

REPORT OF LABORATORY EXAMINATION

Client:	White Shepherd Genetics- (295483) Project - Attn: Judy Huston PO Box 404 Howell, MI 48844	Owner:	Barkley, Beth 7155 Wiltsie Road Lexington MI 48450
Rcvd Date:	4/3/2012 2:08:00 PM	Animal:	FEARGHAS
Admitted By:	Not, Applicable	Species:	Canine
Ordered By:	N/A	Age:	12 years
Encounter:	01339621	Tag/Reg ID:	
CR#:	AP	Other ID:	
		MRN:	
		Breed:	German Shepherd
		Gender:	Male, Castrated

A d d e n d u m R e p o r t

Accession Number:	Received Date/Time:	Verified Date/Time:	Pathologist:
NC-12-0000365	4/3/2012 2:10:00 PM	4/17/2012 8:27:17 AM	Fitzgerald, Scott D.

Additional Findings

Histopathology: Seven sections of brain, 33 sections of spinal cord, and 3 sections of cauda equina were examined. In sections of spinal cord, there was patchy loss of axons and myelin staining, most prominently in peripheral white matter tracts in mid- to caudal thoracic segments. In some sections, dorsal funiculi (fasciculus gracilis) and the dorsolateral portions of lateral funiculi appeared most affected. There was mild to moderate spongiosis in some of these areas as well, and rare axonal spheroids were present. Mild gliosis was present in areas of most severe axon and myelin loss. These changes lessened in severity as one proceeded cranial to and caudal from mid- to thoracic segments, but lesions were present to some degree in all spinal cord sections. Some cauda equina nerve roots demonstrated spongiosis, with occasional gitter cells and/or myelin and axon debris in areas of spongiosis.

In sections of spinal cord containing dura mater, there was multifocal dural thickening by dense fibrous tissue, with foci of osseous metaplasia. Villinodular thickening of the arachnoid by fibrous tissue was noted in a few areas. The leptomeninges on the dorsal aspects of the cerebral hemispheres also were thickened multifocally by fibrous tissue. Many neuronal cell bodies in the spinal cord and scattered neurons in the brain contained lipofuscin pigment. Scattered neuronal cell bodies in the substantia nigra and hypoglossal nuclei were shrunken and hyperbasophilic, and there was mild associated spongiosis.

Diagnosis(es)

spinal cord: mild to severe axon and myelin degeneration, compatible with degenerative myelopathy
spinal cord and brain: moderate neuronal lipofuscinosis
brain and spinal cord meninges: multifocal leptomeningeal and dural fibrosis, with multifocal dural osseous metaplasia (spinal cord)

Comment:

Histopathologic findings in the spinal cord were compatible with degenerative myelopathy, and this disease would explain the dog's weakness in the hind limbs and tail. Other histopathologic changes in the brain and spinal cord were simply age-related, and probably of no clinical significance.

L = Low Result; H = High Result; @ = Critical Result; ^ = Corrected Result; * = Interpretive Data; # = Result Footnote

Admitted By: Not, Applicable
Encounter: 01339621

Species: Canine
Animal: FEARGHAS

MRN:
Owner: Barkley, Beth

A d d e n d u m R e p o r t

Accession Number: NC-12-0000365
Received Date/Time: 4/3/2012 2:10:00 PM
Verified Date/Time: 4/17/2012 8:27:17 AM
Pathologist: Fitzgerald, Scott D.

Jon S. Patterson, DVM, PhD, Dipl ACVP
Anatomic Pathologist

Jon S. Patterson, DVM, PhD, DACVP

(Electronically signed by) JSP

Verified: 04.17.2012 08:27

JSP /JSP

Accession Number: NC-12-0000365
Received Date/Time: 4/3/2012 2:10:00 PM
Verified Date/Time: 4/16/2012 10:01:51 AM
Pathologist: Fitzgerald, Scott D.

Additional Findings

Toxicology:

**GC/MS organic compiound screen on liver: Phenytoin and Pentobarbitol.
PCB on fat tissue: No PCBs detected at test detection limits (<0.500ppm).**

Diagnosis(es)

No toxic compounds detected on toxicology screens

Comments:

The compounds found in the liver by GC/MS are routinely used to euthanize animals; no toxic organic compounds were detected. Nor were any PCB's detected in the adipose tissue. This completes the toxicologic screening assays for this dog.

Scott D. Fitzgerald, DVM, PhD, DACVP, DACPV

(Electronically signed by) SDF

Verified: 04.16.2012 10:01

SDF /SDF

L = Low Result; H = High Result; @ = Critical Result; ^ = Corrected Result; * = Interpretive Data; # = Result Footnote

Admitted By: Not, Applicable
Encounter: 01339621

Species: Canine
Animal: FEARGHAS

MRN:
Owner: Barkley, Beth

N e c r o p s y P r e l i m i n a r y R e p o r t

Accession Number:
NC-12-0000365

Received Date/Time:
4/3/2012 2:10:00 PM

Verified Date/Time:
4/4/2012 1:03:38 PM

Pathologist:
Fitzgerald, Scott D.

History

A 12-year-old, castrated male white German shepherd dog was euthanized on 4/3/12 because of reported regrowth of a tumor that had begun to "block his airways." The tumor was initially detected 2.5 years ago, but the type of tumor was never determined, and its location was not given in the history. The dog is part of the Search and Rescue Dog Necropsy project in which Dr. Scott Fitzgerald participates, and the White Shepherd Genetics project in which Dr. Jon Patterson participates.

Gross Description

A 47.55-kg dog in good nutritional condition and good post mortem condition was presented dead for necropsy. There was a tattoo (224-58-12) in the inguinal region of the right caudoventral abdomen. There was a multilobular, cauliflower-like, 3x1x1-cm, firm, dark gray mass on the right elbow (callus). Multiple movable, soft, fatty, homogeneous, pale tan, generally spherical (and up to 4-5 cm diameter) masses were located in the subcutis on the ventral abdomen and sternum. An irregularly shaped, fairly well demarcated, approximately 8x4x3-cm, multilobular to homogeneous, pale tan mass was present within the skeletal muscle layers on the left side of the head. The caudal extent of the mass was the base of the left external ear canal, with neoplastic tissue surrounding dorsal, cranial, and ventral borders of the canal. The mass extended cranially to the level of the orbit, but did not compress orbital tissues. The mass extended ventrally to cause a 1-cm bulge of the soft palate into the oral cavity in the area of the left tonsil. Skeletal muscle tissues immediately adjacent to the mass were compressed and streaked with pale brown areas (atrophy).

The serosa of the gall bladder was mottled dark red and gray, and the bile was dark brown and thick with mucus. The mucosal surface contained multiple raised, spherical, 2- to 3-mm diameter nodules.

On the margin of the capsular surface of the ventral aspect of the spleen, there was a somewhat rectangular, 3x5-mm, raised (1 mm), yellow tan, firm plaque (hemosiderotic plaque). The capsular surface of the spleen also contained numerous round, 1-mm diameter, soft, white to gray foci (fat, presumptive).

The capsular surface of the liver contained numerous pinpoint, shallow depressions over all lobes; some of these foci were gray white while others were the color of the surrounding hepatic parenchyma.

The entire spinal cord was removed and examined, and all vertebrae were examined. The spinal cord was grossly normal, except for an elliptical, 6x3-mm, gray red, firm plaque in the dura mater at the level of the L3 cord (dural ossification). The vertebrae were normal except for moderate bridging spondylosis at several sites (C7-T1; T7-T8; T12-T13; T13-L1; L1-L2; L2-L3; L3-L4; L6-L7). At all sites, the bony bridge protruded 5-6 mm ventrally from the intervertebral disc space. All intervertebral discs were grossly normal, with gelatinous, gray nucleus pulposus, except for the disc at the L6-L7 joint, which was opaque, yellow tan, and somewhat dry (degeneration).

Gross Diagnosis(es)

intramuscular mass on the head: neoplasm, presumptive
vertebral column: moderate multifocal bridging spondylosis
gall bladder: cystic mucinous hyperplasia
spleen: focal hemosiderotic plaque
liver: hepatopathy

Comment:

The most significant finding was the extensive proliferative lesion on the left side of the head. This is believed to be neoplastic. Histopathologic examination of tissues is in progress.

L = Low Result; H = High Result; @ = Critical Result; ^ = Corrected Result; * = Interpretive Data; # = Result Footnote

Admitted By: Not, Applicable
Encounter: 01339621

Species: Canine
Animal: FEARGHAS

MRN:
Owner: Barkley, Beth

N e c r o p s y P r e l i m i n a r y R e p o r t

Accession Number: NC-12-0000365
Received Date/Time: 4/3/2012 2:10:00 PM
Verified Date/Time: 4/4/2012 1:03:38 PM
Pathologist: Fitzgerald, Scott D.

Misa Komine DVM, PhD
Pathology Resident

Jon S. Patterson, DVM, PhD, Dipl ACVP
Anatomic Pathologist

Jon S. Patterson, DVM, PhD, DACVP

(Electronically signed by) JSP

Verified: 04.04.2012 13:03

JSP /MSK

N e c r o p s y F i n a l R e p o r t

Accession Number: NC-12-0000365
Received Date/Time: 4/3/2012 2:10:00 PM
Verified Date/Time: 4/12/2012 8:34:11 AM
Pathologist: Fitzgerald, Scott D.

Laboratory Findings

Toxicology:

GC/MS Organic toxicant screen on liver: pending

ICP heavy metal screen on liver: No elevation in toxic heavy metals; mild elevation in iron (normal 100-500 ppm; this animal 603 ppm).

ICP heavy metal screen on kidney: No elevation in toxic heavy metals; mildly low levels of manganese.

PCBs on adipose tissue: pending

Microscopic Description

Sections from the face contained a widespread neoplastic mass invading the skeletal muscle and connective tissues. Neoplastic cells were arranged in sheets, occasionally encircling small vessels forming pseudorosettes, with multifocal necrotic areas, some of which were partially mineralized. The neoplastic cell population consisted of small round nuclei with densely clumped chromatin, low mitotic index, and scant amounts of indistinct cytoplasm, with low numbers of fusiform or elongated cells, consistent with a poorly differentiated tumor. Immunohistochemical staining of the neoplastic cells was routinely negative for CD79 (marker for B lymphocytes), routinely negative for CD3 (marker for T lymphocytes), and negative against a cocktail of four different markers for melanomas. Immunohistochemical staining for CD45 (marker for leukocytes/white blood cells) was routinely negative for neoplastic cells, but did positively stain scattered inflammatory cells within the tumor mass. Immunohistochemical staining for vimentin (marker for mesenchymal cells including sarcomas, cells of fibroblast, myocyte, osteocyte origin amongst others) was strongly positive within the cytoplasm of nearly all neoplastic cells.

Both tonsils had reactive hyperplasia of the lymphoid population causing loss of normal follicular architecture, compression and attenuation of the overlying oral squamous epithelium.

L = Low Result; H = High Result; @ = Critical Result; ^ = Corrected Result; * = Interpretive Data; # = Result Footnote

Admitted By: Not, Applicable
Encounter: 01339621

Species: Canine
Animal: FEARGHAS

MRN:
Owner: Barkley, Beth

N e c r o p s y F i n a l R e p o r t

Accession Number:
NC-12-0000365

Received Date/Time:
4/3/2012 2:10:00 PM

Verified Date/Time:
4/12/2012 8:34:11 AM

Pathologist:
Fitzgerald, Scott D.

Sections of one eye were morphologically normal, except for a lymphocytic infiltrate within the lacrimal gland of the third eyelid, which infiltrated between ducts and acini. A cranial lymph node contained moderate amounts of hemorrhage and moderate numbers of hemosiderin-laden macrophages.

Sections of lung had mild accumulations of macrophages filled with dark black pigment (anthracosis) adjacent to small airways. Under polarized light, these same macrophage aggregates contained low numbers of refractile foreign particulate material.

Sections of heart had mild fatty infiltrates within the myocardial interstitium. In addition, medium-sized arteries had degenerative lesions consisting of expansion of the tunica media by pale basophilic material with narrowing of the vascular lumen, consistent with arteriosclerosis.

Sections of both kidneys had mild to moderate irregular thickening of the glomerular tufts, consistent with membranous glomerulonephritis.

Sections of liver were diffusely congested. Sections of spleen were diffusely congested and contained moderate numbers of hemosiderin-laden macrophages. Sections of gall bladder had numerous variably sized epithelial-lined cysts filled with mucinous substance, consistent with cystic mucinous hyperplasia.

Sections of stomach had scattered submucosal lymphoid follicular structures. Sections of small intestine, colon, thyroid, parathyroid, pancreas, adrenal, skeletal muscle, skin and bone marrow were morphologically normal.

Morphologic Diagnosis(es)

- 1) Facial mass: poorly differentiated sarcoma
- 2) Tonsils: reactive lymphoid hyperplasia
- 3) Lacrimal gland of the third eyelid: mild lymphocytic adenitis
- 4) Lungs: mild anthracosis and foreign particulate material
- 5) Heart: mild fatty interstitial infiltrates, and moderate vascular arteriosclerosis
- 6) Kidneys: moderate membranous glomerulonephritis
- 7) Liver and spleen: diffuse congestion
- 8) Gall bladder: moderate cystic mucinous hyperplasia
- 9) Stomach: mild chronic submucosal lymphoid aggregates

Final Diagnosis(es)

Poorly differentiated sarcoma of the facial tissues

Comments:

To date, the immunohistochemical staining performed has not definitively identified the cell of origin of this dog's facial tumor. We have ruled out B cell lymphoma, T cell lymphoma, and malignant melanoma, and leukocytic neoplasia to date. Vimentin was strongly positive for nearly 100% of the neoplastic cell population. This is a marker for mesenchymal origin cells and means this neoplasm is a sarcoma. Unfortunately, there was no differentiation of the neoplasm toward a specific sarcoma such as fibrosarcoma, neurofibrosarcoma, osteosarcoma, rhabdomyosarcoma, etc. This leaves us with a diagnosis of poorly differentiated sarcoma, or spindle cell sarcoma of uncertain primary cell of origin.

L = Low Result; H = High Result; @ = Critical Result; ^ = Corrected Result; * = Interpretive Data; # = Result Footnote

Admitted By: Not, Applicable	Species: Canine	MRN:
Encounter: 01339621	Animal: FEARGHAS	Owner: Barkley, Beth

N e c r o p s y F i n a l R e p o r t

Accession Number: NC-12-0000365 Received Date/Time: 4/3/2012 2:10:00 PM Verified Date/Time: 4/12/2012 8:34:11 AM Pathologist: Fitzgerald, Scott D.

Most of the other histologic lesions would be considered mild or incidental aging lesions; these would include reactive lymphoid hyperplasia in the tonsils, mild lymphocytic adenitis of the 3rd eyelid gland, fatty interstitial infiltrates in the heart, moderate arteriosclerosis of cardiac vessels, membranous glomerulonephritis in the kidneys, cystic mucinous hyperplasia in the gall bladder, and mild lymphoid aggregates in the stomach. The only lesions which may have been directly connected with this dog's Search & Rescue activities are the presence of anthracosis pigments and particulate matter in the lungs; although these changes may also be associated with living in urban or suburban areas and inhaling air pollutants over a prolonged period of time.

There is no evidence of exposure to toxic heavy metals. The mild iron elevation in the liver is commonly encountered in older dogs and of no clinical significance. An addendum report will be issued when additional toxicologic screening tests are completed.

Dr. Jon Patterson is completing his evaluation of the central nervous system of this dog as part of the White German Shepherd Study, and he will release an addendum when his interpretations are completed.

Scott D. Fitzgerald, DVM, PhD, DACVP, DACPV

(Electronically signed by) SDF

Verified: 04.12.2012 08:34

SDF /SDF

T o x i c o l o g y

Toxic Elements

Collected Date/Time (If Provided)	04/03/2012 15:24:00	04/03/2012 15:23:00		
Procedure			Ref Range	Units
Tissue Minerals Specimen	Kidney	Liver		
Antimony	<1.00	<1.00		ppm
Arsenic	<0.50	<0.50		ppm
Barium	<0.05	<0.05		ppm
Boron	<1.00	<1.00		ppm
Cadmium	4.69	0.32		ppm
Calcium	174.7			ppm
Calcium		43.2	[30.0-250.0]	ppm
Chromium	<0.20	<0.20		ppm
Cobalt	<0.10	<0.10		ppm
Copper	5.2			ppm
Copper		51.7	[10.0-100.0]	ppm
Iron	160			ppm

L = Low Result; H = High Result; @ = Critical Result; ^ = Corrected Result; * = Interpretive Data; # = Result Footnote

Admitted By: Not, Applicable	Species: Canine	MRN:
Encounter: 01339621	Animal: FEARGHAS	Owner: Barkley, Beth

T o x i c o l o g y

Toxic Elements

Collected Date/Time (If Provided)	04/03/2012 15:24:00	04/03/2012 15:23:00		
Procedure			Ref Range	Units
Iron		603 H	[100-500]	ppm
Lead	<0.50	<0.50		ppm
Magnesium	103			ppm
Magnesium		121	[90-200]	ppm
Manganese	0.66			ppm
Manganese		1.49	[1.00-5.00]	ppm
Mercury	<2.00	<2.00		ppm
Molybdenum	<0.20			ppm
Molybdenum		0.27	[0.00-0.90]	ppm
Phosphorus	1599			ppm
Phosphorus		2159	[1,710-3,430]	ppm
Potassium	1803			ppm
Potassium		1769	[1,000-3,800]	ppm
Selenium	<2.00	<2.00		ppm
Sodium	2327			ppm
Sodium		1496	[900-1,900]	ppm
Thallium	<2.50	<2.50		ppm
Zinc	18			ppm
Zinc		27	[20-70]	ppm
Tissue Minerals Interpretation	See Below	See Below		

04/03/2012 15:24:00 Tissue Minerals Interpretation

Toxic elements (Sb, As, Cd, Cr, Pb, Hg, Se, Tl) were negative or low. Other elements (Ca, Cu, Fe, Mg, Na, Zn) were in their correct normal ranges, with the exception of manganese which was lower than the reference range of 1.2-1.8 ppm. Note that Mn deficiency is considered rare in dogs, and, if present, the signs include crooked, shortened soft bones. Note that there is apparently some competition between Fe and Mn, such that excess Mn can result in Fe deficiency, and the suspicion may be that the opposite is true as well, since liver results indicate somewhat excessive iron and kidney results suggest reduced Mn. Ref: Mineral Levels in Animal Health, by R. Puls, 1994.

Please be advised that the DCPAH Toxicology Section disposes of all samples 12 months from the date of receipt.

Andreas Lehner, Ph.D.
Analytical Chemist
4/6/2012 4:33 PM

L = Low Result; H = High Result; @ = Critical Result; ^ = Corrected Result; * = Interpretive Data; # = Result Footnote

Admitted By: Not, Applicable	Species: Canine	MRN:
Encounter: 01339621	Animal: FEARGHAS	Owner: Barkley, Beth

T o x i c o l o g y

Toxic Elements

04/03/2012 15:23:00 Tissue Minerals Interpretation

Toxic elements (Sb, As, Cd, Cr, Pb, Hg, Se, Tl) were negative or low. Other elements were in their correct normal ranges, with the exception of iron. Iron values >500 ppm are considered high, but note that iron accumulates in the liver with advancing age. Ref: Mineral Levels in Animal Health, by R. Puls, 1994.

Please be advised that the DCPAH Toxicology Section disposes of all samples 12 months from the date of receipt.

Andreas Lehner, Ph.D.
Analytical Chemist
4/6/2012 4:35 PM

General Toxicology

Collected Date/Time (If Provided)	04/03/2012 15:29:00	04/03/2012 15:23:00		
Procedure			Ref Range	Units
GCMS Specimen		Liver		
GCMS		Negative		
GCMS Interpretation		See Below		
PCB Specimen	Fat			
PCB	<0.500			ppm
PCB Interpretation	See Below			

04/03/2012 15:29:00 PCB Interpretation

The sample was negative for PCBs to the tested limits of detection.

Please be advised that the DCPAH Toxicology Section disposes of all samples 12 months from the date of receipt.

John P. Buchweitz, Ph.D.
Clinical Toxicologist
4/12/2012 12:50 PM

L = Low Result; H = High Result; @ = Critical Result; ^ = Corrected Result; * = Interpretive Data; # = Result Footnote

Admitted By: Not, Applicable
Encounter: 01339621

Species: Canine
Animal: FEARGHAS

MRN:
Owner: Barkley, Beth

T o x i c o l o g y

General Toxicology

04/03/2012 15:23:00 GCMS Interpretation

None of the toxic organic compounds that can be identified by GCMS were present in the sample.

The following compounds were identified by mass spectral library match:

- 1) phenytoin
- 2) pentobarbital

These agents are consistent with euthanasia.

Please be advised that the DCPAH Toxicology Section disposes of all samples 12 months from the date of receipt.

John P. Buchweitz, Ph.D.
Clinical Toxicologist
4/13/2012 5:17 PM

L = Low Result; H = High Result; @ = Critical Result; ^ = Corrected Result; * = Interpretive Data; # = Result Footnote