

CANINE PRACTICE — INFECTIOUS DISEASE

Out of 51 puppies testing positive for parvovirus, 30 were divided into three groups of ten each for antiserum treatment. A significant difference ($p < 0.01$) was found between the survival rate of the antiserum treatment group (83%) and control group (52%). Survival rates were 70, 90, and 90%, in the 1, 2, and 4 ml antiserum per lb treatment groups, respectively. *Escherichia coli* were isolated from 61% of the antiserum-treated puppies, suggesting that gram-negative endotoxins are important contributors to complications of parvoviral infections. No anaphylactic responses were noted.

Clinical Experience With Cross-Protective Anti-Endotoxin Antiserum in Dogs with Parvoviral Enteritis

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Introduction

Anti-endotoxin antibodies have provided cross-protection to swine,¹ cattle,²⁻⁴ and horses^{5,6} suffering from gram-negative septicemias. *Escherichia coli*, *Salmonella* sp., *Shigella* sp., *Klebsiella* sp., *Pseudomonas* sp., *Haemophilus* sp., and other gram-negative bacteria produce deadly endotoxins and are, therefore, serious threats to domestic animals. A recent retrospective study yielded a 90% isolation rate of *E. coli* from the livers and/or lungs of dogs with parvovirus-positive accessions to a state diagnostic laboratory.⁷ Results of that report and the findings of the investigators cited previously led to the hypothesis that the cross-protective antibodies produced in response to a mutant *Salmonella typhimurium* bacterin-toxoid could provide an improvement in the survival rate of puppies suffering from the gram-negative endotoxin complications of canine parvoviral enteritis.

The purpose of this study was to determine if gram-negative organisms were the preponderant secondary invaders following parvoviral enteritis and to investigate whether or not cross-protective antibodies provided by antiserum (Endoserum®:

Immvac®, Inc., Columbia, Mo.) would protect parvoviral enteritis puppies from endotoxins.

Materials and Methods

A total of 51 parvo/enteritis puppies were administered routine therapies always including 10 mg/lb of body weight of intravenously (IV) administered cephalosporin, isotonic lactated Ringer's solution to correct blood volume deficits, the isotonic combination of lactated Ringer's solution and dextrose to correct blood volume and energy source deficits as needed, and 1 mg/kg of body weight of promazine hydrochloride intramuscularly (IM) as needed to reduce intestinal motility.

Thirty of the puppies were randomly divided into three groups of ten each, and were treated with equine-origin anti-endotoxin antiserum as follows:

- Group 1 — 1 ml/lb of body weight;
- Group 2 — 2 ml/lb of body weight; and
- Group 3 — 4 ml/lb of body weight.

The antiserum was diluted at a ratio of at least 1:1 with either lactated Ringer's solution or lactated Ringer's/dextrose solution. Responses of the 30 antiserum-treated puppies were compared to the

21 control-group puppies in terms of whether the puppies lived or died, and whether or not the equine-derived antiserum could be safely administered to dogs. The safety of the antiserum was evaluated by development of any clinical signs of anaphylaxis.

Fecal samples taken via sterile rectal swabs were tested to determine whether or not parvovirus was present via enzyme immunoassay (CITE® Canine Parvovirus Test Semi-Quant™: IDEXX Corp., Portland, Maine) and cultured to identify the species of secondary bacterial invaders.

All of the 28 fecal samples retrieved from the antiserum-treated puppies were examined for frank blood, leukocytes, and ovarian parasites; and homogenized and plated on:

- MacConkey's agar to differentiate lactose fermenting Enterobacteriaceae from the nonlactose fermenters *Salmonella* sp. and *Shigella* sp.;
- xylose-lysine-deoxycholate (XLD) and Hektoen (HE) to differentiate coliforms (especially *E. coli* which appear as yellow lactose fermenting colonies on XLD and salmon-yellow colonies on HE); and
- 5% sheep blood (BA) and phenyl-ethyl-alcohol (PEA) plates to isolate *Streptococci* sp., *Staphylococci* sp., and yeasts.

All plates were streaked for isolation, incubated at 37°C in 6% CO₂, and examined at 24, 48, and 72 hours' incubation. The specimens also were streaked for isolation on Campy blood agar (plates incubated at 37°C and 42°C in Campy-Pak jars for vibrio-forms; and on chenodeoxycholate [CDC] anaerobic blood plates incubated anaerobically for isolation of *Clostridium* sp.).

Species identifications were determined by Gram staining, conventional biochemical tests, and the automated API20E® and AN-IDENT™ systems (API® Analytab Products, Inc., Plainview, N.Y. A division of Sherwood Medical Co., St. Louis).

In terms of statistical analysis, the predetermined, acceptable probability level was 0.05 or less. The death rates (survival rates) versus treatment levels were subjected to logistic regression analysis¹¹ via a JMP® software computer program (SAS Institute, Inc., Cary, N.C.).

Results

There was a statistically significant ($p < 0.01$) relationship between the survival rate and antiserum treatment levels (Fig. 1). Survival rate of the antiserum-treated puppies was 83% (25 of 30) compared to 52% (11 of 21) in the conventionally treated group. Death rate of the antiserum-treated group was 17% (5 of 30) compared to 48% (10 of

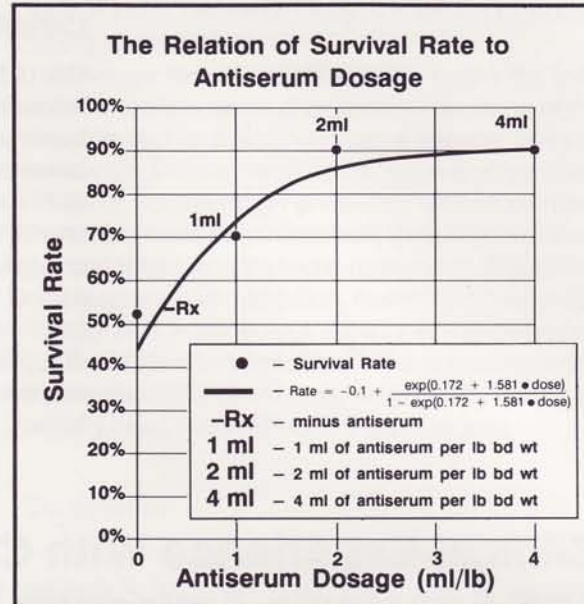


FIG. 1 — The survival rate of puppies was compared to various antiserum dosage levels via logistic regression analysis. A least squares sigmoid curve fitted to the data also suggested a highly significant ($p < 0.01$) relationship, and that the 4 ml/lb dose of antiserum maximized its effect.

21) in the conventionally treated group. There was a 70% survival rate in the group of puppies treated with antiserum at 1 ml/lb, a 90% survival rate in the 2 ml/lb group, and a 90% survival rate in the 4 ml/lb group. There was a 30% (1 ml/lb), 10% (2 ml/lb), and 10% (4 ml/lb) death rate in the respective treatment groups (Table 1).

All puppies in the control and treatment groups tested positive for the presence of parvovirus in their gastrointestinal tracts. *E. coli* was isolated from 61% of the puppies cultured. *Streptococcus* sp. (43%), *Bacteroides fragilis* (22%), *Clostridium* sp. (22%), *Proteus* sp. (3%), and *Shigella* sp. (3%) were also isolated from those puppies cultured. Two species of bacteria were isolated from 11 dogs, and 3 species of bacteria from 2 dogs, of the 28 dogs cultured.

The 1 ml/lb, 2 ml/lb, and 4 ml/lb dosage rates of antiserum did not result in any anaphylactic or anaphylactoid reactions, i.e., there were no incidences of systemic responses or localized heat, swelling, or pain observed in the dogs following antiserum administration.

Discussion

A significant correlation between survival rate and antiserum administration was demonstrated.

TABLE 1
Characteristics of Parvo/Enteritis Puppies

Antiserum Treatment	Case Number	Sex	Weight (lbs)	Age (wks)	Breed (X if Cross)	EC ^a	PR ^b	SH ^c	SP ^d	SF ^e	BF ^f	CL ^g	Days (Hosp)	Final Result
0 ml/lb	1	F	12.00	9	Basset								7	Died
	2	M	9.50	9	Basset								8	Lived
	3	M	7.75	7	Mix								3	Died
	4	F	7.75	10	Rott								6	Died
	5	F	40.00	24	Lab								5	Lived
	6	M	17.00	13	Dobe								5	Died
	7	F	14.00	10	Shep								3	Lived
	8	F	36.20	24	Dalmation								4	Lived
	9	M	10.00	10	Cocker								4	Died
	10	M	3.00	7	Scottie								6	Lived
	11	M	35.00	16	LabX								6	Lived
	12	M	6.00	12	Mix								4	Died
	13	F	1.00	8	Poodle								3	Died
	14	F	28.00	24	LabX								5	Lived
	15	F	8.00	9	Pitbull								4	Died
	16	F	11.00	8	Pitbull								7	Lived
	17	M	14.00	8	Basset								5	Lived
	18	F	25.00	12	DobeX								5	Died
	19	M	7.75	8	LabX								4	Lived
	20	F	58.00	24	Gold Ret								3	Lived
	21	F	15.00	8	ShepX								3	Died
1 ml/lb	1	F	2.50	7	Min Schnau	+	-	-	+	-			5	Died
	2	F	33.50	15	Gt Dane	+	-	-	+	-			5	Lived
	3	M	32.00	16	LabX Shep	+	-	-	-	-			4	Lived
	4	F	25.00	16	LabX Shep	-	-	-	+	-			4	Lived
	5	F	78.00	40	RottX	+	-	-	-	-			4	Lived
	6	M	5.00	12	Aust ShepX	+	-	-	-	-			6	Lived
	7	F	43.00	20	Rott	+	-	+	-	+			3	Lived
	8	F	16.60	11	Boavdeflau	+	-	-	+	-			3	Lived
	9	M	14.50	13	ShepX	-	-	-	+	-			3	Died
	10	M	15.50	32	LhasaX	+	-	-	-	-			4	Died
2 ml/lb	1	F	18.00	12	Shep	+	-	-	-	-	+	-	6	Lived
	2	M	11.00	8	Dobe	+	-	-	-	-	+	-	6	Lived
	3	F	32.00	16	LabX	-	-	-	+	-	-	-	3	Lived
	4	F	9.00	9	Maltese	-	-	-	-	-	-	-	4	Lived
	5	F	4.70	20	Shitzu	-	-	-	+	-	-	+	6	Lived
	6	F	2.00	8	Chi	-	-	-	+	-	-	-	3	Died
	7	M	25.00	12	RottX	+	-	-	-	-	-	-	5	Lived
	8	F	8.50	9.5	Mix	+	+	-	-	-	+	-	4	Lived
	9	F	2.00	8	Chi								5	Lived
	10	F	10.50	16	Mix								5	Lived
4 ml/lb	1	F	13.00	16	CockerX	-	-	-	-	-	-	-	4	Lived
	2	F	35.00	16	Lab	+	-	-	-	-	-	-	5	Lived
	3	F	30.00	16	Lab	-	-	-	-	-	-	+	6	Lived
	4	M	45.00	24	ShepX Lab	+	-	-	+	-	-	-	4	Lived
	5	M	28.00	3.5	Irish Set	+	-	-	-	-	-	-	7	Lived
	6	M	4.00	8	Poodle	-	-	-	+	-	-	+	7	Lived
	7	F	18.00	12	Spitz	+	-	-	+	-	-	+	3	Lived
	8	F	1.50	6	York	-	-	-	+	-	+	-	3	Died
	9	M	12.00	8	BeagleX	-	-	-	-	-	-	-	7	Lived
	10	F	15.00	9	Rott	+	-	-	-	-	-	-	6	Lived

^aEC = *Escherichia coli*
^bPR = *Proteus* sp.
^cSH = *Shigella* sp.
^dSP = *Streptococcus* sp.
^eSF = *Staphylococcus* sp.
^fBF = *Bacteriodes fragilis*
^gCL = *Clostridium* sp.

Analysis of the dosage level data suggested that there was not a significant difference in the survival rate of the group that received 2 ml/lb of antiserum and the group that was administered 4 ml/lb. A dosage recommendation of more than 2 ml/lb of the antiserum does not appear to be necessary; however, the possible therapeutic advantage of repeating the administration of the antiserum at any of the dosage rates was not pursued in this study.

The fact that all of the puppies tested in this study were positive to the enzyme immunoassay test confirmed that parvovirus was constant among the puppies studied. The 61% recovery rate of *E. coli* from the cultured puppies confirms the findings of Turk and coworkers⁷ that coliform organisms are the primary secondary invaders of parvovirus infection in dogs.

Because gram-negative organisms are the most common secondary invaders following parvovirus infections, the chances for developing gram-negative endotoxemia are very high. Therefore, the administration of antiserum containing anti-endotoxin antibodies was rational when one considers that gram-negative endotoxins are produced both during the rapid-growth phase and upon the kill-off of gram-negative bacteria.

The mechanisms responsible for the efficacy demonstrated by the results of this study may not be completely explained by the fact that the antiserum contains cross-protective anti-endotoxin antibodies. For instance, infusion of Tumor Necrosis Factor (TNF) into dogs,⁸ sheep,⁹ and calves¹⁰ has resulted in reproduction of the signs associated with IV administration of endotoxins. The cardiovascular depression characteristic of toxic shock may be induced by either gram-negative or gram-positive toxins inducing effector cells to release TNF-alpha,⁸ and human immunoglobulin has been shown to suppress TNF-alpha production when administered to rabbits subsequently challenged with endotoxin.¹¹ The antiserum used in this study will subsequently be analyzed to determine the level of anti-TNF-alpha antibodies present.

The 1 ml/lb, 2 ml/lb, and 4 ml/lb dosage rates of the antiserum did not result in any anaphylactic or anaphylactoid reactions. However, one should always have ready access to epinephrine and other appropriate therapeutic agents when using equine-origin antiserum or any other biological product.

The simultaneous administration of antiserum and antibiotics is rational from the standpoint of treating the possible gram-positive as well as gram-negative bacterial invaders. When antibiotics kill gram-negative organisms, however, endo-

toxin is often released, thereby initiating or potentiating endotoxemia.

Although repeat administration might be indicated in severe cases of endotoxemia, none of the puppies in this study received a second dose of antiserum. If the administration interval of repeated doses of equine-origin antiserum was 10 or more days after initial administration, then one could expect recipient anaphylactic responses. Repetition of antiserum administration, however, would probably be safe if carried out within 5 to 7 days following the initial administration.¹³ The outcome of any parvoenteritis/endotoxemia emergency case has usually been determined in 7 days or less.

Conclusion

The equine-origin antiserum, as used in this study, appears to be safe and effective in the clinical management of secondary gram-negative endotoxemias associated with parvoviral enteritis in dogs. The cross-protective anti-endotoxin antibodies appear to account for at least part of the efficacy of the antiserum but other factors that may contribute to its effectiveness remain to be determined. ■

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