

Laboratory Report
Addendum
Version 1

*This report supersedes all
 previous reports for this case*

Case #: W1502158
Referral #:
Date Collected: 08/17/2015
Date Received: 08/17/2015
Case Coordinator: Dr. Don Kitchen
Owner:
 Colorado Dept of Wildlife-Drayton Harrison
 151 E. 16 th St.
 DURANGO, CO 81301

Email To: margie.michaels@STATE.CO.US
 Colorado Parks & Wildlife NE Region
 317 West Prospect
 Fort Collins, CO 80526

Electronically Signed and Authorized
By:
 Dr. Don Kitchen
 sent by Alexandria Fenton
 on 8/28/2015 5:49:42PM

Case Contacts

| | | | |
|-----------|-------------------------------------|--------------|--------------------------------|
| Report To | Fox, Karen | 970-472-4318 | karen.fox@colostate.edu |
| Report To | Pabilonia, Kristy | 970-297-4109 | kristy.pabilonia@colostate.edu |
| Submitter | Colorado Parks & Wildlife NE Region | 970-472-4478 | margie.michaels@STATE.CO.US |

Specimen Details

| ID | Taxonomy | Sex | Age |
|----------|----------|-----|-----|
| Beaver 1 | Beaver | | |
| Beaver 2 | Beaver | | |

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Specimens Received: Body; Liver Tissue;

Laboratory Findings/Diagnosis

CLINICAL HISTORY AND NECROPSY FINDINGS:

Two male beavers were submitted by the Colorado Division of Wildlife with a history of being found dead in the Animus River near the site of contamination from old mine tailings. There was concern that the river was contaminated with heavy metals and that the cause of death in these beavers may have been due to heavy metal toxicity.

Two adult male beavers were submitted for necropsy. There is always concern of tularemia in the beaver population and an abbreviated necropsy was performed primarily to collect liver tissue for histopathologic evaluation, identification of possible infection with tularemia and evaluation of the liver for heavy metal content. Liver and spleen were collected and submitted for real-time PCR to determine the presence of Francisella tularensis genetic material. Liver samples were also submitted for microscopic examination even though they were extremely autolytic. No gross lesions were discerned that were consistent with tularemia. However, these animals were severely autolytic and lesions may not have been discernible.

GROSS NECROPSY DIAGNOSIS: NONE, NO LESIONS SUGGESTING TULAREMIA

LABORATORY RESULTS:

Real-time PCR failed to identify Francisella tularensis genetic material. This test was considered to be negative.

Mineral analysis:

The liver was submitted to Michigan State University DCPAH for mineral analysis. Interpretation by the toxicologist stated the following: Beaver "A", toxic elements (arsenic, mercury, selenium and Thallium) were negative or low with the

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exception of cadmium and lead, which were both elevated or high, but most likely not in the toxic range. The following elements appear to be in their normal ranges for this species: zinc, copper, iron and selenium.

Beaver "B"-toxic elements (arsenic, Mercury, selenium and Thallium) were negative or low with the exception of cadmium and lead, which were both elevated or high. Both elements are at a value that border on potential toxic ranges. The following elements appeared to be in their normal ranges for the species: manganese, zinc, copper, iron and selenium. This report is attached.

HISTOPATHOLOGY REPORT:

Slides 1, 2 and 3. Liver and spleen (1 each). Both tissues were examined histologically and found to be extremely autolytic. There was; however, no histologic evidence of inflammatory disease. Evaluation of tissue this autolytic is of little significance in most cases.

HISTOPATHOLOGIC DIAGNOSIS: TOO AUTOLYTIC FOR MEANINGFUL HISTOLOGIC INTERPRETATION.

REMARKS:

Neither of these animals had any evidence of tularemia (gross examination, histopathologic evaluation and PCR results). The mineral analysis, as outlined above, did not suggest that these animals died of exposure to toxic minerals from the contaminated river. It may be helpful to compare these findings to water analysis results from the suspected river. The cause of death in these animals was not due to tularemia and not likely due to exposure to any of the above listed minerals.

Sincerely,
 Don Kitchen, DVM, PhD, DACVP, Director
 Colorado State University WS Diagnostic Laboratory
 425 29 Road, Grand Junction, CO 81504

Case Summary

Neither of these animals had any evidence of tularemia (gross examination, histopathologic evaluation and PCR results). The mineral analysis has outlined above did not suggest that these animals died of exposure to toxic minerals from the contaminated river. It may be helpful to compare these findings to water analysis results from the suspected river. The cause of death in these animals was not due to tularemia and not likely due to exposure to any of the above listed minerals.

BSL3

Francisella tularensis (Tularemia) real-time PCR

| Animal/Source | Specimen | Specimen Type | Result Date | Results |
|---------------|----------|---------------|-------------|---|
| Beaver 1 | Beaver 1 | Tissue Pool | 19-Aug-2015 | Negative Liver & spleen were pooled for testing. |
| Beaver 2 | Beaver 2 | Liver Tissue | 19-Aug-2015 | Negative |

Necropsy

Necropsy Wildlife / Exotics Gross Examination Only

| Animal/Source | Specimen | Specimen Type | Result Date | Results |
|---------------|----------|---------------|-------------|----------|
| Beaver 1 | Beaver | Body | 27-Aug-2015 | Complete |

Tissue Collection

| Animal/Source | Specimen | Specimen Type | Result Date | Results |
|---------------|----------|---------------|-------------|----------|
| Beaver 1 | Beaver | Body | 27-Aug-2015 | Complete |

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Referral Tests

Referral Lab Send Out Test

| Animal/Source | Specimen | Specimen Type | Result Date | Results |
|----------------------|-----------------|----------------------|--------------------|-------------------------|
| Beaver 1 | Beaver 1 | Liver Tissue | 27-Aug-2015 | Complete - See attached |
| Beaver 1 | Beaver 2 | Liver Tissue | 27-Aug-2015 | Complete - See attached |

Appendix - Report Related Images

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MICHIGAN STATE
UNIVERSITY



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Lansing, MI 48910-8104
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REPORT OF LABORATORY EXAMINATION

Client: Colorado State University (29874)
Western Slope Ahd/
425 29 Road
Grand Junction, CO 81501

Owner: CO Division of Wildlife, Drayton Harrison

Report #: C15233025
Received: 08/21/15 11:58
Printed: 08/26/15 16:22

Admit: Kitchen, Dr.
Hard:
Species: Other Mammal

Sex: Unknown
Age:

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| Animal ID | DWR | Reference Range () | Specimen Tissue | Reference Range () | Mercury, Tiss (ug/g dry) | Reference Range (ug/g dry) |
|-----------|-------|--------------------|-----------------|--------------------|--------------------------|----------------------------|
| W1502158A | 0.237 | 0.260-0.340 | Liver | | <0.53 | |
| W1502158B | 0.253 | 0.260-0.340 | Liver | | <0.50 | |

| Animal ID | Copper, Tiss (ug/g dry) | Reference Range (ug/g dry) | Thallium, Tiss (ug/g dry) | Reference Range (ug/g dry) | Lead, Tiss (ug/g dry) | Reference Range (ug/g dry) |
|-----------|-------------------------|----------------------------|---------------------------|----------------------------|-----------------------|----------------------------|
| W1502158A | 12.52 | | <0.11 | | 2.44 | <=3.00 |
| W1502158B | 15.76 | | <0.10 | | 4.85 | <=3.00 |

| Animal ID | Iron, Tiss (ug/g dry) | Reference Range (ug/g dry) | Cobalt, Tiss (ug/g dry) | Reference Range (ug/g dry) | Arsenic, Tiss (ug/g dry) | Reference Range (ug/g dry) |
|-----------|-----------------------|----------------------------|-------------------------|----------------------------|--------------------------|----------------------------|
| W1502158A | 548.00 | | 0.13 | | <0.11 | <=8.00 |
| W1502158B | 841.89 | | 0.13 | | <0.10 | <=8.00 |

| Animal ID | Cadmium, Tiss (ug/g dry) | Reference Range (ug/g dry) | Selenium, Tiss (ug/g dry) | Reference Range (ug/g dry) | Zinc, Tiss (ug/g dry) | Reference Range (ug/g dry) |
|-----------|--------------------------|----------------------------|---------------------------|----------------------------|-----------------------|----------------------------|
| W1502158A | 61.74 | | 1.48 | | 162.72 | |
| W1502158B | 185.13 | | 1.64 | | 166.03 | |

| Animal ID | Molybdenum, Tiss (ug/g dry) | Reference Range (ug/g dry) | Manganese, Tiss (ug/g dry) | Reference Range (ug/g dry) |
|-----------|-----------------------------|----------------------------|----------------------------|----------------------------|
| W1502158A | 2.17 | | 41.09 | |
| W1502158B | 1.41 | | 15.62 | |

▲ = Corrected Result

Owner: Colorado Dept of Wildlife-Drayton Harrison

Appendix - Report Related Images

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Client: Colorado State University (29874)
Report #: C15233025

Owner: CO Division of Wildlife, Drayton Harrison
Herd:

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Admit: Kitchen, Dr.

| Animal ID | Comment, Tissue Mineral | Reference Range |
|-----------|--|-----------------|
| W1502158A | <p>Toxic elements (As, Hg, Se, Tl) were negative or low, with the exceptions of Cd and Pb which were both elevated or high, but most likely not in a toxic range. The following elements appear to be in their normal ranges for this species: Zn, Cu, Fe, Se.</p> <p>Please be advised that the DCPAH Toxicology Section disposes of all samples 12 months from the date of receipt.</p> <p>Andreas Lehner, Ph.D. Analytical Chemist 8/26/2015 4:15 PM</p> | 0 |
| W1602158B | <p>Toxic elements (As, Hg, Se, Tl) were negative or low, with the exceptions of Cd and Pb which were both elevated or high. Both elements are at values that border on potential toxic ranges. The following elements appear to be in their normal ranges for this species: Mn, Zn, Cu, Fe, Se.</p> <p>Please be advised that the DCPAH Toxicology Section disposes of all samples 12 months from the date of receipt.</p> <p>Andreas Lehner, Ph.D. Analytical Chemist 8/26/2015 4:20 PM</p> | |

▲ = Corrected Result

End of Report