella* Dublin inoculation, the proteins with molecular weights 125,000 Da (ceruloplasmin), 45,000 Da (haptoglobin), 40,000 Da (acid glycoprotein) and 34,000 Da were increased in animals of the infected group (Table 1). From 0 to 72 hours after the inoculation of *Salmonella* Dublin, proteins that showed the highest concentration increase was ceruloplasmin (16%, 110% and 182%, respectively), and the proteins with molecular weight 34,000 Da (88%, 226% and 216%, respectively), compared with the baseline concentration in this group (Table 1, Fig.1). Ninety-six hours after experimental infection, the protein with molecular weight 40,000 Da (acid glycoprotein) showed a significant increase (72%), compared with the control group (Table 1, Fig.1). From 96 to 168 hours after inoculation, the protein of molecular weight 34,000 Da showed the highest increase (251% to 277%, respectively) in animals from group 2 (Table 1, Fig.1).

[3] CC-530, CELM, Barueri, São Paulo, Brazil.

[4] CM0225, Oxoid, Basingstoke, Hampshire, England.

[5] Labtest, Labtest Diagnóstica, Lagoa Santa, MG, Brazil.

[6] Labquest, Labtest Diagnóstica, Lagoa Santa, MG, Brazil.

[7] Shimadzu CS 9301, Shimadzu Corporation, Tokyo, Japan.

[8] Sigma Chemical Company, St Louis, MO, USA.

Table 1. Serum protein concentrations (mean±SD), determined by means of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), in healthy calves (Group 1) and in calves with experimental induced infection with *Salmonella* Dublin (Group 2) before and after *Salmonella* Dublin inoculation

Protein and/or molecular weight (MW) and Group (G)	Time after *Salmonella* Dublin inoculation						
	0h	24h	48h	72h	96h	120h	168h
Total serum protein (g/dL)							
G1	6.60±0.66[Aa]	6.53±0.63[Aa]	6.54±0.48[Aa]	6.66±0.52[Aa]	6.62±0.41[Aa]	6.63±0.45[Aa]	6.54±0.39[Aa]
G2	6.56±0.72[Aa]	6.49±0.76[Aa]	6.47±0.48[Aa]	6.44±0.47[Aa]	6.25±0.45[Aa]	6.26±0.49[Aa]	6.52±0.28[Aa]
Ceruloplasmin (MW 125,000; mg/dL)							
G1	18.8±5.07[Aa]	19.0±4.89[Aa]	17.7±5.37[Aa]	18.0±4.66[Aa]	18.4±5.07[Aa]	17.8±4.28Aa	18.2±4.71[Aa]
G2	18.9±6.97[Aa]	22.0±5.28[Aa]	40.1±6.72[Bb]	53.6±5.96[Bc]	50.1±8.39[Bc]	45.1±9.74[Bb]	23.7±7.41[Ba]
Transferrin (MW 81,000; mg/dL)							
G1	318±72[Aa]	326±51[Aa]	332±68[Aa]	321±60[Aa]	315±54[Aa]	320±65[Aa]	328±59[Aa]
G2	331±90[Aa]	313±67[Aa]	314±86[Aa]	310±71[Aa]	329±97[Aa]	312±95[Aa]	287±89[Aa]
Albumin (MW 65,000; mg/dL)							
G1	4,047±232[Aa]	3,953±152[Aa]	3,968±165[Aa]	3,999±172[Aa]	4,010±201[Aa]	3,976±193[Aa]	4,033±203[Aa]
G2	3,909±265[Aa]	3,806±268[Aa]	3,854±289[Aa]	3,873±337[Aa]	3,750±153[Ba]	3,702±186[Bb]	3,668±196[Bb]
IgG heavy chain (MW 57,000; mg/dL)							
G1	1,155±268[Aa]	1,157±316[Aa]	1,151±317[Aa]	1,166±285[Aa]	1,130±260[Aa]	1,124±218[Aa]	1,022±176[Aa]
G2	1,156±340[Aa]	1,105±343[Aa]	1,098±295[Aa]	1,079±300[Aa]	1,006±288[Aa]	1,039±326[Aa]	1,167±199[Aa]
Haptoglobin (MW 45,000; mg/dL)							
G1	70.4±12.3[Aa]	67.3±11.7[Aa]	71.9±13.0[Aa]	66.8±10.6[Aa]	64.3±11.7[Aa]	68.9±13.4[Aa]	72.1±12.8[Aa]
G2	68.5±10.1[Aa]	72.5±11.3[Aa]	88.6±11.8[Bb]	97.9±10.3[Bb]	123±15.4[Bc]	108±13.6[Bc]	99.4±12.5[Bb]
Acid glycoprotein (MW 40,000; mg/dL)							
G1	26.2±5.4[Aa]	29.0±6.1[Aa]	27.6±5.2[Aa]	30.4±4.9[Aa]	28.6±5.4[Aa]	26.0±4.3[Aa]	28.4±6.2[Aa]
G2	28.5±6.2[Aa]	30.1±6.5[Aab]	32.3±6.1[Aab]	35.1±5.7[Ab]	48.3±6.0[Bc]	50.7±7.8[Bc]	54.4±8.2[Bc]
No identified protein (MW 34,000; mg/dL)							
G1	7.18±3.87[Aa]	8.19±5.59[Aa]	8.31±4.05[Aa]	8.20±4.42[Aa]	7.58±5.06[Aa]	6.40±5.38[Aa]	8.45±2.54[Aa]
G2	7.92±3.44[Aa]	15.1±4.34[Bb]	26.1±5.65[Bc]	25.3±4.95[Bc]	28.1±5.78[Bc]	31.1±6.37[Bc]	30.2±6.07[Bc]
IgG light chain (MW 31,000; mg/dL)							
G1	460±244[Aa]	483±212[Aa]	478±192[Aa]	450±222[Aa]	456±204[Aa]	467±183[Aa]	445±154[Aa]
G2	438±201[Aa]	442±195[Aa]	463±202[Aa]	442±198[Aa]	466±211[Aa]	440±189[Aa]	451±193[Aa]

Means followed by the same capital letters in the column and lower case letters in the line do not differ statistically by Tukey's test (P>0.05).
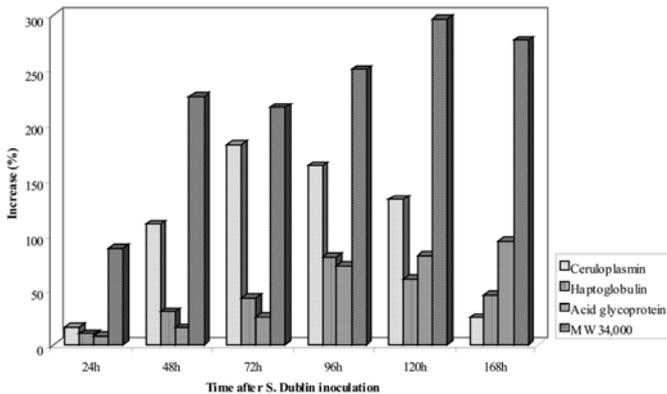
Fig.1. Percentage increase in acute phase protein concentrations, compared with baseline concentrations, in calves experimentally infected with *Salmonella* Dublin (Group 2).

DISCUSSION

The proteinogram obtained by sodium dodecyl sulphate-polyacrylamide gel electrophoresios (SDS-PAGE) allowed to detect 30 different proteins, while in other studies (Keay & Doxey 1982, Fagliari et al. 1991), using agarose gel and cellulose acetate, only 5 to 7 proteins were identified.

In the calves experimentally infected with S*almonella* Dublin, serum concentrations of ceruloplasmin, haptoglobin, acid glycoprotein and protein with molecular weight of 34,000 Da increased 24 hours after *Salmonella* Dublin inoculation. Ceruloplasmin, haptoglobin, acid glycoprotein, and probably protein with molecular weight of 34,000 Da have been identified as acute phase proteins (APP) (Gruys et al. 1994, Godson et al. 1996), and are synthesized through the liver in response to inflammatory cytokines, particularly interleukin-6 (Heinrich et al. 1990). Haptoglobin has been identified as the main acute phase protein in cattle and is an important marker of bacterial infection (Eckersall & Conner 1988). The increase in serum APP indicated that an inflammatory response had developed in the early stage of experimentally induced *Salmonella* Dublin infection.

However, Ávila (2009) found only a significant increase in serum concentration of acid glycoprotein in calves experimentally infected with 10^9 CFU of *S*. Typhimurium, and Garcia (2010) observed significant increase in levels of transferrin, haptoglobin and proteins with molecular weights of 99,000 and 90,000 Da after inoculation of chickens with 10^8 CFU of *S*. Gallinarum.

These results suggest that the inflammatory response to *Salmonella* infections depends on the serotype and animal species.

CONCLUSION

The present investigation showed a significant increase of serum proteins in calves experimentally infected with *Salmonella* Dublin. Such findings encourage further studies about the use of proteinogram to better characterize *Salmonella* infections in cattle. In this sense, the effect of the serotype and the number of infecting organisms should lead to important information towards the understanding of salmonellosis in cattle.

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