Supplemental E-Methods

Haptoglobin Administration and Desensitization Protocol

Clinical grade mixed phenotype haptoglobin (2-1 and 2-2) fractionated from human plasma was provided as a generous gift from CSL Behring (CSL Behring, King of Prussia, PA 19406) in phosphate buffered saline (PBS) solution. In dose-finding experiments, 16 two-year-old, purpose-bred beagles (9.8-13.7 kg) were studied. Fifty percent (4/8) of the animals, receiving a total dose of 1600 mg/kg of human haptoglobin, developed anaphylactic reactions occurring shortly after the initial haptoglobin dose consisting of transient severe hypotension over 30-60 minutes associated with a transient erythematous rash. Twenty-five percent (2/8) of the animals receiving a lower total dose of 800 mg/kg of human haptoglobin still developed transient rashes over 30 to 60 min without hypotension. A desensitization protocol was developed using intravenous administration of twelve sequential dilutions, starting with 0.34 mg (diluted in PBS to a concentration of 0.0068 mg/mL) and finishing with a dose of 66.8 mg (33.4 mg/mL). Following desensitization, the first haptoglobin bolus was infused, subtracting from the dose the amount infused during desensitization. Using this desensitization protocol, only one of 21 subsequent sepsis study animals developed a mild erythematous rash over 30 min. Animals randomized to the control arm (PBS or albumin instead of haptoglobin) received equivalent volumes of PBS during the desensitization phase.

Haptoglobin Dose-finding Experiments

The goal was to achieve plasma haptoglobin levels at least equivalent to the average plasma CFH levels detected in previous transfusion experiments (100 to 150 μM).[11-13,42] Each haptoglobin alpha-beta dimer binds two hemoglobin dimers (four heme groups per haptoglobin dimer). The exact binding ratio in a cross-species study is unknown, however quantitative

measurement of haptoglobin-bound and free hemoglobin were determined by analytical ultracentrifugation (see below). Dosing calculations were based on results with a similar human haptoglobin concentrate in a published guinea pig model[6] and in consultation with a clinical pharmacokineticist. We estimated extracellular volume at 0.15 L/kg and plasma volume at 0.04 L/kg for canines, which was assumed to be the volume of distribution of haptoglobin following intravenous infusion. Based on the molecular weight of haptoglobin (400 kD), the estimated volume of distribution in canines (0.04 L/kg) and the desired haptoglobin plasma levels (100 μM), we calculated an initial dose of 1600 mg/kg of haptoglobin over 48 h.

Initially, 8 of the 16 animals in the dose-finding study received bolus doses of 200 mg/kg every 3 h over the first 10 h followed by a 400 mg/kg bolus every 24 h for 48 h for a total dose of 1600 mg/kg. These non-septic animals were randomized either to exchange-transfusion with 80 mL/kg (divided into four 20 mL/kg doses as previously described) of stored RBCs (n=4) or no transfusion (n=2). Control animals (n=2) were not exchanged-transfused and received no haptoglobin. Since the desired level was exceeded in all animals after every haptoglobin bolus and levels varied markedly over time, the bolus dose was lowered and continuous infusions added. In the other 8 additional animals studied, total haptoglobin dose was lowered to 800 mg/kg, each bolus lowered to 100 mg/kg, the number of bolus doses decreased to two, and continuous infusion of haptoglobin employed until the 48h termination. This dosing protocol achieved and maintained the desired plasma levels, between 100 and 150 μM, in all eight animals receiving the haptoglobin protocol. This temporal pattern mimics the pattern of plasma CFH elevations previously reported with stored RBC exchange-transfusion studies.[11-13,42,46]

Laboratory Measures and Haptoglobin-Hemoglobin Binding Studies

Canine levels of CFH (μmol/L) were determined by Drabkin's method, as previously described.[1,2,4] This assay measures both plasma CFH and CFH bound to haptoglobin. Canine non-transferrin bound iron (NTBI) plasma levels were determined by a commercial laboratory (aFerrix Ltd., Tel Aviv, Israel) using a proprietary assay. Transferrin bound iron (TBI) was measured using electron paramagnetic resonance spectroscopy as previously described.[1,11] Arterial blood gases (Stat profile prime analyzer, Nova Biomedical, Waltham MA) and complete blood counts (Element HT5, Heska, Loveland CO) were determined by laboratory based analyzers. C-reactive protein (CRP) was determined using a commercially available Elisa kit (eBioscience, San Diego, CA). TNFα, IL-6 and IL-10 were determined using a multiplex immunoassay system (Bio-Rad, Hercules, CA) and commercially available kits (Invitrogen, ThermoFisher, Waltham, MA)

Analytical ultracentrifugation (Beckman Optima XL-A with UV/vis optics) was used to determine if human haptoglobin bound canine hemoglobin, and to estimate the proportion of plasma hemoglobin bound to haptoglobin in selected samples.[30] Samples were spun at 45,000 rpm (163,000g). Data were analyzed using DCDT+ (version 6.31) software (J. Philo, Thousand Oaks, CA).[30,31]

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: Haptoglobin Therapy Study Timeline Over the 96-hour duration of the Sepsis Study.

In the first of two sepsis experiments, 24 purpose-bred beagles (18 to 30 months old, 9 to 12.5 kg,) with *S. aureus* pneumonia were randomized to receive intravenous haptoglobin administration (800 mg/kg total dose, in two divided 100 mg/kg bolus doses at 4 and 7 h after infection followed immediately by a 600 mg/kg continuous infusion over 48 h) or an osmotically equivalent volume of human 25% albumin (control) +/- Blood transfusion. In a second sepsis experiment, 18 purpose-bred beagles (18 to 30 months old, 9 to 12.5 kg) with experimental *S. aureus* pneumonia were additionally exchanged-transfused with 80 mL/kg of 7-day-old stored canine universal donor blood in four divided doses (20 mL/kg) given sequentially every 3 h starting 4 h after infection but otherwise treated the same and given haptoglobin therapy as in the first experiment. Critical Care Supportive Treatments (antibiotics, vasopressors, fluids, ventilation) and diagnostic measurements (laboratory and hemodynamics) are serially obtained over the 96-hour duration.

Supplemental Figure 2: Mean (± SE) Arterial Pressure and Norepinephrine Infusion Rates Over the 96-hour duration of the Sepsis Study at Serial Time Points. Individual components of the Shock Score (serial mean arterial pressure and norepinephrine requirements ±SE) are shown plotted over time (96 hours) after *S. aureus* challenge in canines receiving haptoglobin or no haptoglobin with (panels A and C) or without RBC exchange-transfusion (panels B and D). A higher norepinephrine rate requirement means that an animal required more vasopressor for maintenance of MAP. Changes from baseline are shown for each study group

plotted from a common origin the mean value for animals at baseline. P values indicate significance in each group comparison in each panel and are denoted by asterisks (for changes over time) or crosses (comparing haptoglobin vs. no haptoglobin at each time point).

Supplemental Figure 3: Mean (± SE) Alveolar-arterial O_2 Gradient, Mean Pulmonary Artery Pressure, Plateau Pressure, Oxygen Saturation, and Respiratory Rate Measurements Over the 96-hour duration of the Sepsis Study at Serial Time Points.
Individual components of the Lung Injury Score (serially mean AaO_2, mean pulmonary artery pressure, plateau pressure oxygen saturation, and respiratory rate ±SE) are shown plotted over time (96 hours) after *S. aureus* challenge in canines receiving haptoglobin or no haptoglobin with (panels A, C, E, G, I) or without RBC exchange-transfusion (panels B, D, F, H, J). Changes from baseline are shown for each study group plotted from a common origin the mean value for animals at baseline. P values indicate significance in each group comparison in each panel and are denoted by asterisks (for changes over time) or crosses (comparing haptoglobin vs. no haptoglobin at each time point).

Supplemental Figure 4: White Blood Cell Count and Differential Over the 96-hour duration of the Sepsis Study at Serial Time Points.
White blood cell count (WBC) and cellular differential was performed over 96 hours after *S. aureus* challenge in canines receiving haptoglobin or no haptoglobin with (panels A, C, E, G) or without RBC exchange-transfusion (panels B, D, F, H). Changes from baseline are shown for each study group plotted from a common origin the mean value for animals at baseline. In animals with *S. aureus* pneumonia randomized to receive haptoglobin therapy and exchange-

transfused RBCs *vs.* no haptoglobin therapy (septic controls) there were no significant differences in mean circulating WBC counts at all time points studied (panel A). In contrast, animals with *S. aureus* pneumonia and not exchange-transfused RBCs randomized to receive haptoglobin therapy *vs.* no haptoglobin therapy (septic controls) had significant increases in mean circulating WBC counts at 10, 16, 24, and 96 h (Panel B). The circulating WBC levels mean increases with haptoglobin treatment group *vs.* no haptoglobin therapy (septic controls) were significantly greater at 24 and 96 h with *vs.* without RBC exchange-transfusion (interaction). Preservation of WBC with haptoglobin therapy without exchange transfusion was present in varying degrees in all white blood cell lines, including neutrophils (Panel C and D), monocytes (Panel E and F) and lymphocytes (Panel G and H). Notably, this effect on circulating white blood cells seen only without exchange-transfusion of RBCs and thus cannot easily fully explain the similar beneficial effect of haptoglobin therapy during *S. aureus* pneumonia with and without RBC exchange transfusion on survival, shock score and LIS (*i.e.* the preservation of white blood cells was lost with exchange-transfusion, therefore is more likely a beneficial outcome diminished by mixing with exchange-transfusion of RBCs rather than a necessary protective mechanism of haptoglobin therapy during sepsis). The P values indicate significance in each group comparison in each panel and are denoted by asterisks (for changes over time) or crosses (comparing haptoglobin vs. no haptoglobin at each time point).

SUPPLEMENTAL TABLE LEGENDS

Supplemental Table 1: Renal and Hepatic Functional Measurements Over the 96-hour duration of the Sepsis Study at Serial Time Points.

Indices of renal (BUN and creatinine) and hepatic (ALT, AST, LDH, and total bilirubin) function were quantified over 96 hours after *S. aureus* challenge in canines receiving haptoglobin or no haptoglobin with or without RBC exchange-transfusion. P values greater than 0.05 considered non-significant (NS).

Supplemental Table 2: Measures of Cardiac Function Over the 96-hour duration of the Sepsis Study at Serial Time Points.

Cardiac function was assessed using mean left ventricular ejection fraction and mean cardiac output was assessed via echocardiography and pulmonary artery catheter thermodilution measurements over 96 hours after *S. aureus* challenge in canines receiving haptoglobin or no haptoglobin with or without RBC exchange-transfusion. P values greater than 0.05 considered non-significant (NS).

Supplemental Table 3: Serum Electrolyte Measurements Over the 96-hour duration of the Sepsis Study at Serial Time Points.

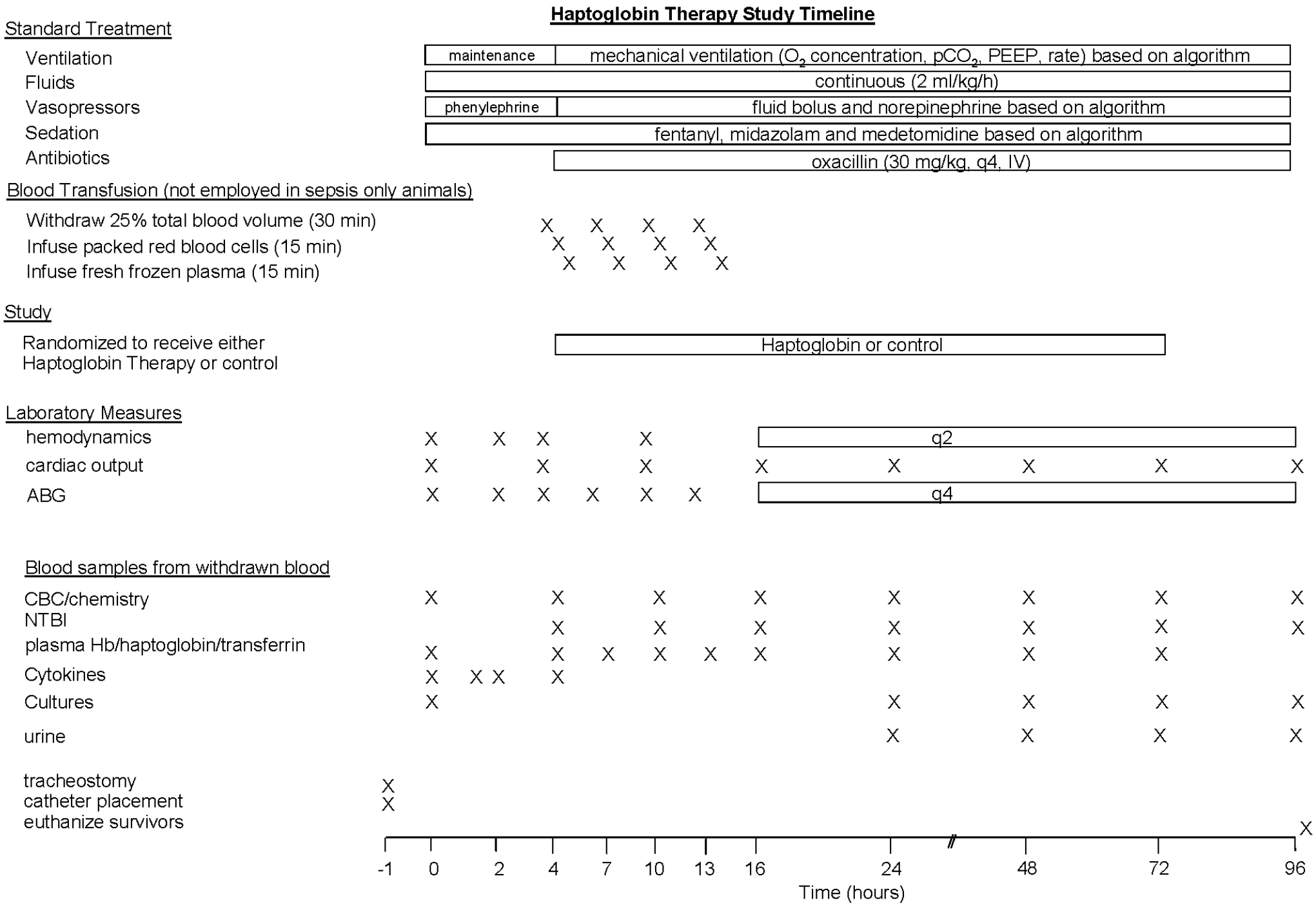
Sodium, potassium, chlorine, and glucose are measured over 96 hours after *S. aureus* challenge in canines receiving haptoglobin or no haptoglobin with or without RBC exchange-transfusion. P values greater than 0.05 considered non-significant (NS).

Supplemental Table 4: Transferrin Bound Iron and Platelet Count Over the 96-hour duration of the Sepsis Study at Serial Time Points.

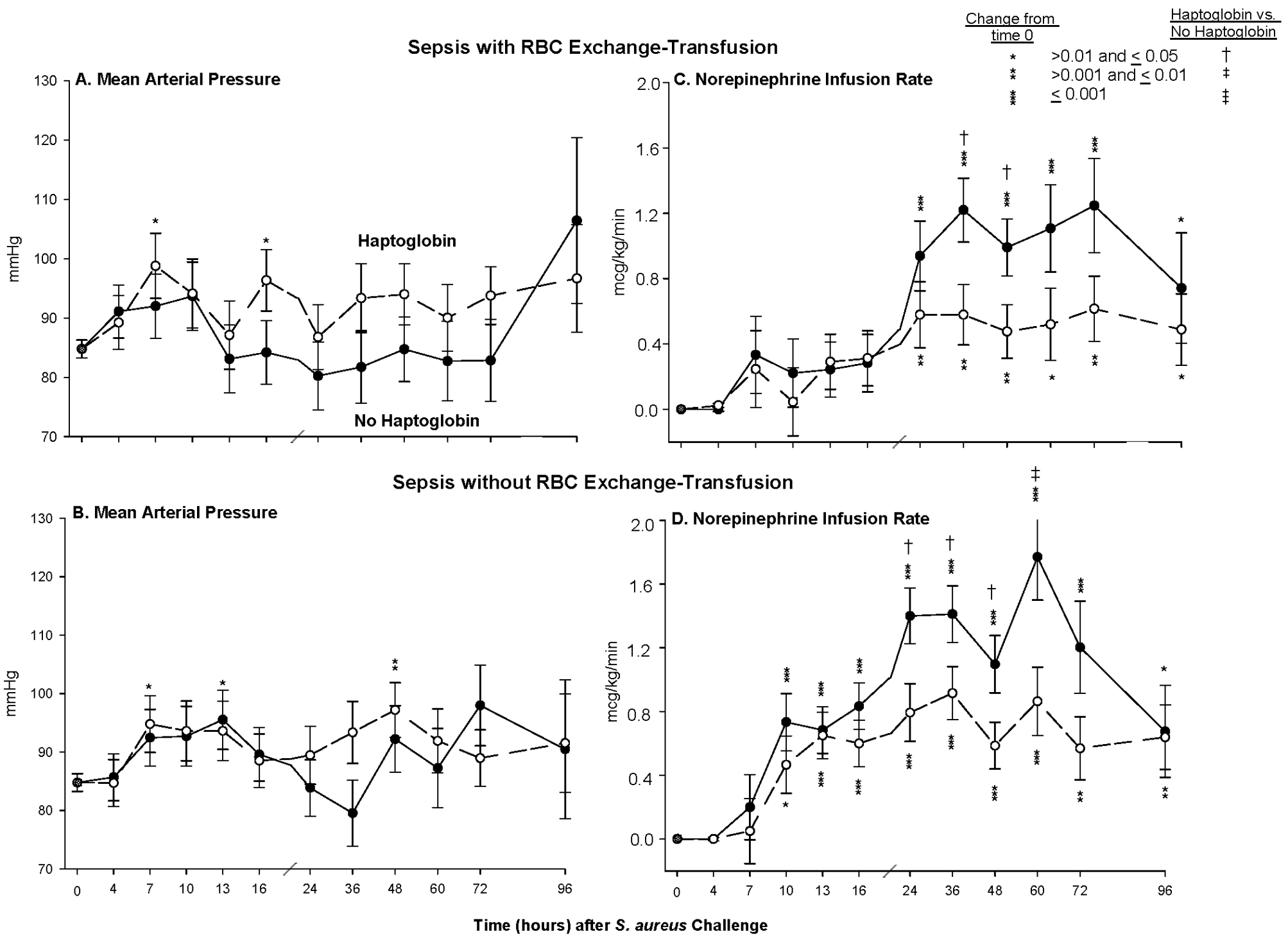
Mean transferrin bound iron and mean platelet counts were quantified over 96 hours after *S. aureus* challenge in canines receiving haptoglobin or no haptoglobin with or without RBC exchange-transfusion. P values greater than 0.05 is considered non-significant (NS).

Supplemental Table 5: Log IL 6, Log IL 10 and Log TNF Levels.

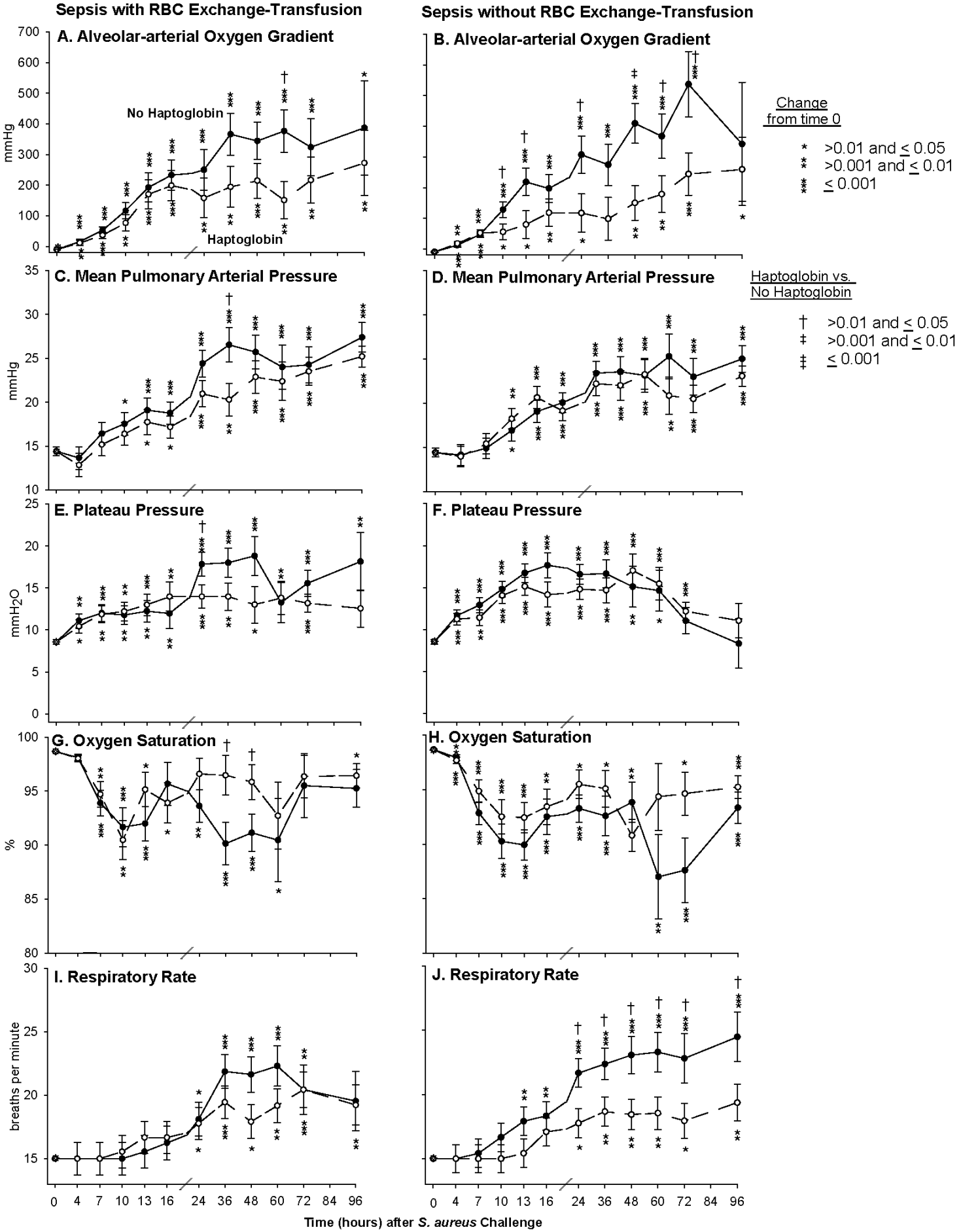
Mean IL 6, IL 10 and TNF levels were quantified over 24 hours after *S. aureus* challenge in canines receiving haptoglobin or no haptoglobin with or without RBC exchange-transfusion. P values greater than 0.05 is considered non-significant (NS).



Supplemental Figure 1: Haptoglobin Therapy Study Timeline Over the 96-hour duration of the Sepsis Study.
In the first of two sepsis experiments, 24 purpose-bred beagles (18 to 30 months old, 9 to 12.5 kg,) with S. aureus pneumonia were randomized to receive intravenous haptoglobin administration (800 mg/kg total dose, in two divided 100 mg/kg bolus doses at 4 and 7 h after infection followed immediately by a 600 mg/kg continuous infusion over 48 h) or an osmotically equivalent volume of human 25% albumin (control) +/- Blood transfusion. In a second sepsis experiment, 18 purpose-bred beagles (18 to 30 months old, 9 to 12.5 kg) with experimental S. aureus pneumonia were additionally exchanged-transfused with 80 mL/kg of 7-day-old stored canine universal donor blood in four divided doses (20 mL/kg) given sequentially every 3 h starting 4 h after infection but otherwise treated the same and given haptoglobin therapy as in the first experiment. Critical Care Supportive Treatments (antibiotics, vasopressors, fluids, ventilation) and diagnostic measurements (laboratory and hemodynamics) are serially obtained over the 96-hour duration.

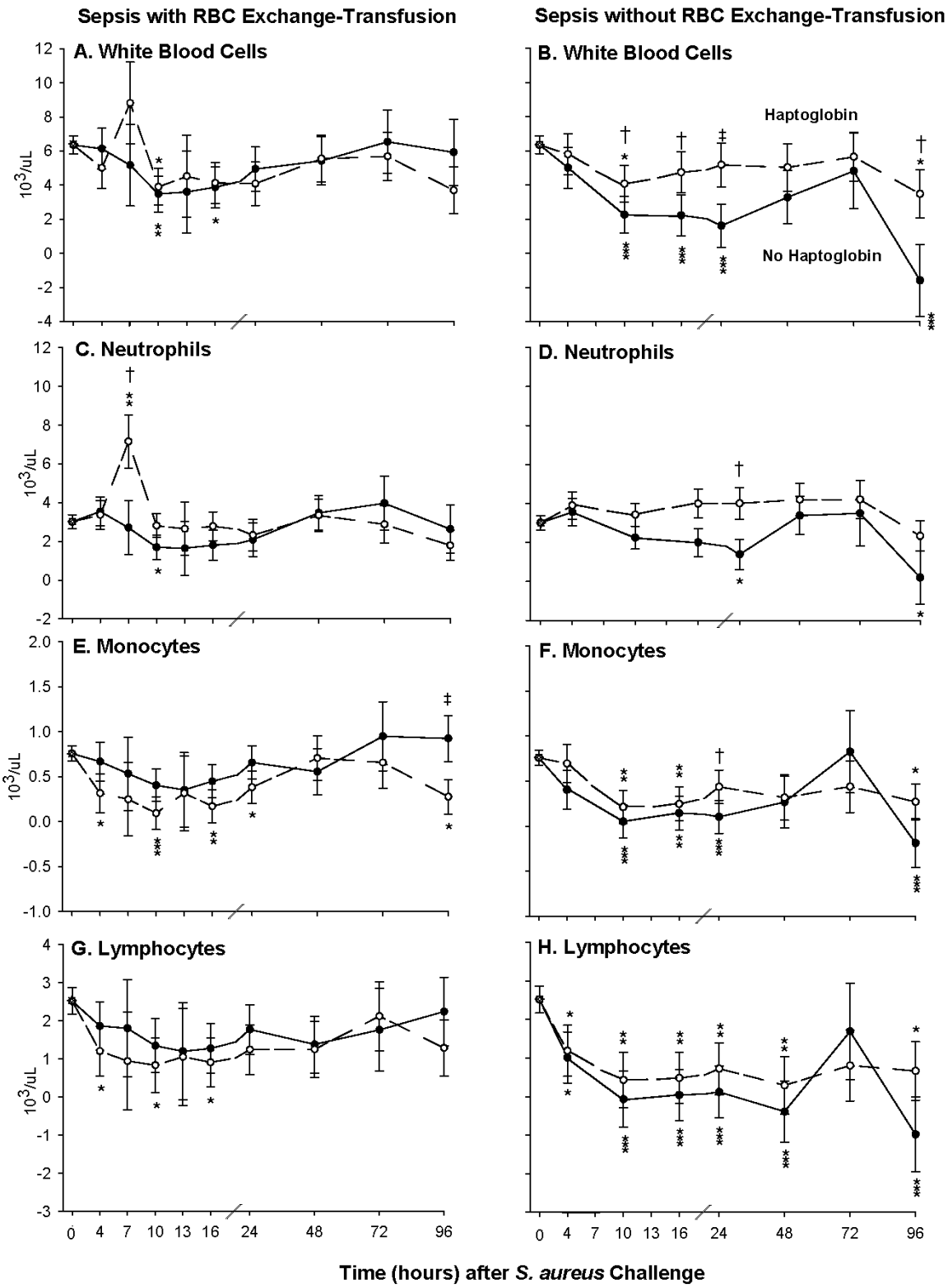


Supplemental Figure 2: Mean (± SE) Arterial Pressure and Norepinephrine Infusion Rates Over the 96-hour duration of the Sepsis Study at Serial Time Points. Individual components of the Shock Score (serial mean arterial pressure and norepinephrine requirements ±SE) are shown plotted over time (96 hours) after S. aureus challenge in canines receiving haptoglobin or no haptoglobin with (panels A and C) or without RBC exchange-transfusion (panels B and D). A higher norepinephrine rate requirement means that an animal required more vasopressor for maintenance of MAP. Changes from baseline are shown for each study group plotted from a common origin the mean value for animals at baseline. P values indicate significance in each group comparison in each panel and are denoted by asterisks (for changes over time) or crosses (comparing haptoglobin vs. no haptoglobin at each time point).



Supplemental Figure 3: Mean (± SE) Alveolar-arterial O2 Gradient, Mean Pulmonary Artery Pressure, Plateau Pressure, Oxygen Saturation, and Respiratory Rate Measurements Over the 96-hour duration of the Sepsis Study at Serial Time Points.

Individual components of the Lung Injury Score (serially mean AaO2, mean pulmonary artery pressure, plateau pressure oxygen saturation, and respiratory rate ±SE) are shown plotted over time (96 hours) after S. aureus challenge in canines receiving haptoglobin or no haptoglobin with (panels A, C, E, G, I) or without RBC exchange-transfusion (panels B, D, F, H, J). Changes from baseline are shown for each study group plotted from a common origin the mean value for animals at baseline. P values indicate significance in each group comparison in each panel and are denoted by asterisks (for changes over time) or crosses (comparing haptoglobin vs. no haptoglobin at each time point).



Supplemental Figure 4: White Blood Cell Count and Differential Over the 96-hour duration of the Sepsis Study at Serial Time Points.
White blood cell count (WBC) and cellular differential was performed over 96 hours after S. aureus challenge in canines receiving haptoglobin or no haptoglobin with (panels A, C, E, G) or without RBC exchange-transfusion (panels B, D, F, H). Changes from baseline are shown for each study group plotted from a common origin the mean value for animals at baseline. In animals with S. aureus pneumonia randomized to receive haptoglobin therapy and exchange-transfused RBCs vs. no haptoglobin therapy (septic controls) there were no significant differences in mean circulating WBC counts at all time points studied (panel A). In contrast, animals with S. aureus pneumonia and not exchange-transfused RBCs randomized to receive haptoglobin therapy vs. no haptoglobin therapy (septic controls) had significant increases in mean circulating WBC counts at 10, 16, 24, and 96 h (Panel B). The circulating WBC levels mean increases with haptoglobin treatment group vs. no haptoglobin therapy (septic controls) were significantly greater at 24 and 96 h with vs. without RBC exchange-transfusion (interaction). Preservation of WBC with haptoglobin therapy without exchange transfusion was present in varying degrees in all white blood cell lines, including neutrophils (Panel C and D), monocytes (Panel E and F) and lymphocytes (Panel G and H). Notably, this effect on circulating white blood cells seen only without exchange-transfusion of RBCs and thus cannot easily fully explain the similar beneficial effect of haptoglobin therapy during S. aureus pneumonia with and without RBC exchange transfusion on survival, shock score and LIS (i.e. the preservation of white blood cells was lost with exchange-transfusion, therefore is more likely a beneficial outcome diminished by mixing with exchange-transfusion of RBCs rather than a necessary protective mechanism of haptoglobin therapy during sepsis). The P values indicate significance in each group comparison in each panel and are denoted by asterisks (for changes over time) or crosses (comparing haptoglobin vs. no haptoglobin at each time point).

e-Table 1	Haptoglobin	P-Value vs. Time (0h)	No Haptoglobin	P-Value vs. Time (0h)	Time(h)	P-Value Haptoglobin vs. No Haptoglobin
Creatine(mg/dL) Mean (±SE)						
RBC Exchange Transfusion	0.53(± 0.02)	NS	0.52(± 0.02)	NS	4	NS
	0.46(± 0.03)	0.0004	0.51(± 0.03)	NS	10	NS
	0.52(± 0.07)	NS	0.52 (± 0.07)	NS	16	NS
	0.44(± 0.13)	NS	0.56(± 0.13)	NS	24	NS
	0.58(± 0.05)	NS	0.68(± 0.05)	0.01	48	NS
	0.49(± 0.06)	NS	0.62(± 0.10)	NS	72	NS
	0.59(± 0.05)	NS	0.45(± 0.08)	NS	96	NS
No RBC Exchange Transfusion	0.48(± 0.02)	<.0001	0.49 (± 0.02)	0.0001	4	NS
	0.45(± 0.02)	<.0001	0.42(± 0.02)	<.0001	10	NS
	0.62(± 0.06)	NS	0.48(± 0.06)	NS	16	NS
	0.67(± 0.11)	NS	0.75(± 0.11)	NS	24	NS
	0.48(± 0.04)	NS	0.54(± 0.05)	NS	48	NS
	0.41(± 0.07)	0.02	0.38(± 0.10)	NS	72	NS
	0.41(± 0.05)	0.003	0.52(± 0.07)	NS	96	NS
Blood Urea Nitrogen (mg/dL) Mean (±SE)						
RBC Exchange Transfusion	9.9(± 0.5)	0.004	9.5(± 0.5)	0.0002	4	NS
	10.0(± 0.8)	NS	9.1(± 0.8)	0.003	10	NS
	13.7(± 1.7)	NS	10.7(± 1.7)	NS	16	NS
	15.0(± 2.9)	NS	11.1(± 3.0)	NS	24	NS
	11.8(± 1.7)	NS	11.3(± 1.8)	NS	48	NS
	10.7(± 2.3)	NS	12.2(± 3.1)	NS	72	NS
	14.9(± 3.0)	NS	14.0(± 4.4)	NS	96	NS
No RBC Exchange Transfusion	9.7(± 0.5)	0.0004	9.4(± 0.5)	<.0001	4	NS
	8.5(± 0.7)	<.0001	7.8(± 0.7)	<.0001	10	NS
	9.1(± 1.5)	NS	7.9(± 1.5)	0.02	16	NS
	11.5(± 2.5)	NS	12.0(± 2.5)	NS	24	NS
	8.8(± 1.5)	NS	9.8(± 1.7)	NS	48	NS
	10.5(± 2.2)	NS	10.0(± 3.1)	NS	72	NS
	12.9(± 2.9)	NS	12.9(± 4.1)	NS	96	NS
Alanine Transaminase (mg/dL) Mean (±SE)						
RBC Exchange Transfusion	31.6(± 7.3)	NS	37.5(± 7.3)	NS	4	NS
	40.2(± 9.7)	NS	33.9(± 9.6)	NS	10	NS
	27.4(± 8.4)	NS	26.9(± 8.6)	NS	16	NS
	24.3(± 8.4)	NS	22.0(± 8.4)	NS	24	NS
	40.6(± 14.1)	NS	24.6(± 14.0)	NS	48	NS
	25.5(± 11.0)	NS	20.1(± 15.2)	NS	72	NS
	39.0(± 27.1)	NS	19.1(± 41.7)	NS	96	NS
No RBC Exchange Transfusion	32.5(± 6.4)	NS	29.4(± 6.4)	NS	4	NS
	35.2(± 8.4)	NS	26.4(± 8.4)	NS	10	NS
	30.7(± 7.5)	NS	21.7(± 7.4)	NS	16	NS
	25.8(± 7.3)	NS	19.7(± 7.1)	NS	24	NS
	23.5(± 11.9)	NS	30.7(± 13.8)	NS	48	NS
	22.4(± 10.9)	NS	19.3(± 15.2)	NS	72	NS
	44.3(± 27.0)	NS	27.6(± 37.9)	NS	96	NS
Asparate Aminotransferase (mg/dL) Mean (±SE)						
RBC Exchange Transfusion	32.3(± 5.0)	NS	34.6(± 5.0)	NS	4	NS
	32.8(± 6.6)	NS	29.2(± 6.6)	NS	10	NS
	24.8(± 5.6)	NS	21.7(± 5.8)	NS	16	NS
	24.9(± 6.8)	NS	17.2(± 6.7)	NS	24	NS
	17.3(± 10.9)	NS	36.7(± 11.3)	NS	48	NS
	16.7(± 9.0)	NS	27.2(± 13.6)	NS	72	NS
	57.7(± 26.9)	NS	38.1(± 43.9)	NS	96	NS
No RBC Exchange Transfusion	33.5(± 4.3)	NS	36.4(± 4.3)	NS	4	NS
	28.0(± 5.7)	NS	33.0(± 5.7)	NS	10	NS
	20.0(± 5.0)	NS	36.5(± 4.8)	NS	16	0.02
	19.1(± 5.7)	NS	34.5(± 5.5)	NS	24	NS
	21.4(± 8.9)	NS	27.7(± 10.9)	NS	48	NS
	20.3(± 9.2)	NS	31.9(± 13.5)	NS	72	NS
	51.1(± 27.2)	NS	34.4(± 39.1)	NS	96	NS
Lactate Dehydrogenase (mg/dL) Mean (±SE)						
	63.6(± 10.7)	NS	70.1(± 10.5)	NS	4	NS

RBC Exchange Transfusion	82.7(± 20.0)	NS	114.7(± 19.9)	0.01	10	NS
	106.5(± 42.2)	NS	181.4(± 44.5)	0.009	16	NS
	80.6(± 15.9)	NS	95.6(± 15.3)	0.04	24	NS
	44.2(± 25.5)	NS	132.4(± 25.6)	0.009	48	0.01
	120.4(±28.9)	NS	239.5(±46.0)	0.0002	72	0.03
	105.1(± 27.3)	NS	120.5(± 46.1)	NS	96	NS
No RBC Exchange Transfusion	45.9(± 9.7)	NS	43.8(± 9.7)	0.04	4	NS
	84.8(± 17.6)	NS	60.1(± 17.6)	NS	10	NS
	77.6(± 38.1)	NS	79.8(± 36.6)	NS	16	NS
	51.5(± 13.4)	NS	39.5(± 13.0)	NS	24	NS
	78.3(± 20.3)	NS	58.4(± 25.5)	NS	48	NS
	75.5(±30.6)	NS	77.7(±46.0)	NS	72	NS
	116.4(± 27.5)	NS	78.6(± 38.9)	NS	96	NS
Total Bilirubin (mg/dL) Mean (±SE)						
RBC Exchange Transfusion	0.17(± 0.04)	NS	0.19(± 0.04)	NS	4	0.03
	0.44(± 0.08)	0.006	0.42(± 0.08)	0.01	10	NS
	0.73(± 0.36)	NS	0.65(± 0.38)	NS	16	NS
	1.09(± 0.31)	0.005	0.62(± 0.32)	NS	24	NS
	1.79(± 0.58)	0.008	1.18(± 0.59)	NS	48	NS
	0.95(±0.25)	0.003	0.90(±0.38)	NS	72	NS
	1.27(± 0.38)	0.006	0.83(± 0.63)	NS	96	NS
No RBC Exchange Transfusion	0.17(±-0.03)	NS	0.27(±-0.03)	NS	4	0.03
	0.27(± 0.07)	NS	0.32(± 0.07)	NS	10	NS
	0.77(± 0.31)	NS	0.47(± 0.31)	NS	16	NS
	0.50(± 0.27)	NS	1.15(± 0.26)	0.0004	24	NS
	0.88(± 0.49)	NS	1.30(± 0.62)	NS	48	NS
	0.46(±0.26)	NS	0.86(±0.38)	NS	72	NS
	0.75(± 0.38)	NS	1.28(± 0.55)	NS	96	NS

Supplemental e-Table 1: Renal and Hepatic Functional Measurements.

Indices of renal (BUN and creatinine) and hepatic (ALT, AST, LDH, and total bilirubin) function were quantified over 96 hours after *S. aureus* challenge in canines receiving haptoglobin or no haptoglobin with or without RBC exchange-transfusion. P values less than 0.05 considered non-significant (NS).

e-Table 2	Haptoglobin	P-Value vs. Time (0h)	No Haptoglobin	P-Value vs. Time (0h)	Time(h)	P-Value Haptoglobin vs. No Haptoglobin
Left Ventricular Ejection Fraction Mean (±SE)						
RBC Exchange Transfusion	50.2(± 2.1)	NS	49.3(± 2.0)	0.03	4	NS
	46.8(± 3.5)	NS	53.6(± 4.3)	NS	24	NS
	46.2(± 4.6)	NS	33.9(± 5.1)	0.0002	48	NS
	43.8(± 4.6)	0.03	38.1(± 6.4)	0.02	72	NS
	44.4(± 6.7)	NS	39.5(± 11.5)	NS	96	NS
No RBC Exchange Transfusion	49.5(± 1.4)	0.006	53.0(± 1.4)	NS	4	NS
	49.1(± 2.6)	NS	42.8(± 2.6)	<.0001	24	NS
	39.7(± 3.6)	0.0002	39.2(± 4.2)	0.0009	48	NS
	42.3(± 3.4)	0.002	41.9(± 5.3)	0.03	72	NS
	42.6(± 4.7)	0.02	32.6(± 6.7)	0.002	96	NS
Cardiac Output (L/min) Mean (±SE)						
RBC Exchange Transfusion	1.5(± .2)	NS	1.7(± .2)	NS	4	NS
	1.4(± .2)	NS	1.7(± .2)	NS	7	NS
	1.4(± .3)	NS	1.6(± .3)	NS	10	NS
	1.7(± .3)	NS	1.4(± .3)	NS	13	NS
	1.7(± .2)	NS	1.5(± .2)	NS	16	NS
	2.0(± .3)	NS	2.0(± .3)	NS	24	NS
	2.0(± .2)	NS	1.9(± .2)	NS	48	NS
	2.0(± .3)	NS	1.4(± .4)	NS	72	NS
	2.2(± .4)	NS	1.6(± .6)	NS	96	NS
No RBC Exchange Transfusion	1.2(± .2)	NS	1.6(± .2)	NS	4	0.006
	1.4(± .2)	NS	1.6(± .2)	NS	7	NS
	1.7(± .3)	NS	1.6(± .3)	NS	10	NS
	1.8(± .2)	NS	1.7(± .2)	NS	13	NS
	1.5(± .2)	NS	1.5(± .2)	NS	16	NS
	1.6(± .2)	NS	1.4(± .2)	NS	24	NS
	1.5(± .2)	NS	1.7(± .3)	NS	48	NS
	1.5(± .3)	NS	2.1(± .4)	NS	72	NS
	1.7(± .4)	NS	1.6(± .5)	NS	96	NS

Supplemental e-Table 2: Measures of Cardiac Function.

Cardiac function was assessed using mean left ventricular ejection fraction and mean cardiac output was assessed via echocardiography and pulmonary artery catheter thermodilution measurements over 96 hours after *S. aureus* challenge in canines receiving haptoglobin or no haptoglobin with or without RBC exchange-transfusion. P values greater than 0.05 considered non-significant (NS).

e-Table 3	Haptoglobin	P-Value vs. Time (0h)	No Haptoglobin	P-Value vs. Time (0h)	Time(h)	P-Value Haptoglobin vs. No Haptoglobin
Sodium (mmol/L) Mean (±SE)						
RBC Exchange Transfusion	148.1(± 1.3)	NS	148.2(± 1.3)	NS	4	NS
	147.7(± 3.1)	NS	148.3(± 3.1)	NS	10	NS
	149.3(± 3.3)	NS	149.0(± 3.4)	NS	16	NS
	148.9(± 3.4)	NS	147.9(± 3.5)	NS	24	NS
	151.1(± 3.2)	NS	148.1(± 3.3)	NS	48	NS
	149.0(± 3.7)	NS	149.8(± 4.4)	NS	72	NS
	145.0(± 7.2)	NS	149.2(± 9.4)	NS	96	NS
No RBC Exchange Transfusion	147.5(± 1.3)	NS	145.9(± 1.3)	NS	4	NS
	145.1(± 2.8)	NS	139.3(± 2.8)	0.003	10	NS
	144.9(± 3.1)	NS	139.8(± 3.1)	0.009	16	NS
	143.9(± 3.1)	NS	142.5(± 3.1)	NS	24	NS
	143.2(± 2.9)	NS	140.7(± 3.1)	0.02	48	NS
	143.6(± 3.6)	NS	138.0(± 4.3)	0.02	72	NS
	142.3(± 7.2)	NS	134.3(± 8.7)	NS	96	NS
Potassium (mmol/L) Mean (±SE)						
RBC Exchange Transfusion	4.0(± .1)	NS	3.9(± .1)	NS	4	NS
	3.8(± .6)	NS	4.6(± .6)	NS	10	NS
	3.5(± .5)	NS	4.4(± .5)	NS	16	NS
	3.4(± .4)	NS	4.0(± .4)	NS	24	NS
	3.7(± .4)	NS	4.6(± .4)	NS	48	NS
	3.7(± .3)	NS	3.7(± .5)	NS	72	NS
	3.4(± 1.1)	NS	3.3(± 1.7)	NS	96	NS
No RBC Exchange Transfusion	3.9(± .1)	NS	4.0(± .1)	NS	4	NS
	4.5(± .5)	NS	3.9(± .5)	NS	10	NS
	4.6(± .4)	NS	4.1(± .4)	NS	16	NS
	4.7(± .4)	NS	4.3(± .4)	NS	24	NS
	4.1(± .3)	NS	5.0(± .4)	NS	48	NS
	4.0(± .3)	NS	3.8(± .5)	NS	72	NS
	6.6(± 1.1)	0.01	4.5(± 1.4)	NS	96	NS
Chlorine (mmol/L) Mean (±SE)						
RBC Exchange Transfusion	122.0(± 1.3)	NS	123.8(± 1.3)	0.05	4	NS
	121.8(± 2.5)	NS	125.3(± 2.5)	NS	10	NS
	123.9(± 3.1)	NS	127.6(± 3.1)	0.04	16	NS
	125.6(± 3.3)	NS	129.3(± 3.4)	0.02	24	NS
	125.9(± 3.2)	NS	130.5(± 3.3)	0.005	48	NS
	123.3(± 3.9)	NS	130.6(± 4.9)	NS	72	NS
	121.2(± 5.2)	NS	128.9(± 7.1)	NS	96	NS
No RBC Exchange Transfusion	122.6(± 1.2)	NS	120.9(± 1.2)	NS	4	NS
	122.4(± 2.2)	NS	118.1(± 2.2)	NS	10	NS
	124.7(± 2.7)	NS	121.6(± 2.7)	NS	16	NS
	126.7(± 2.9)	NS	127.0(± 2.9)	0.049	24	NS
	126.6(± 2.7)	NS	126.5(± 3.)	NS	48	NS
	126.0(± 3.7)	NS	122.6(± 4.7)	NS	72	NS
	124.7(± 4.9)	NS	121.6(± 6.5)	NS	96	NS
Glucose (mg/dL) Mean (±SE)						
RBC Exchange Transfusion	106.9(± 7.4)	NS	114.8(± 7.3)	NS	4	NS
	111.8(± 7.8)	NS	130.7(± 7.7)	0.01	10	0.04
	134.4(± 12.3)	NS	147.4(± 12.7)	0.004	16	NS
	140.0(± 12.)	0.01	148.6(± 12.1)	0.002	24	NS
	158.6(± 15.9)	0.003	169.4(± 15.5)	0.0002	48	NS
	129.9(± 19.2)	NS	172.7(± 28.3)	0.03	72	NS
	125.4(± 14.8)	NS	171.4(± 23.5)	0.01	96	NS

No RBC Exchange Transfusion	119.7(± 6.7)	NS	120.5(± 6.7)	NS	4	NS
	129.0(± 7.1)	0.01	123.2(± 7.1)	NS	10	NS
	148.7(± 10.8)	0.0006	132.3(± 10.8)	0.046	16	NS
	158.2(± 10.5)	<.0001	146.5(± 10.3)	0.0006	24	NS
	160.2(± 13.2)	0.0002	173.6(± 15.8)	<.0001	48	NS
	187.0(± 19.6)	0.0001	188.7(± 28.4)	0.007	72	NS
	161.7(± 14.9)	0.0007	148.8(± 21.)	NS	96	NS

Supplemental e-Table 3: Serum Electrolyte Measurements.

Sodium, potassium, chlorine, and glucose are measured over 96 hours after *S. aureus* challenge in canines receiving haptoglobin or no haptoglobin with or without RBC exchange-transfusion. P values greater than 0.05 considered non-significant (NS).

e-Table 4	Haptoglobin	P-Value vs. Time (0h)	No Haptoglobin	P-Value vs. Time (0h)	Time(h)	P-Value Haptoglobin vs. No Haptoglobin
Transferrin Bound Iron (μM) Mean (±SE)						
RBC Exchange Transfusion	5.0(± 1.6)	NS	6.4(± 1.6)	NS	4	NS
	4.3(± 1.6)	NS	1.8(± 1.6)	0.0007	7	NS
	2.2(± 1.3)	0.0002	0.0(± 1.3)	<.0001	10	0.01
	2.9(± 1.3)	0.002	-1.0(± 1.3)	<.0001	13	0.0003
	1.7(± 1.3)	<.0001	-0.1(± 1.4)	<.0001	16	NS
	6.0(± 2.7)	NS	-0.8(± 2.9)	0.006	24	NS
	1.1(± 1.5)	<.0001	-0.9(± 1.5)	<.0001	36	NS
	1.1(± 1.3)	<.0001	2.5(1.4)	0.001	48	NS
	3.9(± 1.5)	0.03	4.3(± 1.6)	NS	60	NS
	4.5(± 2.5)	NS	5.2(3.4)	NS	72	NS
	8.8(± 1.9)	NS	10.1(± 2.8)	NS	96	NS
No RBC Exchange Transfusion	6.0(± 1.5)	NS	6.3(± 1.5)	NS	4	NS
	5.2(± 1.5)	NS	4.2(± 1.5)	0.048	7	NS
	3.7(± 1.2)	0.006	2.9(± 1.2)	0.0007	10	NS
	3.1(± 1.3)	0.002	2.6(± 1.3)	0.0005	13	NS
	3.2(± 1.3)	0.002	2.6(± 1.3)	0.0005	16	NS
	4.2(± 2.6)	NS	3.3(± 2.4)	NS	24	NS
	4.5(± 1.4)	NS	3.3(± 1.5)	0.01	36	NS
	4.0(± 1.3)	0.02	3.8(± 1.4)	0.02	48	NS
	4.6(± 1.4)	NS	4.7(± 1.6)	NS	60	NS
	6.6(± 2.5)	NS	6.0(± 3.4)	NS	72	NS
	5.1(± 1.9)	NS	5.0(± 2.5)	NS	96	NS
Platelet Counts (103/uL) Mean (±SE)						
RBC Exchange Transfusion	243.9(± 34.8)	0.01	246.9(± 34.2)	0.01	4	NS
	365.5(± 57.1)	NS	226.8(± 56.6)	NS	7	NS
	178.0(± 38.4)	<.0001	234.1(± 37.8)	0.01	10	NS
	157.1(± 56.7)	0.002	233.4(± 56.4)	NS	13	NS
	120.0(± 40.0)	<.0001	195.0(± 40.3)	0.0009	16	NS
	139.0(± 45.8)	<.0001	174.4(± 46.6)	0.0009	24	NS
	105.8(± 35.2)	<.0001	139.0(± 35.8)	<.0001	48	NS
	90.2(± 37.7)	<.0001	132.0(± 45.3)	<.0001	72	NS
	87.8(± 42.0)	<.0001	176.3(± 56.2)	0.006	96	NS
No RBC Exchange Transfusion	287.2(± 34.5)	NS	275.6(± 34.5)	NS	4	NS
	Not Collected				7	NS
	294.1(± 37.7)	NS	332.9(± 37.7)	NS	10	NS
	Not Collected				13	NS
	295.7(± 39.8)	NS	309.5(± 39.5)	NS	16	NS
	220.8(± 45.6)	0.02	274.7(± 44.5)	NS	24	NS
	143.1(± 35.6)	<.0001	180.9(± 38.3)	0.0001	48	NS
	153.4(± 38.2)	<.0001	175.1(± 52.3)	0.003	72	NS
	149.7(± 42.4)	<.0001	169.2(± 61.6)	0.009	96	NS

Supplemental e-Table 4: Transferrin Bound Iron and Platelet Count.

Mean transferrin bound iron and mean platelet counts were quantified over 96 hours after *S. aureus* challenge in canines receiving haptoglobin or no haptoglobin with or without RBC exchange-transfusion. P values greater than 0.05 is considered non-significant (NS).

e-Table 5	Haptoglobin	P-Value vs. Time (0h)	No Haptoglobin	P-Value vs. Time (0h)	Time(h)	P-Value Haptoglobin vs. No Haptoglobin
Log_{10}IL6(pg/ml) Mean (±SE)						
RBC Exchange Transfusion	2.26(±0.25)	NS	2.31(±0.25)	NS	4	NS
	2.55(±0.27)	NS	2.53(±0.27)	NS	16	NS
	2.36(±0.32)	NS	2.49(±0.32)	NS	24	NS
No RBC Exchange Transfusion	2.10(±0.14)	NS	2.17(±0.14)	NS	4	NS
	2.00(±0.15)	NS	2.25(±0.15)	NS	16	NS
	2.27(±0.20)	NS	2.23(±0.19)	NS	24	NS
Log_{10}IL10(pg/ml) Mean (±SE)						
RBC Exchange Transfusion	1.59(±0.17)	NS	1.62(±0.17)	NS	4	NS
	1.59(±0.18)	NS	1.59(±0.18)	NS	16	NS
	1.50(±0.18)	NS	1.69(±0.18)	NS	24	NS
No RBC Exchange Transfusion	1.38(±0.10)	NS	1.63(±0.10)	NS	4	0.04
	1.40(±0.11)	NS	1.71(±0.11)	NS	16	0.02
	1.41(±0.11)	NS	1.72(±0.11)	NS	24	0.02
Log_{10}TNFα(pg/ml) Mean (±SE)						
RBC Exchange Transfusion	1.15(±0.39)	NS	1.23(±0.39)	NS	4	NS
	1.22(±0.37)	NS	1.14(±0.37)	NS	16	NS
	1.10(±0.36)	NS	1.26(±0.36)	NS	24	NS
No RBC Exchange Transfusion	0.85(±0.23)	NS	1.39(±0.23)	NS	4	NS
	0.85(±0.22)	NS	1.36(±0.22)	NS	16	NS
	1.05(±0.22)	NS	1.13(±0.21)	NS	24	NS

Supplemental e-Table 5: Log IL 6, Log IL 10 and Log TNF Levels.

Mean IL 6, IL 10 and TNF levels were quantified over 24 hours after *S. aureus* challenge in canines receiving haptoglobin or no haptoglobin with or without RBC exchange-transfusion. P values greater than 0.05 is considered non-significant (NS).