Characterisation of lipid profiles in dogs with parvoviral enteritis

OBJECTIVE: To characterise the lipid profiles in dogs with parvoviral enteritis.

METHODS: Blood was collected before treatment from 30 dogs that fulfilled the criteria for severe sepsis including hypo- or hyperthermia, hypotension, leucopenia, thrombocytopenia and evidences of organ dysfunction. Canine parvovirus was detected by haemagglutination and indirect fluorescence antibody tests in the faeces. Twenty control dogs were also enrolled on the basis of normal physical examination results, complete blood count and serum biochemistry profiles.

RESULTS: Tachycardia, tachypnoea, hypotension, leucopenia, thrombocytopenia and increased serum markers of tissue injury (alanine aminotransferase, creatinine kinase myocardial isoenzyme [CK-MB], blood urea nitrogen and creatinine) were observed in dogs with parvoviral enteritis. Serum total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol levels were lower, but serum triglyceride level was higher in dogs with parvoviral enteritis than those in control dogs (P<0.001). Circulating tumour necrosis factor α correlated negatively with total cholesterol (r=-0.979; P<0.001) but positively with triglyceride (r=0.953; P<0.001) in dogs with parvoviral enteritis. Serum total cholesterol and high-density lipoprotein cholesterol levels were lower in non-survival (n=9) dogs than in survival dogs (n=21, P<0.001).

CLINICAL SIGNIFICANCE: Serum total cholesterol and high-density lipoprotein cholesterol levels decreased, but serum triglyceride level increased in dogs with parvoviral enteritis. Low serum total cholesterol and high-density lipoprotein cholesterol levels may be used as an index of the severity of parvoviral enteritis.

INTRODUCTION

Canine parvovirus (CPV) is a well-known causative agent of worldwide endemics of severe haemorrhagic enteritis in dogs (Cohn and Langdon 2003, Prittie 2004). Most dogs will live if they can be supported long enough. No definitive treatment has been established, and mortality rates of 4 to 40 per cent have been reported despite aggressive supportive care. Survival in affected dogs has recently been shown to vary depending on the place of treatment, with higher survival rates reported in tertiary care hospitals versus private practices (Prittie 2004). However, very young puppies, dogs in septic shock and certain breeds seem to be more severely affected and may have a more guarded prognosis (Willard 2003). Despite the fact that the treatment of dogs with parvoviral enteritis (PVE) is often successful, some may die because of complications such as sepsis, systemic inflammatory response syndrome (SIRS), endotoxaemia or disseminated intravascular coagulation (Turk and others 1990, 1992, Nappert and others 2002, Cohn and Langdon 2003, de Laforcade and others 2003, Willard 2003, Prittie 2004, Mantione and Otto 2005).

Lipopolysaccharide (LPS) or endotoxin is released from the outer membranes of Gram-negative bacteria and is thought to be an important trigger for the host response known as sepsis (Amersfoorth and others 2003, Nguyen and others 2006, Wu and others 2006). In addition to their roles in cholesterol and lipid transport, plasma lipoproteins provide an important host mechanism for controlling responses to LPS. Lipoproteins bind the bioactive lipid A portion of the molecule and prevent it from stimulating monocytes, macrophages and other LPS-responsive cells (Hudgins and others 2003, Kitchens and others 2003, Sprong and others 2004). LPS and LPS-binding protein or lipoprotein complexes on cells trigger the production and release of a cascade of cytokines (Amersfoorth and others 2003, Wu and others 2006). Tumour necrosis factor α (TNF-α) has an integral role during PVE (Otto and others 1997, Mantione and Otto 2005) and endotoxaemia in dogs (Ilcol and others 2005).

In experimental models, raising plasma lipoprotein levels has decreased the stimulatory effects of LPS and increased host survival during endotoxaemia (Levine and others 1993, Viktorov and Iurkiv 2006) or Gram-negative bacterial infection (Read and others 1993, Spong and others 2004). Conversely, lowering
lipoprotein levels renders animals more susceptible to LPS-induced lethality (Feingold and others 1995). Hypocholes-
teraemia has been a consistent finding in human beings with infection or critical illness (Khovidhunkit and others 2000, Bonville and others 2004, Viktorov and Iurkiv 2006).

Several reports have shown strong cor-
relation between low plasma cholesterol and mortality in critically ill or infected patients (Crook and others 1999, Gordon and others 2001, Bonville and others 2004, Berbee and others 2005). Additionally, Quezado and others (1995) studied the effects of reconstituted human high-
density lipoprotein (R-HDL) on survival, endotoxaemia, cytokine production and pathophysiologic and metabolic events in an animal model of septic shock and suggested that dogs treated with R-HDL had lower levels of circulating endotoxin and TNF-α. These data show that endotoxaemia is associated with rapid and marked declines in serum cholesterol level and that high-density lipoprotein cholesterol (HDL-C) has a protective role against LPS in human beings and animals. To the best of our knowledge, there is limited information on serum lipids in dogs with sepsis or endotoxaemia (Quezado and others 1995, Icöl and others 2005). Thus, this study was performed for the characterisation of serum lipids in dogs with PVE.

The body temperature, heart and respir-
atory rates, capillary refill time (CRT) and peripheral pulse quality were recorded for each of the animals. The haematological parameters included white blood cell (WBC) and platelet counts, haemocrit, and differential WBC. A diagnosis of CPV infection was suspected on the basis of clinical and haematological findings and was confirmed by the haemagglutini-
ation inhibition and immunofluorescence antibody tests (Yilmaz and others 2005a). After the serologic investigation, CPV negative cases were excluded from the study protocol.

Dogs were also tested for coccidial oocysts on faecal hyperosmolar sugar floata-
tion and for blood parasites (Babesia, Ehrlichia or Hepatozoon species) on stained peripheral blood smears and confirmed to be negative before the study. Giardiasis was excluded in all dogs by the absence of trophozoites on a faecal "wet mounted" slide at admission and two consecutive negative zinc sulphate flotation tests on the first two days of hospitalisation.

Twenty control dogs were also enrolled on the basis of normal physical examination results, complete blood count and serum biochemistry profiles.

Definitions. All dogs with CPV ful-
filled the criteria for severe sepsis within 24 hours of admission (Fig 1). Dogs were classified as septic if serological confirmation of infection was available and if two of the following criteria (SIRS) were met: hypothermia or hyperthermia (temperature less than 37.8°C or more than 39.4°C, respectively), tachycardia (heart rate more than 140 beats/minute), tachypnoea (respira-
tory rate more than 30 breaths/minute), leucopenia (WBC count less than 5500/µl) or leucocytosis (WBC count more than 12.500/µl) (Fig 1). Severe sepsis was defined as the presence of sepsis and at least three organ dysfunctions.

Dogs that fulfilled the criteria for severe sepsis were further subdivided into two groups for the purpose of analysis: survival (n=21) and non-survival dogs (n=9).

Sample collection and measurement

At the time of first admission and before any treatment, 5 to 10 ml of venous blood samples were collected into vacutainer tubes with and without ethylenediamine
were assessed by measuring the alterations in serum levels of alanine aminotransferase (ALT), a specific marker for hepatic parenchymal injury in dogs. Renal injury and dysfunction were assessed by measuring the rises in serum concentration of Creatinine, an indicator of reduced glomerular filtration rate, and hence renal failure, and urea, an indicator of impaired excretory function of the kidney and/or increased protein catabolism. Cardiac injury was assessed by measuring the rises in serum concentration of creatinine phosphokinase myocardial isoenzyme (CK-MB), as described in our previous study (Ilcol and others 2005). The changes in the lipid profile were assessed by measuring the alterations in serum concentrations of total cholesterol, triglyceride (Tg), HDL-C, low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein (VLDL). Serum TNF-α was measured by enzyme-linked immunosorbent assay (ELISA) using an enzyme-linked immunosassay kit (Rat TNF-α ELISA kit; Biosource International Inc.) as reported earlier (Ilcol and others 2005, Senturk 2005) and was expressed as picograms per millilitre.

**Standard treatments**

All dogs were hospitalised separately for a minimum of seven days in heated cages in the VAH infectious diseases isolation unit. The dogs, at the first admission day, were rehydrated over six hours using Lactated Ringer solution (Lakatli Ringer; Eczacibas-Baxter Ltd) added with dextrose 5 per cent (Delstroz per cent 5; Eczacibas-Baxter Ltd) and 20 mEq/l potassium chloride (Kadeks-40; Eczacibas-Baxter Ltd). Fluid therapy was administered at a fairly rapid rate until normal mucous membrane colour was restored and CRT was restored to 1 to 1.5 seconds. Further fluid requirements were maintained based on the clinical assessments. Intravenous 10 mg/kg hydroxyethyl starch bolus (Ilohe per cent 6; Eczacibas-Baxter Ltd) was administered if adequate crystalloid resuscitation failed to correct hypovolaemia. Dobutamine (Dobutrex 250 mg; Lilly), a synthetic sympathomimetic, in 5 per cent dextrose was administered at the dose of 1 to 10 μg/kg/minute, as a continuous rate infusion, if fluid (crystalloid and/or colloid) resuscitations were not effective to restore circulatory function. After the normalisation of CRT, mucous membrane colour, and heart beat and peripheral pulse qualities, dobutamine infusion was ceased. Intramuscular 10 mg/kg methylprednisolone (Prednol 250 mg; Mustafa Nevzat) was injected once, as a potent anti-inflammatory agent, with a broad spectrum antibiotic combination. The antibiotic therapy consisted of intravenous 22 mg/kg ampicillin (Ampisina 250 to 500 mg/Flk; Mustafa Nevzat) every eight hours, until vomiting ceased for 24 hours, followed by oral 30 mg/kg ampicillin every 12 hours for 10 days; intravenous 4 mg/kg gentamicin (Gentamin 20 mg/amp; Fako) every 24 hours for five days, initiated once euhydration had been achieved; and oral 25 mg/kg metronidazole (Nitazol 200 mg/5 ml; I.E. Ulhaguy) every 12 hours for five days. Subcutaneous 0.4 mg/kg metcloromide (Metpamid 10 mg/amp; Yeni) every eight to 12 hours was administered as an antemetic until vomiting had ceased (Weerden and Muir 1992, Cohn and Langdon 2003, Prittie 2004).

**RESULTS**

Dogs with PVE were of various weights (11.8±2.4 kg), breeds (14 mixed breeds, five Anatolian shepherd dogs, seven dobermanns and four German shepherd dogs), sex (18 males and 12 females) and ages (between two and six months; mean±SE: 3.9±1.3 months). Control dogs were between two and six months (3.6±1.2 months) of age, were of various weights (12.9±3.4 kg) and breeds of either sex (10 males and 10 females). All healthy dogs and 16 of 30 dogs suffering from PVE had been vaccinated at least once with commercial parvovirus-containing vaccine. Seven dogs with PVE had been vaccinated two times, at three week intervals, with commercial polyvalent vaccines against CPV, canine distemper virus, infectious canine hepatitis and leptospriosis. Nine dogs with PVE had been vaccinated only once with monovalent vaccine against parvovirus infection. The remaining sick dogs (n=14) had not been vaccinated previously.

Facial samples from 30 dogs with haemorrhagic diarrhoea were positive for parvovirus detected using haemaggglutination and indirect fluorescence antibody tests. Severe sepsis was defined by the observation of typical clinical and haematological responses, as shown in Fig 1. Anamnesis from all dogs with PVE included vomiting, bloody diarrhoea, mental depression and anaortexia. Physical examination revealed severe hypovolaemia, hypothermia or pyrexia, pale mucous membranes, prolonged CRT, tachycardia, tachypnoea and weak peripheral pulse quality with hypotension (Table 1). Leucopenia and thrombocytopenia were observed in routine haemogram. Mean length of survival was 2.4±0.3 days for the non-survival dogs (n=9), whereas the remaining dogs (n=21) survived. WBC and platelet counts in survival and non-survival dogs with PVE were lower (P<0.01 to P<0.001) than those in healthy controls (Table 1). Serum biochemical analysis showed that...
Table 1. Selected clinical and laboratory parameters in healthy dogs and in dogs with PVE

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy (n=20), mean±SE</th>
<th>Survival (n=21), mean±SE</th>
<th>Non-survival (n=9), mean±SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>38.4±0.8</td>
<td>38.1±0.5</td>
<td>37.4±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate (beats/minute)</td>
<td>132±24</td>
<td>168±19</td>
<td>173±21</td>
<td>NS</td>
</tr>
<tr>
<td>Respiration (breaths/minute)</td>
<td>32±12</td>
<td>42±8</td>
<td>48±14</td>
<td>NS</td>
</tr>
<tr>
<td>CRT (seconds)</td>
<td>1.0±0.0</td>
<td>3.3±0.2*</td>
<td>3.5±0.2*</td>
<td>0.05</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>134±24</td>
<td>66.4±3.2*</td>
<td>61.5±4.*</td>
<td>0.01</td>
</tr>
<tr>
<td>WBC count (x10^3/µl)</td>
<td>8.7±1.2</td>
<td>3.9±1.0*</td>
<td>3.4±0.8*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Neutrophil count (x10^3/µl)</td>
<td>3.66±0.8</td>
<td>1.45±0.33*</td>
<td>0.97±0.42*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Haematocrit (per cent)</td>
<td>32±4</td>
<td>30±3</td>
<td>26±4</td>
<td>NS</td>
</tr>
<tr>
<td>Platelet count (x10^5/µl)</td>
<td>25.8±8</td>
<td>147±34*</td>
<td>105±42*</td>
<td>0.001</td>
</tr>
<tr>
<td>ALT (iu/l)</td>
<td>68±11</td>
<td>154±42*</td>
<td>168±37*</td>
<td>0.001</td>
</tr>
<tr>
<td>CK-MB (iu/l)</td>
<td>2.56±7.8</td>
<td>18±4.8*</td>
<td>26±8*</td>
<td>0.001</td>
</tr>
<tr>
<td>BUN (mmo1/l)</td>
<td>6±1.6</td>
<td>152±15*</td>
<td>164±26*</td>
<td>0.05 to 0.01</td>
</tr>
</tbody>
</table>

PVE Parvoviral enteritis, SE Standard error, NS Not significant, CRT Capillary refill time, MAP Mean arterial pressure (measured non-invasively), WBC White blood cell, ALT Alanine aminotransferase, CK-MB Creatinine kinase myocardial isoenzyme, BUN Blood urea nitrogen

*Significant difference between dogs with PVE and healthy dogs
†Significant difference between survival and non-survival dogs with PVE (Student’s t test)

- Serum activities of ALT, CK-MB, blood urea nitrogen (BUN) and creatinine in CPV-infected dogs were higher (P<0.05 to 0.001) than those in healthy dogs (Table 1).
- Mean TNF-α levels in peripheral circulation were high in dogs with CPV compared with that in the healthy controls (247 to 271 pg/ml versus 32.5 pg/ml, respectively; P<0.001).

Lipid profile is given in Table 2. The mean serum total cholesterol, HDL-C and LDL-C concentrations were lower, but the mean serum Tg was higher in dogs with PVE when compared with that in the controls (P<0.001). VLDL levels were not significantly different among the groups. In the ROC report, the criterion values corresponding with the highest accuracy (minimal false-negative and false-positive results) of total cholesterol, Tg and HDL-C levels in dogs with PVE were 2.9 mmol/l or less, 1.4 mmol/l or less and more than 0.9 mmol/l, respectively (Table 3). Total cholesterol and HDL-C yielded large area (0.984 to 0.989) under the ROC curve, with 95 per cent confidence intervals ranging from 0.855 to 1.00.

DISCUSSION

The data presented here showed that serum cholesterol level was significantly lower in dogs with CPV infection than in control dogs at time of admission to hospital. Initial clinical signs associated with CPV infection are non-specific and include anorexia, depression and fever. Most affected puppies begin vomiting and develop small bowel diarrhoea within 24 to 48 hours of initial clinical signs (Cohn and Langdon 2003, Willard 2003, Prittie 2004). In this study, clinical findings such as fever, bloody diarrhoea, vomiting and hypotension in dogs with PVE were in line with the reported clinical findings for CPV infection. Mucosal intestinal injuries during CPV infection result in haemorrhagic enteritis and give rise to secondary bacterial invasion from the intestines to blood and organs. In a study carried out using 88 dogs that died of severe PVE, *Escherichia coli* was isolated from the lungs and/or liver in 90 per cent of the dogs (Turk and others 1990). Secondary bacterial infection with clostridia, *Campylobacter* species and salmonellae has also been reported to occur with CPV infection (Turk and others 1990, 1992, Macintire and Smith-Carr 1997). As a part of the systemic defence mechanism, macrophages and other blood cells have been stimulated by serum activities of ALT, CK-MB, blood urea nitrogen (BUN) and creatinine in CPV-infected dogs were higher (P<0.05 to 0.001) than those in healthy dogs (Table 1).

- There was a negative correlation (r=-0.979; P<0.001) between the serum TNF-α and total cholesterol levels and a positive correlation (r=0.953; P<0.001) between the serum TNF-α and Tg levels in dogs with CPV (Fig 2).

Table 2. Serum lipid and TNF-α levels in dogs with PVE and in healthy dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy (n=20), mean±SE</th>
<th>Survival (n=21), mean±SE</th>
<th>Non-survival (n=9), mean±SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total chol (mmo1/l)</td>
<td>5.28±0.4</td>
<td>3.51±0.3*</td>
<td>2.40±0.2*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tg (mmo1/l)</td>
<td>0.81±0.1</td>
<td>1.34±0.1*</td>
<td>1.41±0.3*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C (mmo1/l)</td>
<td>3.13±0.3</td>
<td>1.89±0.2*</td>
<td>1.15±0.1*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C (mmo1/l)</td>
<td>1.79±0.05</td>
<td>1.21±0.06*</td>
<td>1.01±0.2*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLDL-C (mmo1/l)</td>
<td>0.36±0.3</td>
<td>0.40±0.2</td>
<td>0.42±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>TNF-α (ng/ml)</td>
<td>32.5±2.1</td>
<td>247±38*</td>
<td>271±51*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

PVE Parvoviral enteritis, SE Standard error, NS Not significant, Chol Cholesterol, Tg Triglyceride, HDL-C High-density lipoprotein cholesterol, LDL-C Low-density lipoprotein cholesterol, VLDL-C Very-low-density lipoprotein cholesterol

*Significant difference between dogs with PVE and healthy dogs
†Significant difference between survival and non-survival dogs with PVE (Student’s t test)
Table 3. Some cut-off values of total cholesterol, HDL-C and triglyceride and respective SENS and SPEC of the mortality prediction in parovirus-infected dogs complicated with severe sepsis

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>SENS (per cent) (95 per cent confidence intervals)</th>
<th>SPEC (per cent)</th>
<th>PPV (per cent)</th>
<th>NPV (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤2.6</td>
<td>66.7 (30.1 to 92.1)</td>
<td>76.0</td>
<td>91.5</td>
<td></td>
</tr>
<tr>
<td>≤2.8</td>
<td>88.9 (51.7 to 98.2)</td>
<td>81.9</td>
<td>96.2</td>
<td></td>
</tr>
<tr>
<td>≤2.9</td>
<td>100.0 (66.2 to 97.0)</td>
<td>61.0</td>
<td>98.0</td>
<td></td>
</tr>
<tr>
<td>≤1.3</td>
<td>77.8 (40.1 to 96.5)</td>
<td>97.5</td>
<td>91.3</td>
<td></td>
</tr>
<tr>
<td>≤1.4</td>
<td>92.1 (66.2 to 94.3)</td>
<td>95.5</td>
<td>95.4</td>
<td></td>
</tr>
<tr>
<td>≤2.2</td>
<td>94.0 (66.2 to 98.0)</td>
<td>43.0</td>
<td>96.0</td>
<td></td>
</tr>
<tr>
<td>&gt;0.8</td>
<td>44.6 (26.2 to 54.5)</td>
<td>61.0 (58.1 to 94.4)</td>
<td>52.2</td>
<td>48.0</td>
</tr>
<tr>
<td>&gt;0.9</td>
<td>53.9 (51.7 to 68.2)</td>
<td>63.0 (53.7 to 67.0)</td>
<td>55.5</td>
<td>56.5</td>
</tr>
<tr>
<td>&gt;1.6</td>
<td>59.0 (44.0 to 63.8)</td>
<td>65.3 (63.7 to 68.0)</td>
<td>58.1</td>
<td>63.0</td>
</tr>
</tbody>
</table>

HDL-C High-density lipoprotein cholesterol, SENS Sensitivity, SPEC Specificity, PPV Positive predictive value, NPV Negative predictive value

gram-negative microorganisms, resulting in endotoxin elaboration from invading bacteria into peripheral circulation (Turk and others 1990, 1992, Batmaz and others 2003, Cohn and Langdon 2003, Prittie 2004). Thus, secondary bacterial infections during PVE may directly result in septicemia, SIRS or endotoxaemia (Nappert and others 2002, Prittie 2004, Mantiene and Otto 2005).

It is difficult to select a uniform patient population in any study of naturally occurring sepsis. In human beings, specific definitions have been established for sepsis, septic shock and severe sepsis to categorize the patients according to the severity of the inflammatory response (Balk and Bone 1989, Levy and others 2003, Nguyen and others 2006). A classification system is also in use for dogs with sepsis (de Laforcade and others 2003) and septic shock or endotoxaemia (Weeren and Muir 1992, Yilmaz 2000, Yilmaz and others 2002, Ilcol and others 2005). The definition sepsis used in this study is based on the evidence of SIRS along with the documented infection. SIRS was described as a clinical phenomenon in dogs (Fig 1); it included two or more non-specific variables with the presence of CPV infection. Severe sepsis was defined as the presence of sepsis and evidence of dysfunction in three organs (Fig 1). In the present study, increased serum levels of ALT, CK-MB, BUN and creatinine were regarded as diagnostic indicators for liver, myocardium and kidney injuries (Nguyen and others 2006). Observed increases in tissue injury markers in dogs with PVE may be attributable to hepatic hypoxia, hypovolaemia, hypotension (Macintire and Smith-Carr 1997) and specific localisation of parovirus-infected cells (Nho and others 1997, Prittie 2004). Furthermore, excessive
production and release of TNF-α during CPV infection may have a role to initiate widespread tissue injury, which can result in organ dysfunction (Levy and others 2003).

Data regarding septic shock (Yilmaz 2000, Yilmaz and others 2002) or endotoxaemia in dogs (Batmaz and others 2003, Yilmaz and others 2005b, 2006) show that the effects of endotoxin on clinical and laboratory parameters mimic the signs of severe sepsis. Thus, leucopenia, thrombocytopenia and MODS caused by CPV may be aggravated by secondary bacterial sepsis and subsequent endotoxin elaboration. This concept is supported by the studies reporting that WBC and platelet counts decreased within one hour after intravenous infusion of LPS (Batmaz and others 2003, Ilcol and others 2005, Yilmaz and others 2005b, 2006) or *E. coli* in dogs (Hardie and others 1988, Yilmaz 2000). Leucopenia, especially neutropenia, is common in severe disease. Up to 86 per cent of PVE may become leucopenic during the first four days of hospitalisation. Neutropenia has been suggested to be primarily the result of net consumptions of neutrophils at the injured intestinal mucosa rather than a primary failure of granulopoiesis, as infection and destruction of granulocyte precursor cells is an inconsistent feature of CPV infection (Cohn and others 1999). Some investigators have found a significant correlation between the degree of leucopenia at admission and poor prognosis (O'Sullivan and others 1984, Mason and others 1987), while others were unable to reveal such a correlation (Jacobs and others 1988, Sonl and others 1999). In this study, even though marked leucopenia and neutropenia (P<0-01) were observed in non-survival dogs, the severity of leucopenia was not correlated with the outcome. Because of this discrepancy, it may be suggested that leucopenia and neutropenia may indicate the presence of more severe disease, but neutrophil count may be used as a prognostic indicator in clinical settings (Cunneen and Cartwright 2004).

Several studies showed a positive correlation between the TNF-α level and the severity of sepsis and fatality (Miyamoto and others 1996, Zhang and others 2001). In our study, the mean serum TNF-α level was much higher in dogs with PVE than in healthy controls (P<0-001) but did not differ significantly between the survival and non-survival dogs, suggesting that it is not a valuable tool to predict the prognosis of CPV-infected dogs in the study. A reason for this may be that all the dogs in the study were already at a late stage of CPV infection when presented. Other findings such as prolonged CRT, pale mucous membranes, hypothermia and evidence of MODS provided clinical and laboratory evidences for a hyperdynamic sepsis (late phase) rather than a hyperdynamic sepsis (early phase) (Weeren and Muir 1992).

Despite advances in supportive care, severe sepsis remains one of the leading causes of death in critically ill patients. Thus, recent studies have focused on early diagnosis, treatment and prophylactic strategies against the inflammatory condition related to sepsis or endotoxaemia (Ilcol and others 2005, Senturk 2005, Yilmaz and others 2005b, 2006). Lipid profile is increasingly used for the prognostic evaluation of patients with sepsis (Crook and others 1999, Barton and others 2000, Bonville and others 2004, Viktorov and Iurkiv 2006). Total cholesterol, LDL-C and HDL-C levels have been shown to be substantially decreased as part of an acute-phase response during infection (Kitchens and others 2003, Viktorov and Iurkiv 2006). Although Crook and others (1999) reported that hypocholesterolaemia might be a useful predictor of mortality in hospital patients, Kitchens and others (2003) suggested that in critically ill patients, serum cholesterol was a poor indicator of the capacity of lipoproteins to bind LPS, owing to the loss of esterified cholesterol from the core of lipoprotein particles. In this study, in accordance with the study of Crook and others (1999), we found that serum total cholesterol, LDL-C and HDL-C levels were lower (P<0-001) in dogs with severe sepsis than in control animals (Table 2). In vitro lipoproteins have been found to bind LPS and neutralise its toxic effects (Read and others 1993, Kitchens and others 2003). In patients with sepsis, however, the concentrations of lipoproteins, especially of the high-density lipoprotein (HDL) phospholipid, are decreased (Gordon and others 2001, Hudgins and others 2003, Berbee and others 2005), and the observed changes are strongly related to the severity of the infection (Sammalkorpi and others 1990). In parallel, HDL level in non-survival dogs with PVE was significantly lower (P<0-01, 1-15±0-1) than that in survival (1-89±0-2) and control dogs (3-13±0-3). For serum total cholesterol and HDL-C cut-offs of 2-8 mg/dl or less and 1-3 mg/dl or less, estimated PPV was high (81-0 and 97-5 per cent, respectively) but SENS was low (66-7 and 77-8 per cent, respectively), showing that serum total cholesterol and HDL-C may have a stronger predictive value on survival in this disease. These results may be supported by the study of Levine and others (1993) who reported that transgenic mice with increased HDL-C concentrations had fourfold survival rates following endotoxin infusions than that of otherwise identical mice with lower HDL-C concentrations. Moreover, infusion of a chemically defined R-HDL preparation has been shown to blunt LPS-induced TNF-α production and protect against the consequences of LPS administration in mice (Levine and others 1993) and rabbits (Hubsch and others 1993).

Tg level appears to respond differently than other lipids and lipoproteins during the acute phase of an illness. The most typical change in lipoprotein metabolism during infection and inflammation is hypertriglyceridaemia (Berbee and others 2005, Aspichueta and others 2006). Acute-phase response is accompanied by alterations in lipid metabolism, which include increased serum Tg and decreased HDL levels (Hudgins and others 2003). In the present study, as compared with healthy dogs, serum Tg showed significant increases in CPV-infected dogs. However, serum Tg level was similar in survival and non-survival dogs. In parallel, serum Tg with different cut-off values (more than 1-5 to more than 1-6 mg/dl) had lower SPEC and SENS, compared with total cholesterol and HDL-C, indicating lower predictive value for mortality. The increase in Tg level is thought to be mediated by TNF-α and other cytokines, which rapidly stimulate hepatic fatty acid synthesis (Krauss and others 1990, Feingold and others 2003).
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**References**


APROUCHA, P., PEREZ-AGOTE, B., PÉREZ, S., ODOS, B. & FRESNEDO, O. (2006) Impaired response of VLDL and HDL-C levels decrease and serum total cholesterol (r= -0.979; P<0.001) and HDL-C levels decrease and serum total cholesterol (r= -0.979; P<0.001) or serum Tg (r=0-953; P<0.001) in dogs with VLDL particles, respectively (Fig 2). This observation is consistent with the study reporting that low cholesterol concentrations correlate with high concentrations of cytokines (Bonville and others 2004).

In conclusion, the present study demonstrates that the serum total cholesterol and HDL-C levels decrease and serum Tg level increases in dogs with VLDL. More studies are needed to understand whether hypcholesterolaemia has only diagnostic and prognostic implications or whether it may also contribute to worsening of the disease.

**Acknowledgement**

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