DIAGNOSTIC SERVICES SECTION

FINAL REPORT

SOUTHEASTERN COOPERATIVE WILDLIFE DISEASE STUDY (SCWDS) COLLEGE OF VETERINARY MEDICINE THE UNIVERSITY OF GEORGIA **ATHENS, GEORGIA 30602-7393**

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CASE NUMBER	CC18-699
DATE RECEIVED	December 17, 2018
DATE OF REPORT	April 26, 2019

SPECIES (NO.)*	(7)	_SEX	* AGE_	*WEIGHT
SCWDS Number	Species	Sex	Age	Weight (grams)
CC18-699 A	Herring gull	Male	Juvenile	650
CC18-699 B	Common eider	Female	Juvenile	1090
CC18-699 C	Brown pelican	Male	Juvenile	5230
CC18-699 D	Northern gannet	Male	Adult	4010
CC18-699 E	Laughing gull	Male	Adult	485
CC18-699 F	Laughing gull	Male	Adult	415
CC18-699 G	Laughing gull	Female	Adult	505

CASE HISTORY: The carcasses of seven birds (multiple species) were submitted by Mr. James Dillman of the South Carolina Department of Natural Resources (SCDNR). Over 30 birds of multiple species, including juveniles and adults, were found dead or dying on North Myrtle Beach over a two day period. Some birds were actively feeding at the time of death. Some of the public and an officer reported a "smell" in the air. Additional dead and dying birds were reported in Garden City, SC, at the same time. The carcasses were received by SCWDS on December 17, 2018, and were necropsied the following day, once the carcasses were thawed.

FINAL DIAGNOSIS: CC18-699 A-G: Cause of mortality event undetermined

STATE SC COUNTY Horry AREA North Myrtle Beach

CC18-699 A: Multi-organ granulomas (ceca, intestine, liver, lung) with acid fast bacteria, presumed Mycobacterium spp.; bacterial bursitis; Brevetoxin detected

CC18-699 B: Brevetoxin detected

CC18-699 C: Hepatitis; bacterial tracheitis; brevetoxin detected

CC18-699E: Bacterial ventriculitis

COMMENTS: Numerous toxins and infectious agents were ruled out as the cause of this multi-species outbreak. Numerous birds had evidence of varied bacterial infections (but not consistently among the birds), suggesting weakened immune systems. For example, CC18-699 A had microscopic lesions consistent with *Mycobacterium* spp. infection in numerous organs, including the intestine; several other birds had evidence of localized bacterial infections (e.g., inflammation in the trachea of CC18-699C and in the ventriculus of CC18-699E). No Salmonella spp. were isolated from CC18-699 A-C. A general toxicology screening test in a pooled sample from CC18-699 A-G for common organic compounds was run and yielded no significant results. Low levels of brevetoxin were detected in CC18-699 A-C; however, this is suspected to be incidental and not to be the cause of this mortality event. No domoic acid was detected from CC18-699 A-C. No abnormalities in heavy metal or cholinesterase levels were detected in samples from CC18-699 D-F. The potential role of severe weather events as well as more long-term environmental factors in mortality events in birds and other wildlife is difficult to document. These types of events often result in minimal gross and microscopic evidence of disease. Many of the birds in the present outbreak were in good nutritional condition and appeared to be eating when they died, suggesting death was very acute.

Mr. Dillman was notified of receipt of the carcasses on December 17, 2018, an update on December 29, 2018, February 15, 2019, April 3, 2019, and a final diagnosis on April 15, 2019, via electronic mail.

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WILDLIFE IMPLICATIONS: Various Mycobacterium spp. have been isolated from numerous domestic and wild birds.

PUBLIC HEALTH IMPLICATIONS: There are numerous Mycobacterium spp., all of which have varying levels of pathogenicity in humans. The most common Mycobacterium spp. in birds are unlikely to cause disease in an immunocompetent adult human; however, children and immunosuppressed individuals pose a higher risk. Care should always be taken when handling infected tissue, and animals infected with Mycobacterium spp. should not be consumed.

DOMESTIC ANIMAL IMPLICATIONS: Various *Mycobacterium* spp. have been isolated from numerous domestic animals, some of which play an important role in agriculture.

DIAGNOSTICIAN

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DISTRIBUTION: SCWDS File, Dillman, Ruth, Cantrell

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GROSS LESIONS:

CC18-699 A: The juvenile, male herring gull is in fair nutritional condition with mild postmortem autolysis. Pinpoint, yellow foci are scattered throughout the mucosal aspect of the proventriculus. Red fluid (suspect blood mixed with liquid) is in the distal ileum and large intestine. A 1 cm, slightly raised, light tan plaque is on the mucosal aspect of the mid-large intestine.

CC18-699 B: The juvenile, female common eider is in poor nutritional condition with mild postmortem autolysis. Occasional, 1 mm diameter, tan to yellow nodules are scattered along the serosal aspect of the small intestine. These nodules are associated with numerous, orange parasites (likely *Acanthocephalan* sp.) that are firmly attached to the small intestinal mucosa, extending from the jejunum to the ileum.

CC18-699 C: The juvenile, male brown pelican is in excellent nutritional condition with moderate postmortem autolysis. A 6 cm diameter, chronic, ulcer is along the left side of the oral cavity, and multiple, small chronic, ulcers are along the length of the throat pouch and the proventriculus and the ventriculus. A 2 cm diameter, chronic, superficial abrasion is approximately midway along the length of the left side of the neck. Mild hemorrhage is along the ventral neck. The lungs are diffusely inflated with air with clotted blood scattered throughout the parenchyma. Numerous fish are within the oral cavity, the throat pouch, and the proventriculus and the ventriculus. Numerous roundworms are in the proventriculus, and a moderate number of tapeworms are scattered throughout the small intestine.

CC18-699 D: The adult, male northern gannet is in good nutritional condition with mild postmortem autolysis. The 4th digit of the left foot has multiple, superficial, round, yellow plaques. Several roundworms, a single fish, several shrimp, and fish eggs are in the proventriculus and ventriculus. Numerous tapeworms are within the small intestine.

CC18-699 E: The adult, male laughing gull is in excellent nutritional condition with mild postmortem autolysis. The liver is diffusely mottled pale. Numerous fish are within the oral cavity, proventriculus, and ventriculus, and light tan liquid fills the small and large intestines.

CC18-699 F: The adult, male laughing gull is in good nutritional condition with mild postmortem autolysis. The lungs are diffusely congested and emphysematous, and the liver is slightly rounded and mottled pale. The ventriculus contains a scant amount of fish bones and clear, light green fluid, and the intestines contain orange to dark brown liquid.

CC18-699 G: The adult, female laughing gull is in good nutritional condition with mild postmortem autolysis. Numerous fish are within the proventriculus and ventriculus. The intestines are empty.

MICROSCOPIC FINDINGS:

CC18-699 A:

Ceca and intestine: Multiple granulomas, characterized by a central area of necrosis surrounded by a thick, circular band of Langhan's multinucleated giant cells, numerous of which contain clear vacuoles, eosinophils, lymphocytes, plasma cells, rare heterophils and histiocytes, and a thin band of fibrous connective tissue, are scattered throughout the much of the submucosa. Abundant lymphocytes and plasma cells spill from the granulomas into the adjacent lamina propria. Occasional, acid-fast, short bacilli are detected by Ziehl-Neelsen acid-fast bacteria (AFB) stain within the granulomas (consistent with *Mycobacterium* spp.).

Liver: Two to three, small granulomas, characterized by a thick, circular band of Langhan's multinucleated giant cells, numerous of which contain clear vacuoles, eosinophils, rare histiocytes, and a thin band of fibrous connective tissue, are scattered throughout the hepatic parenchyma. Occasional dark tan, globular structures are scattered throughout the parenchyma (consistent with hemosiderin).

Lung: Similar granulomas as those described in the liver are rarely scattered throughout the pulmonary parenchyma. These granulomas occasionally have brightly eosinophilic, necrotic debris centrally. Long, filamentous, brown yeast or fungi are detected within the granulomas by Grocott's methanemine silver (GMS) stain. These filamentous

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organisms do not stain with the Lillie Twort gram stain. Mixed bacterial colonies, including gram positive chains of cocci and gram negative short bacilli, are detected by Lillie Twort Gram stain within the granulomas. No acid-fast bacteria are detected by acid-fast bacteria (AFB) stain.

Cloacal bursa: Abundant necrotic, eosinophilic debris, admixed with viable and degenerate eosinophils and mats of bacteria, are within the bursal lumen, adjacent to plicae. One follicle is distended by central necrotic debris among an aggregate of heterophils; myriad bacteria are within the debris. Bacteria within the lumens are gram-negative short bacilli, evident by Lillie Twort Gram stain. Occasional eosinophils are scattered throughout the interstitium and are within follicles. Few follicles contain clear vacuoles with a small amount of lightly basophilic, wispy material (consistent with mucoid debris).

Crop: Scattered eosinophils infiltrate the superficial lamina propria.

No significant lesions are identified in sections of trachea, ventriculus, heart, kidney, pancreas, and brain.

CC18-699 B:

Lung: Rare parabronchi contain a small number of extravascular erythrocytes (hemorrhage). Diffusely, there is vascular congestion.

Skeletal muscle (peritracheal): Occasional extravascular erythrocytes (hemorrhage) are between myofibers.

Intestine: Small numbers of lymphocytes, plasma cells, and eosinophils are scattered throughout the mucosa. Gravid, adult trematodes are within the intestinal lumens, and approximately 200-µm to 300-µm diameter larvae are within the mucosa (suspect *Acanthocephalen* sp.).

Liver: Occasional bile ducts contain a moderate amount of granular, eosinophilic material (bile plugs).

No significant lesions are identified in sections of ovary, crop, kidney, oviduct, peripheral nerve, trachea, air sac, spleen, pancreas, and brain.

CC18-699 C:

Trachea: The lamina propria is moderately to markedly expanded by abundant viable and degenerated heterophils intermixed with pyknotic debris as well as markedly congested blood vessels. The overlying epithelium is markedly hyperplastic, and infiltrated by occasional heterophils. Numerous short, bacterial bacilli are scattered along and adhered to the surface of the epithelium within the tracheal lumen. Diffusely, there is vascular congestion.

Intestine: Rare lymphocytes, plasma cells, and eosinophils are scattered throughout the mucosa. Occasional cross-sections and tangential sections of trematodes, characterized by a lightly eosinophilic cuticle, gastrointestinal tract, reproductive tract, lacking a body cavity, and often containing operculated eggs, are within the lumen and the intestinal mucosa.

Lung: Scattered throughout the interstitium, most prominently within air capillaries and occasionally within blood vessels, there are variably-sized colonies of short, wide bacilli, which are gram-negative by Lillie Twort Gram stain (presumed postmortem overgrowth). Diffusely, there is vascular congestion.

Cloacal bursa: The follicles are large and contain a mostly uniform population of lymphocytes and fewer plasma cells; the follicles lack cortical and medullary distinction.

Liver: Moderate numbers of cells of erythroid and myeloid cell lines of varied maturity surround numerous vessels scattered throughout the parenchyma (extramedullary hematopoiesis). No organisms are detected by Lillie Twort Gram stain.

No significant lesions are identified in sections of heart, kidney, spleen, brain, and ventriculus.

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CC18-699 D:

Duodenum: Numerous heterophils, and variable proportions of lymphocytes and plasma cells, infiltrate the lamina propria. Crypt lumens within these areas contain degenerated heterophils and pyknotic debris (abscesses).

Liver: Rare lymphocytes, plasma cells, and fewer histiocytes surround occasional blood vessels.

Lung: Degenerated histiocytes, lymphocytes, and plasma cells infiltrate the skeletal muscle adjacent to the parabronchi and spill into the parabronchial lumens. Long bacilli are within blood capillaries and often scattered along the surface of the parabronchi (presumed postmortem overgrowth).

Esophagus: Occasional, degenerated lymphocytes and plasma cells infiltrate the superficial submucosa and occasionally surround blood vessels.

Kidney: Multifocal, nodular aggregates of cells of mixed erythroid and myeloid cell lines of varied maturation expand the interstitium (consistent with extramedullary hematopoiesis). The tubular epithelium is multifocally attenuated and compresses adjacent tubules.

No significant lesions are identified in sections of small and large intestines, heart, crop, spleen, pancreas, bile duct, and brain.

CC18-699 E:

Ventriculus: There is a focal area of koilin degeneration and necrosis with a small number of degenerated mononuclear inflammatory cells. The surface of the koilin is irregular and invaded by scattered colonies of large, coccoid bacteria, which extend approximately halfway through the layer.

Kidney: A few small aggregates of lymphocytes and plasma cells mildly expand the interstitium. Fluke eggs are embedded within a single focus of renal tissue (possibly postmortem processing artifact).

Liver: Small to moderate sized aggregates of degenerated, mononuclear cells surround occasional blood vessels.

Lung: Scattered throughout the interstitium, most prominently within air capillaries, there are variably-sized colonies of short, wide bacilli (similar to Bird A). Long bacilli are within blood capillaries and often scattered along the surface of the parabronchi (suspect postmortem overgrowth). Diffusely, there is vascular congestion. A focus along the pleura contains necrotic cell debris and abundant bacterial bacilli.

No significant lesions are identified in sections of brain, small and large intestines, proventriculus, and heart.

CC18-699 F:

Liver: Small numbers of lymphocytes and plasma cells, and rare heterophils occasionally surround blood vessels. Numerous hepatocytes contain clear, round vacuoles (lipid).

Kidney: Rare lymphocytes and plasma cells are scattered throughout the interstitium.

Esophagus: Small numbers of lymphocytes, plasma cells, and rare heterophils surround a few blood vessels within the adipose tissue along the serosal aspect of the esophagus.

Lung: Scattered throughout the interstitium, most prominently within air capillaries, there are mixed bacterial colonies (short, wide bacilli, large cocci and long bacilli). Occasional foamy macrophages are scattered throughout alveolar capillaries (presumed postmortem overgrowth). Blood vessels are diffusely congested.

No significant lesions are identified in sections of small and large intestines, brain, proventriculus, ventriculus, trachea, esophagus, and heart.

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CC18-699 G:

Kidney: Small numbers of lymphocytes and plasma cells are scattered throughout the interstitium. Small, nodular aggregates of lymphocytes and plasma cells infiltrate the wall of the ureter.

Lung: Scattered throughout the interstitium, most prominently within air capillaries, there are variably-sized colonies of short, wide bacilli (similar to Bird A). Mixed bacteria, predominantly short bacilli, are within blood capillaries and often scattered along the surface of the parabronchi (suspect postmortem overgrowth). Diffusely, there is vascular congestion.

No significant lesions are identified in sections of small and large intestines, brain, heart, ovaries, and liver.

MORPHOLOGIC DIAGNOSES:

CC18-699 A:

Ceca and intestine: Submucosal granulomas, multifocal, severe, chronic, with intra-granulomatous acid-fast bacilli (consistent with *Mycobacterium* spp.)

Liver: Eosinophilic granulomas, multifocal, mild, chronic

Lung: Eosinophilic granulomas, multifocal, mild, chronic, with intra-granulomatous gram-positive bacilli (suspect *Mycobacterium* spp.)

Cloacal bursa: Necrotizing, eosinophilic bursitis, multifocal, severe, chronic with intralesional gram-negative bacilli

CC18-699 B:

Lung: Parabronchial hemorrhage, multifocal, mild

Skeletal muscle: Hemorrhage, multifocal, minimal

Intestine: Eosinophilic enteritis, multifocal, mild, chronic, with intraluminal and intramucosal trematodes (suspect *Acanthocephalen* spp)

CC18-699 C:

Trachea: Necrotizing, heterophilic tracheitis, diffuse, moderate, subacute, with epithelial hyperplasia and intralesional bacterial bacilli

Intestine: Lymphoplasmacytic, eosinophilic enteritis, multifocal, mild, chronic, with intraluminal and intramucosal trematodes

CC18-699 D:

Duodenum: Eosinophilic enteritis with crypt abscesses, multifocal, mild, subacute

Liver: Lymphoplasmacytic, perivascular hepatitis, multifocal, minimal, chronic

Lung: Histiocytic parabronchitis, multifocal, mild, chronic

Esophagus: Lymphoplasmacytic esophagitis, multifocal, mild, chronic

CC18-699 E:

Ventriculus: Necrotizing ventriculitis, focal, severe, with intralesional bacterial cocci

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Kidney: Lymphoplasmacytic, interstitial nephritis, multifocal, mild, chronic

Liver: Hepatitis, multifocal, minimal, chronic

CC18-699 F:

Liver: Hepatic lipidosis, diffuse, mild

CC18-699 G:

Kidney: Lymphoplasmacytic, interstitial nephritis, multifocal, minimal, chronic

BACTERIOLOGY:

CC18-699 A, B, C: Samples of liver were submitted to the Athens Veterinary Diagnostic Laboratory (AVDL) for *Salmonella* spp. testing. No *Salmonella* spp. were isolated in bacterial culture.

CC18-699 C, D, E: Samples of lung were submitted to the AVDL for aerobic bacterial culture. Heavy growth of *Moraxella ovis/lacunata* and *Corynebacterium* spp. were isolated from CC18-699 C. Moderate growth of *Acinetobacter Iwoffii* and *Corynebacterium* spp. were isolated from CC18-699 D. Moderate growth of *Vagococcus fluvialis* and *Streptococcus* sp. (alpha) were isolated from CC18-699 E.

TOXICOLOGY:

CC18-699 A, B, C: Samples of liver with gallbladder, kidney, ventriculus contents, intestinal contents, and brain were submitted to the Greenwater Laboratories in Palatka, FL, for brevetoxin and domoic acid testing. No significant domoic acid levels were detected. Low levels of brevetoxin were detected in the gallbladder and ventriculuar contents of CC18-699A, the liver, gallbladder, and intestinal contents of CC18-699B, and the ventricular contents of CC18-699C. Low brevetoxin levels are difficult to interpret, but are unlikely to have caused this mortality event. No other significant brevetoxin levels were noted.

CC18-699 D:

A sample of brain was submitted to the California Animal Health and Food Safety Laboratories (CAHFSL) in Davis, CA, for cholinesterase testing. The cholinesterase level reported was 17 uM/g/min, which is considered normal for this species.

A sample of liver was submitted to CAHFSL in Davis, CA, for heavy metal testing. No heavy metals of significance were detected.

Heavy Metal Screen

Analyte	Result in parts per million (PPM)	Reporting limit (PPM)	
Lead	Not detected	1	
Manganese	5.1	0.1	
Iron	180	1	
Mercury	Not detected	1	
Arsenic	Not detected	1	
Molybdenum	0.47	0.4	
Zinc	25	0.3	
Copper	8.6	0.3	
Cadmium	Not detected	0.3	

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CC18-699 E:

A sample of brain was submitted to the California Animal Health and Food Safety Laboratories (CAHFSL) in Davis, CA, for cholinesterase testing. The cholinesterase level reported was 19 uM/g/min, which is considered normal for this species.

A sample of liver was submitted to CAHFSL in Davis, CA, for heavy metal testing. No heavy metals of significance were detected.

Heavy Metal Screen

Analyte	Result in parts per million (PPM)	Reporting limit (PPM)
Lead	Not detected	1
Manganese	3.6	0.1
Iron	460	1
Mercury	Not detected	1
Arsenic	Not detected	1
Molybdenum	0.71	0.4
Zinc	28	0.3
Copper	5.6	0.3
Cadmium	Not detected	0.3

CC18-699 F:

A sample of brain was submitted to the California Animal Health and Food Safety Laboratories (CAHFSL) in Davis, CA, for cholinesterase testing. The cholinesterase level reported was 15 uM/g/min, which is considered normal for this species.

A sample of liver was submitted to CAHFSL in Davis, CA, for heavy metal testing. No heavy metals of significance were detected.

Heavy Metal Screen

Analyte	Result in parts per million (PPM)	Reporting limit (PPM)
Lead	Not detected	1
Manganese	3.5	0.1
Iron	230	1
Mercury	Not detected	1
Arsenic	Not detected	1
Molybdenum	0.62	0.4
Zinc	29	0.3
Copper	5.2	0.3
Cadmium	Not detected	0.3

CC18-699 A-G: A pooled sample of liver was submitted to CAHFSL in Davis, CA, for general toxin screening. P,p'-DDE was detected by gas chromatography-mass spectrometry (GC/MS). P,p'-DDE is a metabolite of DDT, and is not a significant finding. No toxic compounds were detected by liquid chromatography-mass spectrometry (LC/MS) organic chemical screen.