

- c. Clements, Sydney, Australia.
- d. Merck, Johannesburg, South Africa.
- e. Nikon, Tokyo, Japan.

References

1. Campbell TW, Coles EH: 1986, Avian hematology and blood chemistry. *In: Veterinary clinical pathology*, ed. Coles EH, 4th ed. pp. 279–301. WB Saunders, Philadelphia, PA.
2. De Villiers OT: 1938, Blood of ostrich. *Onderstepoort J Vet Sci Anim Ind* 11:419–503.
3. Fallaw SA, Jones JE, Hughes BL: 1976, Hematocrit, erythrocyte and hemoglobin values for male and female guineas at various ages. *Poult Sci* 55:814–816.
4. Gessaman JA, Johnson JA, Hoffman SW: 1986, Hematocrits and erythrocyte numbers for Cooper's and sharp-skinned hawks. *Condor* 000:95–96.
5. Hartman FA, Lessler MA: 1963, Erythrocyte measurements in birds. *Auk* 80:467–473.
6. Hawkey C, Hart MG, Samour HJ, et al. 1984, Hematological findings in healthy and sick captive flamingos (*Phenicopterus ruber ruber*). *Avian Pathol* 18:163–172.
7. Hawkey C, Hart MG, Samour HJ: 1984, Age related hematological changes and hematological changes and hemopathological responses in Chilean flamingos (*Phoenicopterus chilensis*). *Avian Pathol* 18:223–229.
8. Hawkey C, Samour JH, Ashton DG, et al.: 1983, Normal and clinical hematology of captive cranes (Gruiformes). *Avian Pathol* 12:73–84.
9. Jain J: 1986, Hematologic techniques. *In: Schalm's veterinary haematology*, ed. Jain NC, 4th ed., pp. 20–86. Lea & Febiger, Philadelphia, PA.
10. Jain NC, Kono CS: 1975, Erythrocyte sedimentation rate in the dog and cat. Comparison of two methods and influence of packed cell volume, temperature and storage of blood. *J. Small Anim Pract* 16:671–678.
11. Kocan RM, Pitts SM: 1976, Blood values of the canvasback duck by age, sex and season. *J Wildl Dis* 12:341–346.
12. Leonard JL: 1982, Clinical laboratory examinations. *In: Diseases of cage and aviary birds*, ed. Petrak ML, pp. 269–303. Lea & Febiger, Philadelphia, PA.
13. Levi A, Perelman B, Waner T, et al.: 1977, Dimensions of erythrocytes of birds. *Ibis* 119:533–535.
14. Lucas AM, Jamroz C: 1961, Atlas of avian hematology. Agriculture Monograph 25. US Department of Agriculture, Washington, DC.
15. Maxwell MH: 1993, Avian blood leucocyte responses to stress. *Poult Sci* 49:34–42.
16. Nirmalan GP, Robinson GA: 1971, Hematology of the Japanese quail (*Coturnix coturnix japonica*). *Br Poult Sci* 12:475–481.
17. Oyewale JO: 1987, Hematological studies on apparently healthy Nigerian domestic chickens (*Gallus domesticus*). *Bull Anim Health Prod Afr* 35:108–112.
18. Palomeque J, Pinto D, Viscor G: 1991, Hematologic and blood chemistry values of the Masai ostrich (*Struthio camelus*). *J Wildl Dis* 27:34–40.
19. Peinado VI, Polo FJ, Viscor G, Palomeque J: 1992, Hematology and blood chemistry values for several flamingo species. *Avian Pathol* 21:55–64.
20. Stoskopf MJ, Beall FB, Ensley PK, Neeley G: 1982, Immobilization of large raptives: blue necked ostrich (*Struthio camelus australis*) and double wattled cassowary (*Casuarius casuarinus*)—with hematological and serum chemistry data. *J Zoo Anim Med* 13:160–168.
21. Stoskopf MK, Neeley E, Mangold B: 1983, Avian hematology in clinical practice. Part 1. *Mod Vet Pract* 64:629–632.
22. Woerpel RW, Roskopf WJ: 1984, Clinical experience with avian laboratory diagnostics. *Vet Clin North Am Small Anim Pract* 14:249–272.

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An outbreak of enterocolitis due to *Campylobacter* spp. in a beagle colony

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Over a 6-week period in spring 1997, 8 beagle dogs from a commercial colony were presented to the Athens Veterinary Diagnostic Laboratory with a history of chronic diarrhea and failure to thrive. Animals ranged in age from 6 weeks to 8 months and included both males and females. All 8 animals were in poor body condition, with rough hair coats. Gross intestinal abnormalities were noted in all, with the most frequent finding being flaccid small and large intestines containing frothy, malodorous, and sometimes mucoid ingesta. Histologically, all had similar changes in the

small intestine, with the most dramatic changes noted in the distal jejunum and ileum. Changes included varying degrees of fusion or blunting of villi, rare evidence of crypt epithelial cell degeneration, and lymphoplasmacytic infiltrates in the lamina propria. Colons were examined histologically from only 7 of the 8 dogs. Changes consisted of superficial erosions, increased numbers of lymphocytes and plasma cells in the lamina propria, varying degrees of crypt epithelial hyperplasia, and occasional crypts packed with filamentous bacteria that became more evident with silver staining (Fig. 1).

A variety of ancillary diagnostic tests were performed (Table 1). All 8 dogs were negative by fluorescent antibody (FA) testing for canine parvovirus and canine distemper virus. One (dog 4) of the 8 dogs was positive by FA for canine coronavirus; all others were negative. An FA test for *Cryp-*

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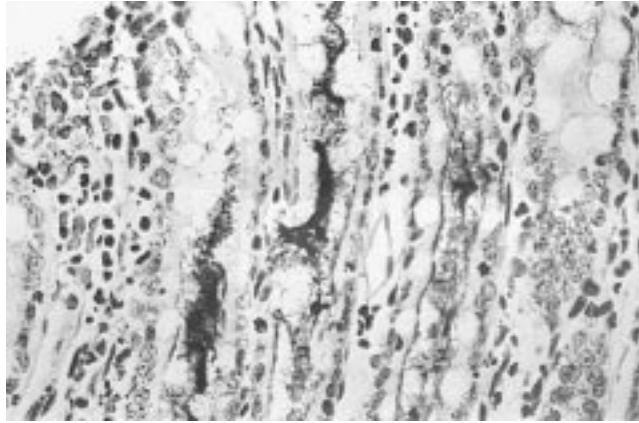


Figure 1. Colon; dog 3. Colonic crypts filled with filamentous organisms. Van Orden silver stain.

tosporidium was done on 5 dogs; all results were negative. Fecal flotations were performed for 7 of the 8 dogs; 1 dog (no. 2) yielded *Cystoisospora*. Verotoxin assay was performed on intestinal contents from 7 of the dogs, and all were negative. *Escherichia coli* was cultured from intestines of all puppies. Selective isolation for *Salmonella* for 7 of the dogs was negative. Specific culturing for *Campylobacter* spp. was done on the last 3 dogs only. Selective isolation consisted of streaking inoculum on *Campylobacter*-selective medium^a incubating under microaerophilic conditions at 42 C, and identifying colonies at 48 hours using an API *Campylobacter* test strip.^b *Campylobacter* organisms were isolated from intestine of 2 of the 3 dogs. Unfortunately, these cultures were discarded and unavailable for identification to species.

As a result of the positive *Campylobacter* spp. cultures, immunohistochemistry (IHC) for *Campylobacter* antigen was performed on intestinal tissues of all dogs. Formalin-fixed, paraffin-embedded sections were cut at 3 μm, deparaffinized, and rehydrated. No antigen retrieval methods were utilized. Endogenous hydrogen peroxide activity was quenched with 3% H₂O₂, and sections were blocked with 2% normal rabbit serum and then incubated with goat anti-*Campylobacter* antibody,^c diluted 1:15,000. Secondary antibody was biotinylated rabbit anti-goat immunoglobulin, followed by avidin–biotin–peroxidase and diaminobenzidine substrate. Sections were counterstained lightly with hematoxylin. Tissues from 3 dogs with no history of diarrhea were also stained in this manner as negative controls. One of these dogs was a kennel cohort that died of another cause, severe anemia.

Positive IHC staining was found in all 8 of the dogs tested and was present most consistently in colon; all 7 of the available colons had distinct clusters of bacteria that were labeled immunohistochemically (Fig. 2). Antigen was most dense within the deeper portions of the crypts but was present at all levels. Positive IHC staining was also seen in the small intestine of 2 of the 8 dogs; it was confined to the ileum and had the same pattern as that seen in the large intestine, i.e., accumulations of bacteria within crypts or adjacent to the epithelial surface of villi. IHC staining of both large and

Table 1. Results of laboratory testing and immunohistochemical (IHC) staining on 8 dogs with diarrhea.

Dog no.	Age	Fecal flotation	Bacteriology (intestine)		Fluorescent antibody tests*			IHC for <i>Campylobacter</i>			
			<i>Salmonella</i>	<i>Campylobacter</i>	CPV	CCV	CDV	<i>C. parvum</i>	Verotoxin assay	Small intestine	Large intestine
1	6 wk	—	ND†	ND	—	—	—	—	—	—	+
2	7 wk	<i>Cystoisospora</i>	—	ND	—	—	—	—	—	—	+
3	7 mo	—	—	ND	—	—	—	—	—	—	+
4	6 mo	—	—	ND	—	+	—	—	—	—	+
5	8 mo	ND	—	ND	—	—	—	—	—	—	+
6	8 mo	—	—	+	—	—	—	—	—	—	+
7	8 mo	—	—	+	—	—	—	—	—	—	+
8	8 mo	—	—	—	—	—	—	ND	—	—	ND
							ND	—	—	—	+

* CPV = canine parvovirus; CCV = canine coronavirus; CDV = canine distemper virus.

† ND = not done.

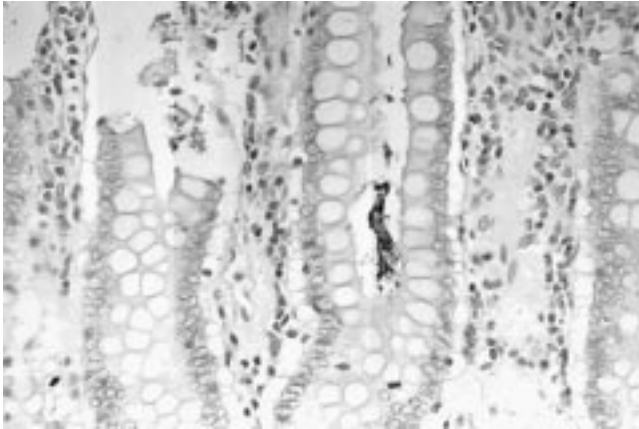


Figure 2. Colon; dog 3. *Campylobacter* antigen within crypts. IHC, hematoxylin counterstain.

small intestine from dogs with no history of diarrhea was negative.

Both *Campylobacter jejuni* and *C. coli* are common causes of diarrhea in humans. Various *Campylobacter* species have been associated with diarrhea in animals, including *C. jejuni*, *C. coli*, and *C. fetus*. In dogs, *Campylobacter* may be carried chronically without clinical disease, and dogs kept in communal situations or dogs <6 months of age have a higher rate of carriage.^{1,5,6} Nevertheless, *Campylobacter* infection has been associated with diarrhea in dogs.^{3,7} In a Swedish study, *Campylobacter* was isolated from 29.6% of dogs with diarrhea and was considered the primary cause of the diarrhea in dogs in more than half of these cases because no other etiologic agents were detected.⁷ In a situation similar to that described here, 10 of 18 3.5-month-old beagles in a laboratory colony became diarrheic. *Campylobacter jejuni* was cultured from 9 of these dogs and was considered the primary cause of the diarrhea.³ Some authors have suggested that *Campylobacter* species may be opportunistic or synergistic with other disease agents and thus more likely to cause disease when other agents have already disrupted the mucosa, allowing for more effective colonization.^{2,3,7}

In a study involving the experimental inoculation of dogs with *C. jejuni*, 3-week-old gnotobiotic beagle puppies experienced malaise, loose feces, and tenesmus. Intestinal lesions consisted of a moderate, superficial erosive colitis progressing to a proliferative response, with numerous bacteria evident on the surface and in the lumen of the upper half of the crypts. Ultrastructural examination revealed organisms morphologically consistent with *Campylobacter* spp. established in largest numbers in the colon but also present in lower numbers in cecum and small intestine.³ In a previous study,⁴ 19 6–8-week-old conventional puppies were inoculated with *C. jejuni*, monitored clinically, and euthanized at intervals up to 10 days postinoculation. Diarrhea that varied from fluid to mucoid was seen in all the puppies. Grossly, the only lesion noted was enlargement of mesenteric lymph nodes. Although there was no microscopic evidence of in-

flammation, both histologically and ultrastructurally, numerous bacteria were present on the mucosa of the cecum and colon, with lesser numbers in the small intestine.

In the present study, the 8 beagle puppies from a laboratory colony had similar gross and histologic findings consisting of flaccid and fluid-filled intestines and enterocolitis, which was most prominent in the ileum. Based on routine ancillary diagnostic testing, infectious causes of diarrhea were limited to coccidia in 1 dog and canine coronavirus in another. Special procedures for *Campylobacter* isolation were performed for 3 dogs; 2 of the 3 were positive. Positive evidence of colonization of intestinal mucosa by *Campylobacter* was seen immunohistochemically in all 8 dogs, with no evidence of similar intestinal colonization in 3 negative control dogs. These results indicate that *Campylobacter* infection was a significant, if not the primary, cause of disease in these animals, although definitive proof was not obtained. According to the manufacturer's specifications, the primary antibody, goat anti-*Campylobacter*, will react with a broad spectrum of *Campylobacter* species, including *C. jejuni*, *C. coli*, *C. fetus*, *C. concisus*, and *C. mucosalis*; the species of *Campylobacter* infecting these dogs could not be determined. The use of IHC methods is helpful in delineating the presence of large numbers of these organisms in the gut.

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Sources and manufacturers

- Remel Co., Lenexa, KS.
- Biomerieux, Hazelwood, MO.
- Kirkegaard and Perry, Gaithersburg, MD.

References

- Bruce D, Zochowski W, Fleming GA: 1980, *Campylobacter* infections in cats and dogs. *Vet Rec* 107:200–201.
- Dillon AR, Boosinger TR, Blevins WT: 1987, *Campylobacter* enteritis in dogs and cats. *Comp and Cont Ed Pract Vet* 9:1176–1183.
- Fox JG, Maxwell KO, Ackerman JI: 1984, *Campylobacter jejuni* associated diarrhea in commercially reared beagles. *Lab Anim Sci* 34:151–155.
- Macartney L, Al-Mashar RR, Taylor DJ, et al.: 1988, Experimental infection of dogs with *Campylobacter jejuni*. *Vet Rec* 122: 245–249.
- Malik R, Love DN: 1989, The isolation of *Campylobacter jejuni/coli* from pound dogs and canine patients in a veterinary hospital. *Aust Vet Pract* 19:16–18.
- Nair GB, Sarkar RK, Chowdhury S, et al.: 1985, *Campylobacter* infection in domestic dogs. *Vet Rec* 116:237–238.
- Olson P, Sandstedt K: 1987, *Campylobacter* in the dog: a clinical and experimental study. *Vet Rec* 121:99–101.
- Prescott JF, Barker IK, Manninen KI, et al.: 1981, *Campylobacter jejuni* colitis in gnotobiotic dogs. *Can J Comp Med* 45:377–383.