

Historical Microscopic Control Data of the Central and Peripheral Nervous System in Beagles

Samantha Hicks-Peña,^{1,2} Charlotte Hollinger,¹ Molly Liepnieks,¹ Sarah Coe¹

¹. Inotiv, Kalamazoo, MI, USA ². Texas A&M University CVM, College Station, TX, USA

Introduction

Beagles (*Canis familiaris*) play a vital role in drug development as a standard large animal model for non-clinical toxicity studies. Establishing historical control data (HCD) aids in distinguishing spontaneous background findings from potentially test article-related effects. While background findings in the nervous system of beagles are described in the literature,¹⁻⁵ it is also important to have an understanding of contemporaneous background findings at the test facility to account for potential differences in environment and husbandry between facilities and changes over time. The goal of this retrospective HCD review was to identify and collate spontaneous findings in the central and peripheral nervous system of control beagles from recent studies at Inotiv.

Methods

Histopathology data from the brain, spinal cord, and sciatic nerve of control beagles was compiled from recent Inotiv studies using the following inclusion criteria: the study was performed according to Good Laboratory Practices (GLP), the study was finalized between 2022-2025, and the test facility was Inotiv-Evansville, IN. As background nervous system findings are uncommon, studies with a variety of lengths (1-39 weeks) and routes of administration, including oral gavage (15), intravenous injection (1), subcutaneous injection (1), and intra-articular injection (2) were included to compile as robust a data set as possible. No studies had direct delivery into the CNS. Data was compiled from 68 male and 67 female control animals from 19 studies with the age of the animals at necropsy ranging from 5 to 18 months.

Results

Minimal extramedullary hematopoiesis (EMH) was observed in the choroid plexus in 2 males (1.5% overall incidence, 3% in males only), and there was 1 instance of minimal focal gliosis in 1 male (0.7% overall incidence, 1.5% in males only). Minimal perivascular mononuclear cell infiltrates were found in the meninges in 1 female (0.7% overall incidence, 1.5% in females only). These findings are summarized in Table 1 and demonstrated in Figure 1. No spontaneous findings occurred in the spinal cord or the sciatic nerve.

Table 1: Spontaneous Microscopic Findings in Control Animals

	Sex	Male	Female
	Number Examined	68	67
Extramedullary hematopoiesis; choroid plexus			
	Minimal	2	0
Gliosis, focal			
	Minimal	1	0
Mononuclear cell infiltrate, perivascular; meninges			
	Minimal	0	1

Figure 1: Spontaneous Microscopic Findings in Beagle CNS

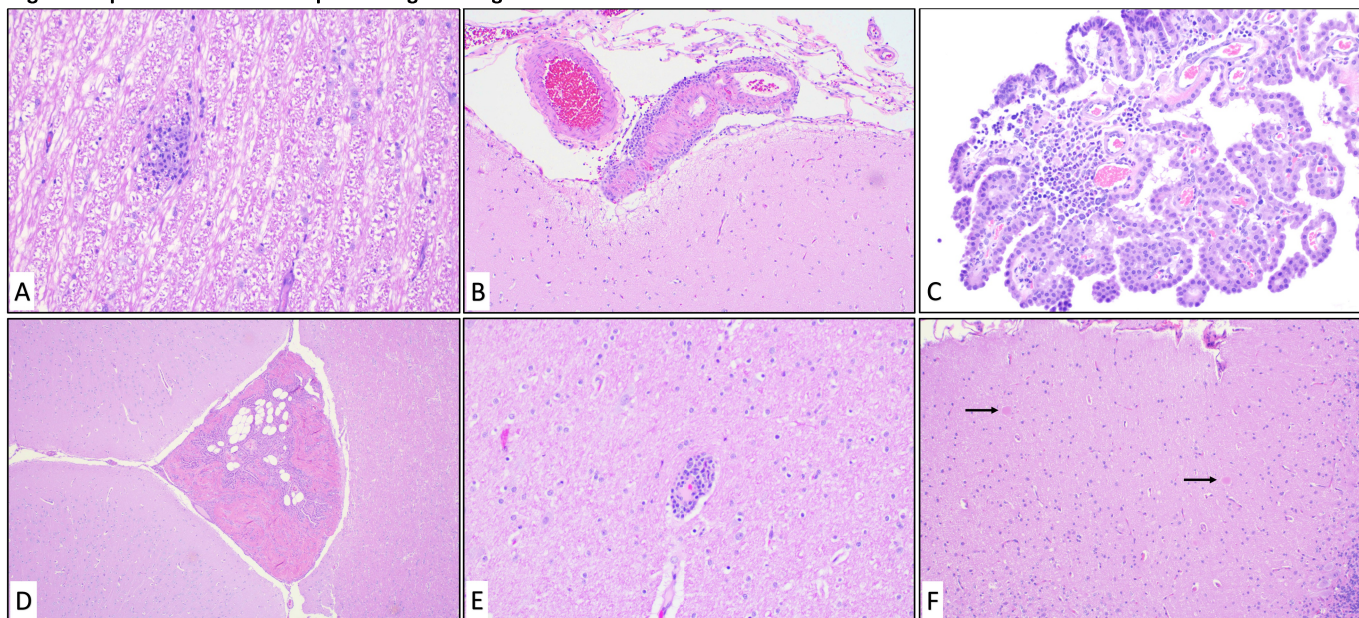


Figure 1: Images A-C demonstrate findings noted in this review. Images D-F demonstrate other reported background findings in beagles that were not reported in this review. A) Minimal, focal gliosis consisted of small aggregates of glial cells (likely microglia and/or astrocytes) in the white matter. H&E. B) Minimal perivascular mononuclear cells infiltrating the meninges of the brain. H&E. C) Focal area of extramedullary hematopoiesis within the choroid plexus consisting of erythroid and myeloid precursor cells. H&E. D) Ectopic choroid plexus consists of densely-packed excess collagenous tissue (sclerosis) and adipocytes amid the choroid plexus. H&E. E) Perivascular mononuclear infiltrates in Virchow-Robbins space. H&E. F) Axonal dystrophy characterized by focal round smooth eosinophilic material with an absence of surrounding tissue response (e.g., inflammation). H&E.

Discussion

In keeping with the 3Rs of animal research, the reduction, refinement, and replacement of animal models leads to relatively small cohorts for toxicity studies, especially for large animals. Due to natural biologic variance, background findings can present a challenge to pathologists in interpreting microscopic findings and their relation to test article administration. This challenge is even greater in tissues where background findings may be rare, and in organ systems where a potentially test article-related finding may have great regulatory impact to a drug development program, such as in the nervous system. Historical control data serves as a reliable reference for spontaneous background findings to utilize as part of a weight of evidence assessment for whether a given finding on a study is test-article related. In this study, the most common finding was EMH in the choroid plexus with males more commonly affected, though any differences between the sexes were likely related to very low incidence and limited animal numbers rather than a true sex-related difference.

As spontaneous findings are rare in the central and peripheral nervous system of beagles, not every reported background finding in beagles was represented in this HCD review. These include ectopic tissue in the choroid plexus, perivascular infiltrates in Virchow-Robbins space, axonal dystrophy, cysts, and canine polyarteritis, as well as occasional axonal degeneration and/or Renault bodies in peripheral nerves.¹⁻⁵ In polyarteritis, clinical signs include recurrent fever, body weight loss, and stiff gait with kyphotic posture due to cervical pain. Histologically, lesions include transmural neutrophilic infiltrates with fibrinoid necrosis, histiocytic-lymphocytic periaxonal infiltrates, and cardiac intimal hyperplasia and medial hypertrophy with variable severity ruptured elastic laminae.^{2,3} Small and medium-sized arteries are most commonly affected, particularly the cervical meninges, extramural branches of the right coronary artery, and cranial mediastinum. Axonal dystrophy can occur in the caudal brain and spinal cord, with an age-related increase in the brain around 5 years old.⁴ Ectopic choroid plexus with variable amounts of fibrovascular connective tissue, adipose, and rests of ependymal cells is reported in Beagles, but no genetic or epigenetic influence has been established.⁵

Conclusions

- Spontaneous findings are rare in the central and peripheral nervous system of beagles, both in the literature and within Inotiv HCD
- The findings in this review were consistent with findings previously reported in beagles.
- This updated contemporaneous HCD will aid in distinguishing test-article-related effects from spontaneous finding in future toxicity studies.

References

1. Clemo FAS, Evering WE, Snyder PW, Albassam MA. Differentiating Spontaneous from Drug-Induced Vascular Injury in the Dog. *Toxicol Pathol.* 2003;31: 25-31.
2. Snyder PW, Kazacos EA, Scott-Moncrieff JC, et al. Pathologic features of naturally occurring juvenile polyarteritis in beagle dogs. *Ver Pathol.* 1995;32(4):337-345.
3. Son WC. Idiopathic canine polyarteritis in control beagle dogs from toxicity studies. *J. Vet. Sci.* (2004), 5(2), 147-150.
4. Pardo IO, Otis D, Ritenour RH, Bailey S, Mashek-Hammerman K, Dowdy HV, Bolon B, Palazzi X. Spontaneous Axonal Dystrophy in the Brain and Spinal Cord in Naive Beagle Dogs. *Toxicol Pathol.* 2020;48(5): 694-701.
5. Pardo IO, Sholeb AM, Gorman R, Virsik M, Santos R, Houle C. Spontaneous Ectopic Choroid Plexus with Sclerosis in Adult Beagle Dogs. *Toxicol Pathol.* 2018;46(5): 608-609.
6. Pardo IO, Weber K, Cramer S, et al. Atlas of Normal Microanatomy, Procedural and Processing Artifacts, Common Background Findings, and Neurotoxic Lesions in the Peripheral Nervous System of Laboratory Animals. *Toxicologic Pathology.* 2020;48(1):105-131.

Acknowledgements

This project was made possible thanks to the generosity of the STP Student Travel Grant and Student Externship scholarship. Special thanks to Nick Vetter and the Inotiv histology and necropsy labs for their support.