DIAGNOSTIC CASE REPORT

U. S. GEOLOGICAL SURVEY-BIOLOGICAL RESOURCES DIVISION NATIONAL WILDLIFE HEALTH CENTER-HONOLULU FIELD STATION P. O. BOX 50167, 300 ALA MOANA BLVD., Rm. 8-132 HONOLULU, HAWAII 96850

Tel: 808-792-9520, Fax: 792-9596, Email: thierry_work@usgs.gov

Case Number: 25287

Submitter Name:

Species submitted (n):

Turtle: Green (6)

Dr. Bethany Doescher

Sea Life Park

41-202 Kalanianaole Hwy Suite 7

Waimanalo, Hawaii 96795

United States

SPECIMENS SUBMITTED: Carcass-Fixed formalin

These 6 turtles were collected from Sea Life Park between August 2015 and February 2016. The common history to these turtles, with some variation (see attached reports) included doing well, then going off feed acutely, becoming lethargic, and dying. A detailed necropsy report accompanied Accession 5.

Acc	Date_Coll	Age	Sex	TagNo	Diagnosis
1	08/04/2015	Immature	Unknown	10	Bacterial enteritis
2	10/06/2015	Immature	Unknown	25	Pneumonia
3	10/16/2015	Immature	Unknown	26	Bacterial esophagitis
4	01/16/2016	Immature	Unknown	22	Bacterial enteritis
5	01/20/2016	Immature	Female	ML 2014 44	Bacterial enteritis
6	2/24/2016	Immature	Female	2014 Turtle1	Bacterial enteritis

Comments: All these turtles died from varying severity of bacterial enteritis other than one that died from pneumonia. It is likely there was local sepsis because many organs had thrombosed vessels. One turtle had a moderate case of pulmonary mycosis, probably secondary to immunosuppression. If samples were stored frozen, it might be informative to send them for bacterial culture to identify the organisms.

Management: It is unclear why these turtles died from bacterial enteritis over such a prologned time period. Questions to consider might include whether there were changes in management of animals (tanks, feed, regimens) or whether these animals were part of a particular clutch or came from a particular female.

Report Date (mm/dd/yyyy): 3/31/2016 Necropy report: Enclosed

Copies of this report sent to:

Mr. George Balazs (NOAA) Dr. Todd Jones (NOAA)

If you have questions regarding this case, contact Thierry M. Work MS, DVM, MPVM at 808-792-9520. Include above Case Number. Diagnostic findings may not be used for publication without the pathologist's knowledge and consent.

NOTE: Information in this report supersedes any information from previous reports regarding this case.

Submitter Name:

Dr. Bethany Doescher

Sea Life Park

41-202 Kalanianaole Hwy Suite 7

Waimanalo, Hawaii 96795

United States

Prosector: Pathologist:

Case:
Accession:

25287

2/11/2016

T. M. Work
T. M. Work

Date Collected: 08/04/2015

Date Examined:

Signalment-Morphometrics-History

CONDITION: (Fair) POSTMORTEM: (Good) EUTHANASIA: (Not euthanized)

TAG TYPE: (Reference Number) TAG NO.: (10)

SPECIES:(Turtle: Green) AGE:(Immature) SEX:(Unknown)

COLLECTION-SITE: (Sea Life Park) AREA: (Honolulu) STATE: (Hawaii) COUNTRY: (United

States)

HISTORY: This is a Sea Life Park captive hatchling turtle that had normal appetite and growth until it acutely went off feed and was lethargic for two days and then died on 4 Aug 2015. Hatch date was 6 July 2015.

External/Internal

EXTERNAL:

INTERNAL:

PRELIMINARY DIAGNOSIS: Undetermined.

Samples

SECIMENS RECEIVED: Carcass-Fixed formalin.

HISTO: Kidney, Brain (A); Lung, Liver (B); Intestine small, Esophagus, Spinal cord, Skeletal muscle (C); Skin, Skeletal muscle (D).

Laboratory Results

HISTOPATHOLOGY

Intestine small: Diffusely, there is full thickness necrosis of mucosa and smooth muscle wall associated with microcolonies of bacteria.

Liver: Diffusely, sinusoids are markedly distended with red cells.

Lung: Diffusely, smooth muscle walls are markedly atrophied.

All other Organs: No remarkable lesions are seen.

COMMENTS: None

Final Diagnosis (in order of importance)

Diagnosis Topog Morpho Etiol Funct Dis Link

1. Bacterial enteritis (T50500)(M42100)(E10012)()()()

Diagnostic findings may not be published without the knowledge and consent of the

25287-1 pathologist.

Page 2 of 2

Milt Code: (Infectious disease)

Submitter Name:

Dr. Bethany Doescher

Sea Life Park

41-202 Kalanianaole Hwy Suite 7

Waimanalo, Hawaii 96795

United States

Case: 25287 Accession: 2

Date Collected: 10/06/2015 Date Examined: 2/11/2016

Prosector: T. M. Work Pathologist: T. M. Work

Signalment-Morphometrics-History

CONDITION: (Fair) POSTMORTEM: (Good) EUTHANASIA: (Not euthanized)

TAG TYPE: (Reference Number) TAG NO.: (25)

SPECIES:(Turtle: Green) AGE:(Immature) SEX:(Unknown)

COLLECTION-SITE: (Sea Life Park) AREA: (Honolulu) STATE: (Hawaii) COUNTRY: (United

States)

HISTORY: This is a Sea Life Park captive hatchling turtle that had normal appetite and growth until it acutely went off feed and was lethargic for a day and then died on 6 October 2015. Hatch date was 7 September 2015.

External/Internal

EXTERNAL:

INTERNAL:

PRELIMINARY DIAGNOSIS: Undetermined.

Samples

SECIMENS RECEIVED: Carcass-Fixed formalin.

HISTO: Yolk sac, Liver, Brain (A); Esophagus, Kidney, Heart, Lung (B); Stomach, Intestine small, Intestine large (C); Skin, Skeletal muscle (D).

Laboratory Results

HISTOPATHOLOGY

Esophagus: Diffusely, mucosal cells exfoliate into the lumen.

Lung: Prominent histiocytic infiltrates are within smooth muscle walls.

All other Organs: No remarkable lesions are seen.

COMMENTS: None

Final Diagnosis (in order of importance)

Diagnosis Topog Morpho Etiol Funct Dis Link
1. Pneumonia (T28000)(M42100)()()()()()

Diagnostic findings may not be published without the knowledge and consent of the pathologist. Milt Code: (Other)

Submitter Name:

Dr. Bethany Doescher

Case:

Aggestion:

Sea Life Park
41-202 Kalanianaole Hwy Suite 7

Accession: 3
Date Collected: 10/16/2015

41-202 Kalanianaole Hwy Suite 7 Waimanalo, Hawaii 96795

United States

Signalment-Morphometrics-History

25287

2/11/2016

T. M. Work

T. M. Work

Date Examined:

Prosector:

Pathologist:

CONDITION: (Fair) POSTMORTEM: (Good) EUTHANASIA: (Not euthanized)

TAG TYPE: (Reference Number) TAG NO.: (26)

SPECIES:(Turtle: Green) AGE:(Immature) SEX:(Unknown)

COLLECTION-SITE: (Sea Life Park) AREA: (Honolulu) STATE: (Hawaii) COUNTRY: (United

States)

HISTORY: This is a Sea Life Park captive hatchling turtle that had normal appetite and growth until 7 Oct 2015 and then refused normal feed stuff, but did eat fish/clam/krill for a week. It did not eat and was lethargic for two days and then died on 16 Oct 2015. Hatch date was 7 September 2015.

External/Internal

EXTERNAL:

INTERNAL:

PRELIMINARY DIAGNOSIS: Undetermined.

Samples

SECIMENS RECEIVED: Carcass-Fixed formalin.

HISTO: Brain, Liver, Yolk sac (A); Heart, Kidney, Lung (B); Bronchus, Esophagus,

Proventriculus (C); Skin, Skeletal muscle (D).

Laboratory Results

HISTOPATHOLOGY

Esophagus: Diffusely, there is full thickness necrosis of mucosa and portions of underlying muscularis associated with occasional clumps of bacteria.

Proventriculus: Diffusely, squamous mucosa is overlaid by sheets of necrotic debris.

Liver: Diffusely, there is marked decrease in internuclear distance between hepatocyte nuclei.

All other Organs: No remarkable lesions are seen.

COMMENTS: None

Final Diagnosis (in order of importance)
Diagnosis Topog Morpho Etiol Funct Dis Link

25287-3 Page 2 of 2

1. Bacterial esophagitis (T62000)(M40000)(E10012)()()()
Diagnostic findings may not be published without the knowledge and consent of the pathologist.

Milt Code: (Infectious disease)

Submitter Name:

Dr. Bethany Doescher Case: 25287
Sea Life Park Accession: 4

Sea Life Park
41-202 Kalanianaole Hwy Suite 7

Accession: 4
Date Collected: 01/16/2016

Waimanalo, Hawaii 96795

United States

Date Examined: 2/11/2016

Prosector: T. M. Work

Pathologist: T. M. Work

Signalment-Morphometrics-History

CONDITION: (Fair) POSTMORTEM: (Good) EUTHANASIA: (Not euthanized)

TAG TYPE: (Reference Number) TAG NO.: (22)

SPECIES: (Turtle: Green) AGE: (Immature) SEX: (Unknown)

COLLECTION-SITE: (Sea Life Park) AREA: (Honolulu) STATE: (Hawaii) COUNTRY: (United

States)

HISTORY: This is a Sea Life Park captive juvenile turtle that had normal appetite and growth until 15 January 2016. The following morning it was found to be extremely lethargic and appeared bloated. It died an hour after being examined. Hatch date was 7 September 2015.

External/Internal

EXTERNAL:

INTERNAL: A brief gross exam by the submitter revealed a gas distended stomach and bowel. Much of the serosal surface of the intestine was bright red. One segment was dark red in color and was not gas distended.

PRELIMINARY DIAGNOSIS: Undetermined.

Samples

SECIMENS RECEIVED: Carcass-Fixed formalin.

HISTO: Spleen, Pancreas, Heart (A); Liver, Kidney (B); Lung, Intestine small (C); Yolk sac, Stomach, Intestine small (D); Skin, Skeletal muscle (E).

Laboratory Results

HISTOPATHOLOGY

Stomach: Diffusely, there are bacteria infiltrating smooth muscle wall.

Yolk sac: There is marked vascular engorgement of smooth muscle wall.

Intestine small: There is marked vascular engorgement of mucosa. In one section, mucosa is effaced by red cells, and basophilic rod are seen invading mucosal cells. In another section, villi are markedly congested.

Lung: Multiple variably sized nidi of giant cells some of which contain a core of eosinophilic debris and surrounded by lymphoid cells are present. In some of those necrotic centers are segmented filamentous bulbous brown structures (fungal hyphae).

Heart: Within ventricle are isolated mild histiocytic infiltrates.

25287-4 Page 2 of 2

All other Organs: No remarkable lesions are seen.

COMMENTS:None

Final Diagnosis (in order of importance)										
Diagnosis	Topog	Morpho	Etiol	Funct	Dis	Link				
1. Bacterial enteritis	(T50500)	(M42100)(E10012) () () ()				
2. Pulmonary mycosis	(T28000)	(M42100)(L40000) () () ()				
Diagnostic findings may not be published without the knowledge and consent of the										
pathologist. Milt Code: (Infectious disease)										

Submitter Name:

Dr. Bethany Doescher Case: 25287
Sea Life Park Accession: 5

41-202 Kalanianaole Hwy Suite 7

Date Collected: 01/20/2016

Waimanalo, Hawaii 96795

United States

Date Examined: 2/11/2016

Prosector: B. Doescher

Pathologist: T. M. Work

Signalment-Morphometrics-History

CONDITION: (Good) POSTMORTEM: (Good) EUTHANASIA: (Not euthanized)

TAG TYPE: (Reference Number) TAG NO.: (ML 2014 44)

SPECIES: (Turtle: Green) AGE: (Immature) SEX: (Female)

MORPHOMETRICS: Weight carcass (7.6 kg), Straight carapace length (36.7 cm).

COLLECTION-SITE: (Sea Life Park) AREA: (Honolulu) STATE: (Hawaii) COUNTRY: (United

States)

HISTORY: 2014 Turtle #44 was hatched on the turtle lagoon beach at SLPH (originally identified at SLPH as 2014 Turtle #21). She was transferred to Mauna Lani on 11-26-14. Weight obtained on 01-02-16 was 16.1#. Growth and feeding patterns were unremarkable until 01-10-16 when Pi'I Laeha reported a "golf ball" size cloacal prolapse and the turtle was moved to a quarantine pool. Within an hour she had prolapsed about 5 inches of intestine. Upon examination the turtle was BAR, swimming and diving. The prolapsed tissue was in relatively good condition (red in color, but had a smooth, uniform mucosal surface throughout). There were several twists along the length and some fine strands of fibrin loosely overlying the mucosal surface in some areas. The most distal portion of the protrusion was darker red in color. There was a torsion in this area causing an about 1.5" ball of intestine. Once it was unrotated, the color returned to the same red as the other tissue. There was quite a lot of intestine out, maybe 20+ inches if it had been flat. The prolapsed was reduced manually and a purse string suture was placed to close the cloaca to a 1 cm diameter. A red rubber tube was passed up into the colon almost the full length to help ensure no intussusception was present. The turtle was put on Amikacin. Over the next 5 days the turtle remained active, swimming and diving, but did not eat. Nothing was noted protruding from the cloaca. Due to the persistent anorexia, on 01-14-16 the turtle was transferred back to SLPH and placed in a quarantine pool. A blood sample indicated a mild leukocytosis with heterophilia and lymphopenia. Radiographs revealed some gas filled pockets and some mineralized material was present. The turtle received intracoelomic fluids, and oral fish mash and mineral oil every other day as well as continuing the course of Amikacin. The turtle remained anorexic. On the 18th , she was observed to pass some dark green liquid feces in the morning and oil residue was seen on the water in the afternoon. On the 19th small rocks were found scattered on the bottom of the pool. During treatments it was noted that some brown firm material was observed at the cloacal opening. The purse string suture was removed and brown leathery bowel was extruded. The decision was made to attempt surgical resection. A 4 cm incision was made in the right prefemoral fossa. Approximately 500 ml of brown fluid flowed from the incision when the coelomic cavity was punctured. Small intestines and proximal large intestine were exteriorized; however, we were unable to visualize the affected tissue. The turtle died spontaneously early the next morning.

External/Internal

EXTERNAL: The turtle appears to be in good body condition with adequate fat pads. There is a foul smell coming from the mouth and from the cloaca. Reddish brown foul

smelling liquid is occasionally discharged from the cloaca. The oral cavity was unremarkable. Some mild irritation (erythema) was present around the glottis secondary to intubation.

INTERNAL: The coelomic cavity contained approximately 20 cc of yellowish brown liquid. Dark red blood clots was present along the length of the left main bronchus and into the small airways. The airways on the right side were normal. The lungs were a fairly uniform pink color on cut surface. The heart and vasculature appeared normal, except the right atrium which was darker red in color that the rest of the parenchyma. The thyroids were reddish brown in color. The pancreas had a homogenous pinkish tan appearance with a smooth surface. A 4 cm wad of green ingesta was present in the distal esophagus. The material had a foul odor, but was soft and easily spread (like a thick paste). This material was not consistent with the pale gray shrimp mash that had been tube fed over the past several days. A 20 \times 4 mm cylindrical rock and several irregular shaped flat rocks (Max diameter 6 mm) were recovered within this material. The esophageal mucosa was a normal pink color at the proximal end and the distal half was yellowish green. Aside from the color change, the mucosal surface appeared normal. The stomach had small amounts of the green ingesta scattered throughout. The pylorus (6 cm) was packed with more than 30 small rocks (3-13 mm in diameter) surrounded by green ingesta. The mucosa throughout the stomach, including the pyloric region, was slightly mottled pale pink to light tan. No mucosal lesions were seen that would indicate trauma secondary to the rock foreign bodies. The mucosa of the small intestines was fairly uniform pale tan in color with occasional small patches of erythema. No rocks were detected in the small intestine. Ingest in the small intestine was a thick liquid and the color ranged from light brown in the duodenum to creamy mustard yellow in the jejunum. There were green feces in the colon with some areas of formed stool. Some of the stool contained small rocks which were encased in layers of fibrin. In one area that was distended with stool, the mucosa is a pale grayish tan color with some patchy erythema. In a second area, the colon is distended but not with stool and there is a large emphysematous patch $(3.7 \times 10.6 \text{ cm})$ within the mucosa. The color of the mucosa in this area is pale yellowish tan, consistent with the surrounding tissue. The serosal surface in this area is unremarkable (pale pink and smooth). A second segment with formed stool that contained fibrin covered rocks has a mucosal surface similar to the first are noted above. It is approximately 10 cm proximal to a sharp transverse demarcation of the mucosa from pale pinkish tan (proximally) to mottled yellow and grayish tan. The mucosa of the colon distal to the transverse line is covered with increasingly thickening layers of fibrin as you move distally. One area of apparently normal looking pink mucosa (oval-7 \times 50 mm) is present approximately 7 cm from the transverse line in a longitudinal orientation. It appears the fibrin is missing in this area. Just distal to this area is where the thicker layers of fibrin starts. The serosal surface in this area (approximately 9.5 cm segment) was darker red and the tissue was firm due to the fibrin layers lining the mucosa. Immediately distal to this was an intussusception involving a 20 cm segment of the distal most colon (length not accounting for the thick bunching of the intussceptum) . The tip of the intussusceptum was approximately 5 cm from the cloacal opening. The outer most layer of the colon was thin, pale pink (serosal) and pale red (mucosal), but had a glistening appearance. Internal to this the intussusceptum was medium brown colored and had a leathery texture

(consistent with the tissue seen protruding from the cloaca the previous day). The proximal $\frac{1}{2}$ of the intussusceptum was covered with thick layers of fibrin up to 1 cm thick. The inner most layer of intestine was a thin tubular structure that was pale to dark red in color, except for a

focal segment that was deep to the fibrin layers where the tissue was pale tan in color. The liver had a mottled yellow and red cobblestoned appearance over 2/3 of the serosal surface. The other 1/3 was a smoother darker reddish brown color. The cut surfaces showed similar

changes. The gall bladder was unremarkable. Bladder was pink and the mucosal surface of the bladder was uniformly .smooth. The kidneys appear symmetrical in size. The spleen is a dark red in color and was uniform in texture. The brain was

not examined.

PRELIMINARY DIAGNOSIS: Intussusception.

Samples

SECIMENS RECEIVED: Tissue-Fixed formalin.

HISTO: Pancreas, Liver (A); Spleen, Heart (B); Kidney (C); Lung (D); Intestine small (E); Intestine large, Esophagus (F); Intestine large (G); Intestine small (H); Intestine large, Lung, Stomach (I); Intestine large, Lung (J); Trachea (K).

Laboratory Results

HISTOPATHOLOGY

Intestine large: In one section (G), diffusely, mucosa is ablated and occupied by eosinophilic cell debris. In another section (I), there is full thickness necrosis of mucosa with colonization of bacteria, and underlying muscularis near the serosal surface is hemorrhaged and diffusely necrotic secondary to thrombosed vessels.

Intestine small: In one section (E), mucosa is ablated, and large bullae are within smooth muscle wall. In another section (J), there is full thickness necrosis of mucosa with colonization of bacteria, and underlying muscularis near the serosal surface is hemorrhaged and diffusely necrotic secondary to thrombosed vessels. In another section, there is diffuse coagulation necrosis of mucosa associated with bacteria and diffuse hemorrhage, necrosis, and thrombosis within underlying muscularis.

Kidney: Scattered proximal tubules manifest cytoplasmic hypereosinophilia and fragmentation.

Heart: Within atrium are large nidi of what appear to be extramedullary hematopoiesis. Occasional large pleomorphic nuclei are scattered about ventriclle.

Liver: A thrombus is present.

All other Organs: No remarkable lesions are seen.

COMMENTS: None

Final Diagnosis (in order of importance)
Diagnosis Topog Morpho Etiol Funct Dis Link

1. Bacterial enteritis (T50500) (M42100) (E10012) () () ()
Diagnostic findings may not be published without the knowledge and consent of the pathologist.

Milt Code: (Infectious disease)

Submitter Name:

Dr. Bethany Doescher

Sea Life Park

41-202 Kalanianaole Hwy Suite 7

Waimanalo, Hawaii 96795

United States

Case: 25287 Accession: 6

Date Collected: 2/24/2016
Date Examined: 3/4/2016

Prosector: T. M. Work
Pathologist: T. M. Work

Signalment-Morphometrics-History

CONDITION: (Unknown) POSTMORTEM: (Good) EUTHANASIA: (Not euthanized)

TAG TYPE: (Reference Number) TAG NO.: (2014 Turtle1)

SPECIES: (Turtle: Green) AGE: (Immature) SEX: (Female)

COLLECTION-SITE: (Sea Life Park) AREA: (Honolulu) STATE: (Hawaii) COUNTRY: (United

States)

HISTORY: Formalin-fixed tissues were submitted from this turtle that died at Sea

Life Park on 24 February 2016. Turtle identification is 2014 Turtle 1.

External/Internal

EXTERNAL:

INTERNAL:

PRELIMINARY DIAGNOSIS: Undetermined.

Samples

SECIMENS RECEIVED: Tissue-Fixed formalin.

HISTO: Liver (A); Heart, Pancreas, Spleen (B); Adrenal, Kidney, Ovary (C); Lung, Skeletal muscle, Trachea (D); Esophagus, Liver, Stomach (E); Intestine small (F); Intestine large, Heart (G); Intestine large (H).

Laboratory Results

HISTOPATHOLOGY

Intestine large: DIffuse areas of mucosa manifest coagulation necrosis associated with clumps of bacteria.

Liver: DIffusely, hepatocytes are distended with multiple variably sized ill-defined vacuoles giving them a lacy appearance.

Kidney: There are localized areas where proximal tubule cells are fragmented with nuclear karyolysis and intact basement membranes.

All other Organs: No remarkable lesions are seen.

COMMENTS: None

Final Diagnosis (in order of importance)

Diagnosis Topog Morpho Etiol Funct Dis Link

25287-6 Page 2 of 2

1. Bacterial enteritis (T50500)(M42100)(E10012)())()()() Diagnostic findings may not be published without the knowledge and consent of the pathologist. Milt Code: (Infectious disease)