Vet Res Commun (2008) 32:333–339
DOI 10.1007/s11259-008-9036-z

ORIGINAL ARTICLE

Serum sialic acid and oxidative stress parameters changes in cattle with leptospirosis

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Accepted: 14 January 2008 / Published online: 5 February 2008
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Abstract This study was designed to disclose some indicators of oxidative stress and inflammation in natural cases of bovine leptospirosis. For this purpose, 12 bulls exhibiting clinical signs of leptospirosis and 10 healthy bulls were used. Animals were subjected to thorough clinical examination and the clinical signs were recorded. All animals were blood sampled in order to determine serum total sialic acid (TSA), lipid bound sialic acid (LBSA), malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO), uric acid (UA), total protein (TP), albumin and glucose. Urine samples were collected from each animal and examined under dark-field microscope to observe spirochetes. Diseased animals exhibited clinical signs suggesting leptospirosis and the diagnosis was supported by positive dark-field microscope examination. Mean TSA (mmol/L), LBSA (mmol/L), TP (g/dl), albumin (g/dl), glucose (mg/dl), MDA (μmol/L), GSH (mg/dl), NO (nmol/ml), and UA (mg/L) levels were 1.63±0.02, 0.40±0.10, 7.18±0.24, 3.23±0.5, 64.96±1.88, 5.71±0.11, 78.68±0.72, 7.94±0.34, and 8.75±0.41 in healthy bulls, and 2.50±0.05, 0.70 ±0.2, 9.27±0.17, 2.55±0.62, 107.93±2.52, 8.82±0.14, 47.85±1.85, 14.57±0.63 and 15.85 ±0.80 in leptospirosis cases, respectively. The differences between the two groups were statistically significant ($P<0.001$). Increased TSA, LBSA, MDA, NO, UA, TP, glucose and decreased GSH and albumin concentrations were suggestive of inflammation and oxidative stress in diseased bulls. The results obtained may suggest that oxidative damage along with other mechanisms might have taken part in the pathogenesis of bovine leptospirosis and further detailed studies are needed to fully understand the mechanism(s) of the disease.

Keywords Cattle · Leptospirosis · Sialic acid · Oxidative stress

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Abbreviations

WHO	World Health Organisation
SA	sialic acid
LBSA	lipid bound sialic acid
MDA	malondialdehyde
GSH	reduced glutathione
NO	nitric oxide
iNOS	inducible nitric oxide synthase
TP	total protein
UA	uric acid

Introduction

Leptospirosis is a zoonotic disease with world-wide distribution. The disease is caused by various serovars of *Leptospira* spp. and is presumed to be the most widespread zoonosis (WHO 1999). The disease is sustained in nature by maintenance hosts namely infected animals. Human and animals may acquire disease by direct or indirect contact with materials contaminated with excretions of the infected animals such as urine, uterus discharge (Radostits et al. 1994).

Although much remains to be determined in the pathogenesis and clinical expression of leptospirosis, several virulent factors such as lipopolysaccharide, glycolypoprotein, sphingomyelinase, hemolysin, known as phopholipase have been proposed to be involved (Lee et al. 2000). Following invasion of the organism the kidney and liver are the major parenchymatous organs affected and cytotoxic factors take part (Thompson and Manktelow 1989; Bharti et al. 2003). Renal colonisation occurs as the organism replicates and persists in renal tubular epithelial cells resulting in varying degree of interstitial nephritis with inflammatory cells surrounding the tubules leading to renal failure (Sitprija et al. 1980; Thompson and Manktelow 1989; Yang et al. 2001). Similarly, liver is also invaded by the bacteria resulting in liver disorganisation and hepotocyte apoptosis (Plank and Dean 2000), and hepatic central-lobular necrosis (Lomar et al. 2000). Additionally, haemolysin and vasculitis also play important role in the pathogenesis and clinical expression in cattle as haemolysin causes extensive intravascular erythrolysis resulting in haemoglobinuria and vasculitis results in petechial haemorrhage in mucosa membranes (Radostits et al. 1994). These events are determined via various indicators of cellular damage and inflammation.

Sialic acid, an acetylated derivative of neuroaminic acid, increases rapidly following the inflammatory and injury process (Schauer 1982; Haq et al. 1993). Therefore the detection of total sialic acide (TSA) particularly lipid bound sialic acid (LBSA) concentrations may be a valuable indicator of inflammatory diseases (Motoi et al. 1984). Previous studies already indicated increased serum SA concentrations during the course of many diseases (Singh et al. 1980; Stenefelli et al. 1985; Thougaard et al. 1998) including bovine leptospirosis (Keles et al. 2000).

Leptospirosis is considered a toxin-mediated disease leading to lipid peroxidation as lypopolysaccaride of its membrane plays role in the cytotoxicity (Alves et al. 1991; Yang et al. 2000, 2001; Levett 2001). The induction of lipid peroxidation gives rise to an increase in malondialdehyde (MDA) content. This procedure activates cell-protective antioxidant

defence mechanisms such as glutathione, uric acid (UA) (Frei et al. 1988). The measurement of UA, albumin, reduced glutathione (GSH) and MDA concentrations can therefore be used as indicators of oxidative stress (Kalaiselvi and Panneerselvam 1998) in diseases like leptospirosis but no studies previously determined the oxidative stress in bovine leptospirosis. In inflammatory conditions, nitric oxide (NO) production increases through stimulation of inducible nitric oxide synthase (iNOS) via activation of pro-inflammatory cytokines and causes NO mediated tissue injury by reacting with superoxide to generate peroxynitrite, a powerful cytotoxin (Carrillo-Vico et al. 2005). A previous study by Maciel et al. (2006) already demonstrated a marked increase in NO level in human with severe leptospirosis. Studies have been conducted to disclose the pathogenesis of leptospirosis in human but there is limited number of documents dealing with the pathogenesis in cattle, an important maintenance host.

This study was therefore designed to determine changes in TSA, LBSA, MDA, GSH, UA, and some biochemical parameters in bulls with leptospirosis.

Materials and methods

The study comprised of 12 bulls, aged between 18–36 months, suffering from leptospirosis and 10 clinically healthy bulls of similar age. All animals were from Kars district, Turkey and were subjected to similar management conditions. A complete physical examination was performed on each animal.

Blood samples were collected from all animals via jugular vein into plain tubes and tubes with anticoagulant and carried to laboratory immediately. Sera were collected by centrifugation at 3000 g for 10 minutes at room temperature and kept frozen (−25°C) until analysis. All serum samples were analysed within 15 days. Serum TSA and LBSA levels were measured calorimetrically according to the method detailed by Sydow (1985) and Katopodis and Stock (1980), respectively. Serum MDA concentration was determined by the Thiobarbituric acid (TBA) reactivity method (Yoshoiko and Kawada 1979). NO was determined according to the method of Miranda et al. (2001). The blood GSH content was measured according to the method of Beutler et al. (1963). Other parameters (serum UA, total protein (TP), albumin and glucose) were determined on a spectrophotometer (UV–1201, Shimadzu, Japan) using commercial kits (Bio-Merieux, France).

Dark-field microscopy of urine samples

Approximately 15–20 ml of urine samples were collected from each animal and centrifuged at 10.000 rpm for 10 min. The supernatant was discarded and the pellet was re-suspended with 0.2 ml of phosphate–buffered saline (pH 7.2). One or two drops of the suspension were examined under dark-field microscope to observe spirochetes (Quinn et al. 1994).

Statistical analyses

Statistical analysis was performed using the SPSS statistical program. Normal distribution of the data was determined using Anderson- Darling Normality test. Values were expressed as mean±standard error. Duncan-ANOVA test was used to compare the parameters between the groups. Significant level was set at $P<0.05$.

Table 1 Changes in oxidative and biochemical parameters in bulls with leptospirosis

Parameters	Groups	
	Healthy (n=10)	Leptospirosis (n=12)
Total sialic acid (mmol/L)	1.63±0.02b	2.50±0.05a
Lipid bound sialic acid (mmol/L)	0.40±0.10b	0.70±0.20a
Total protein (g/dl)	7.18±0.24b	9.27±0.17a
Albumin (g/dl)	3.23±0.5a	2.55±0.62b
Glucose (mg/dl)	64.96±1.88b	107.93±2.52a
Malondialdehyde (μmol/L)	5.71±0.11b	8.82±0.14a
Reduced glutathione (mg/dl)	78.68±0.72a	47.85±1.85b
Nitric oxide (nmol/ml)	7.94±0.34b	14.57±0.63a
Uric acid (mg/L)	8.75±0.41b	15.85±0.80a

Values are expressed as mean±SE

a, b refers to statistical significance between the groups ($P<0.001$)

Result

Clinical findings

Cases had high body temperature, anorexia, excitation, diarrhoea, anaemia, icterohemoglobinuri, jaundice in conjunctiva and mucosa membranes and dark red urine. Clinical signs were indicative of leptospirosis infection. This was supported by the presence of spirochetes on dark-field microscopy examination of urine samples.

The results of biochemical parameters examined for diseased and healthy animals are shown in Table 1. Mean TSA (mmol/L), LBSA (mmol/L), TP (g/dl), albumin (g/dl), glucose (mg/dl), MDA (μmol/L), GSH (mg/dl), NO (nmol/ml), and UA (mg/L) levels were 1.63±0.02, 0.40±0.10, 7.18±0.24, 3.23±0.5, 64.96±1.88, 5.71±0.11, 78.68±0.72, 7.94±0.34, and 8.75±0.41 in healthy bulls, and 2.50±0.05, 0.70±0.2, 9.27±0.17, 2.55±0.62, 107.93±2.52, 8.82±0.14, 47.85±1.85, 14.57±0.63 and 15.85±0.80 in leptospirosis cases, respectively. The differences between the two groups were statistically significant ($P<0.001$). Mean albumin and GSH levels were lower in leptospirosis while all other parameters were considerable high (Table 1).

Discussion

This study tried to disclose some indicators of oxidative stress and inflammation in natural cases of leptospirosis in cattle. Although the pathogenesis of leptospirosis is of complex nature and the underlying factors are not yet fully understood, several mechanisms have been studied; role of toxins released by the organism, bacterial attachment, inflammation and/or immune mediated organ dysfunction.

Clinical signs determined in this study were in agreement with those reported for leptospirosis (Radostits et al. 1994) and this was supported by the presence of spirochetes on dark filed microscope examination (Quinn et al. 1994).

Our study revealed a marked increase in TSA and LBSA in leptospirosis cases as reported previously by Keles et al. (2000). Sialic acid is reported to increase in human and animals during a number of pathological situations where the contributory event is either of tissue damage, tissue proliferation or inflammation (Haq et al. 1993). In these circumstances, rise in TSA and LSBA is attributed to liberation of sialic acid from cell membrane into circulation (Haq et al. 1993; Stefenelli et al. 1985; Taniuchi et al. 1981; Thougaard et al. 1998) as sialic acid is abundantly present in all biological membranes. This may have been the case in this study as haemolysin released by the organism causes damage in erythrocyte and other cells where sialic acid is liberated from glycoproteins of cell membrane (Keles et al. 2000). Increased TP and decreased albumin may also indicate inflammation in this study as TP is documented to increase due especially to hyperfibrinogemia (Sitprija et al. 1980) and albumin, a negative acute phase protein and an antioxidant, is reported to decrease in acute inflammation (Kaneko et al. 1997). Another indicator of cellular damage during the course of leptospirosis may be increased MDA, an indicator of lipid peroxidation and decreased GSH pool, an indicator of antioxidant response. These findings may suggest the production of free radicals and of lipid peroxidation. This hypothesis is supported by increased UA and NO, and decreased albumin concentration in leptorpirosis cases. Uric acid, an antioxidant molecule, also significantly increased in this study as reported in cases of ischemia-reperfusion stroke and liver injury in human where its increase was regarded as a response to oxidative damage (Waring 2002; Glantzounis et al. 2005). Uric acid is well documented to markedly increase during acute oxidative stress and ischemia and its increased concentrations might be a compensatory mechanism that protects tissue against free radicals (Waring 2002). This might have been the case in our study as leptospira causes tissue damage in various organs via different mechanisms. Decreased albumin may also indicate oxidative stress in this study as albumin among its many biological functions, possesses a significant antioxidant activity which is predominantly related to its capacity to bind metal ions and to scavenge free radicals (Bourdon and Blache 2001) and a previous study already stated antioxidant effect of albumin against free radical induced blood haemolysis (Bourdon et al. 1999). This property of albumin is reported to be hindered by the high glucose concentration (Bourdon et al. 1999). Glucose concentration is reported to increase in human leptospirosis with pancreatitis involvement (Daher et al. 2003) this may have also been the case in this study but we have no data to confirm the involvement of pancreatitis. However, increased glucose in this study might have aggravated oxidative stress by inhibiting albumin's antioxidant activity (Bourdon et al. 1999). Increased NO, a gaseous free radical, in this study is in agreement with the study of Maciel et al. (2006) where high serum NO was determined in human patients with severe leptospirosis. Leptospira cellular elements such as lipopolysaccharide and glycolipoprotein, have been reported to activate leukocytes and stimulate the production of pro-inflammatory cytokines (Alves et al. 1991; Werts et al. 2001; Yang et al. 2001; Diament et al. 2002; Marangoni et al. 2006) which induces production of NO through activation of inducible nitric oxide synthase (iNOS). This finding may add credence to that NO may play role in the pathogenesis of leptospirosis (Maciel et al. 2006).

The results of this study may suggest that oxidative damage to tissues along with other mechanisms might have taken part in the pathogenesis of bovine leptospirosis and further detailed studies at cellular level are needed to fully understand the pathogenesis and clinical expression of the disease in cattle, an important source of infection.

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