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Submitting Agency:

Arkansas Game and Fish Commission

Submitter:

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Specimen: DOA

Diagnostic Services

Diagnostic Services Section

State:	AR
County:	Washington
Area:	Adjacent to the Eco Vista Landfill, Tontitown
Taxonomic group:	Avian-Passerine
Species:	European Starling
Age:	Multiple

Case History

The frozen carcasses of six European starlings were submitted by Jennifer Ballard of the Arkansas Game and Fish Commission (AGFC). These starlings were collected over a two day period around December 20, 2021 by private citizens and AGFC biologists and were part of a mortality event. The carcasses were frozen until shipping on January 5, 2022 and received by SCWDS on January 6, 2022. A gross necropsy was performed the following day.

Carcasses A and B were collected by a private citizen and carcasses C-F were collected by AGFC biologists. Individual information is listed below:

W22-006A: 81.0g, adult, male, necropsy W22-006B: 89.7g, adult, male, necropsy W22-006C: 77.0g, adult, female, necropsy W22-006D: 79.0g, adult, sex undetermined, necropsy W22-006E: 80.8g, adult, sex undetermined, carcass frozen W22-006F: 79.2g, adult, sex undetermined, carcass frozen

Final Diagnosis: Caffeine and theobromine exposure: W22-006 A-D; W22-006 E-F (presumed)

Diagnostic Services Section

Comments

Both caffeine and theobromine were detected in a pooled liver sample from starlings W22-006 A-D by a general toxin screening test. This combination of methylxanthine compounds is commonly found in chocolate. While these are occasionally detected in free-ranging scavenger/opportunistic avian species and often considered incidental, detection of these compounds is less common in starlings. Fatal chocolate intoxication has been reported in avian species, however, these reports have typically been limited to parrot species and their role as a significant toxin of passerines has not been determined. Furthermore, the toxin screen test does not quantify the level of each compound and it cannot be determined if the concentrations detected were above the threshold reported in fatal cases in one or all of these birds. The necropsied birds did appear to be in good general health, including good nutritional condition and adequate microscopic fat stores, which supports a more acute or rapidacting disease process. Microscopic lesions suggestive of toxic changes are not identified on examination although these lesions are often subtle and postmortem tissue deterioration may have obscured subtler lesion patterns. No essential oils or other toxic compounds were detected in the pooled liver sample, however, death associated with other toxins cannot be completely ruled out based on this test. There was no evidence of significant trauma or infections, including Mycoplasma sp. or arboviruses (i.e. West Nile virus). Other potential causes or contributors to death that are challenging to document postmortem include environmental events, such as severe weather, or physiological or metabolic derangements (such as acute stress) that may lead to sudden death without overt lesions. Please submit additional birds if the mortality event continues or recurs.

Veterinary toxicologists at the California Animal Health and Food Safety Laboratory (CAHFS) at UC-Davis were consulted on this case.

Confirmation of sample receipt was sent to Jennifer Ballard on January 6, 2022 and an update was provided on February 2, 2022 via electronic mail.

Wildlife Implications

Wildlife exposure to theobromine can result from consumption of livestock feed, cocoa byproducts, or through exposure to confectionaries (and waste products) that are used to feed or attract wildlife. The sensitivity of various wildlife species to theobromine toxicity is variable and largely unknown. Young or small animals may be especially vulnerable because toxicosis can result from consumption of smaller quantities. Wildlife species reported with theobromine toxicosis include the red fox, European badger, coyote, raccoon, and black bear.

Public Health Implications None apparent.

Domestic Animal Implications

Theobromine and caffeine have been shown to be responsible for chocolate toxicosis in domestic dogs. Theobromine-related mortality also has been noted in domestic pigs, poultry, calves, horses, cats, rabbits, and rodents.

Diagnostician: Caitlin Burrell, DVM, MS, Dipl. ACVP

Laboratory Results

Necropsy

Necropsy

Gross Findings

Four European starlings were necropsied.

W22-006A: The carcass of a European starling is received for necropsy in good nutritional condition with adequate adipose stores and skeletal muscle and fair to poor postmortem condition. The proventriculus and ventriculus are empty and the small and large intestines contain a small amount of yellow tan mucoid digesta.

W22-006B: The carcass of a European starling is received for necropsy in good nutritional condition with adequate adipose stores and skeletal muscle and fair to poor postmortem condition. The proventriculus and ventriculus contain a small amount of grit and the small and large intestines contain a small amount of yellow tan mucoid digesta.

W22-006C: The carcass of a European starling is received for necropsy in good nutritional condition with adequate adipose stores and skeletal muscle and fair to poor postmortem condition. The skin overlying the crop has a 2×3 mm, ovoid, full-thickness laceration with no apparent hemorrhage. The small and large intestines contain a small amount of yellow tan mucoid digesta.

W22-006D: The carcass of a European starling is received for necropsy in good nutritional condition with adequate adipose stores and skeletal muscle and fair to poor postmortem condition. Free in the coelom immediately under the keel is a thin, white, nematode. The intestinal walls are diffusely pale pink-red.

Necropsy

Microscopic Findings W22-006A:

Lung: Focally, a single blood vessel contains a large aggregate of small coccobacilli.

Trachea: Diffusely, the mucosa is obscured by large superficial mats of coccobacilli. The submucosa has long chains of bacilli (suspect postmortem overgrowth).

No significant lesions are identified in sections of: brain, spleen, kidney, adrenal gland, testes (inactive), liver, heart, ventriculus, proventriculus, small intestine, large intestine, esophagus, skeletal muscle, and peripheral nerve.

W22-006B:

Trachea: The mucosa has few small aggregates of mononuclear cells.

No significant lesions are identified in sections of: brain, lung, spleen, kidney, liver, lung, adipose (coelomic, epicardial), ventriculus, small intestine, pancreas, cloaca, heart, esophagus, ovary, and adrenal gland.

W22-006C:

Skeletal muscle: Rare myocytes have a thin-walled, intrasarcoplasmic protozoal cyst containing myriad $5 \times 2 \mu m$, crescentic bradyzoite (*Sarcocystis* sp., presumptive). Focally, the interstitium has a small aggregate of necrotic mononuclear cells.

No significant lesions are identified in sections of: spleen, bone marrow, ovary, brain, lung, liver, adipose (coelomic, subcutaneous), trachea, oviduct, cloaca, heart, and ventriculus.

W22-006D:

Lung: The mucosa of a single large, distal bronchus has multiple, roughly nodular aggregates of mononuclear cells and scant cellular debris.

Liver: The parenchyma has a single, small focus of cellular debris, mononuclear cells, and few extravasated erythrocytes (hemorrhage). Many hepatocytes have scant to moderate, tan, finely granular, intracytoplasmic material.

Coelom: Free on a slide and corresponding to the coelomic parasite identified on gross exam is a 1 mm wide nematode with a thick cuticle, platymyarian musculature, vacuolated lateral chords, and paired reproductive tracts containing sperm and ova with a thick-yellow tinged, refractile wall.

No significant lesions are identified in sections of: spleen, adipose (coelomic, peritracheal), brain, kidney, small and large intestine, ventriculus, skeletal muscle, peripheral nerve, esophagus, trachea, thymus, and skin.

Advanced autolysis and freeze-thaw artifact may have obscured subtle lesion patterns in all four birds. Copious postmortem bacterial bacilli are scattered among multiple tissue sections.

Necropsy

Morphologic Diagnoses W22-006A:

None.

W22-006B:

None.

W22-006C:

Skin: Focal, full-thickness perforation (incidental)

Skeletal muscle: Few protozoal cysts (Sarcocystis sp., presumptive, incidental)

W22-006D:

Lung: Minimal, multifocal, chronic mononuclear inflammation

Coelom: Minimal nematodiasis (incidental)

Bacteriology

A swab of the conjunctiva from each bird W22-006 A-D was submitted to the SCWDS Molecular Laboratory for mycoplasma testing. No *Mycoplasma* spp. DNA was detected via polymerase chain reaction (PCR) testing.

Virology

Individual pooled samples of brain, heart, and kidney from birds W22-006 A-D were submitted to the SCWDS Virology Laboratory for arthropod-borne virus (West Nile virus, eastern equine encephalitis virus) testing. No arthropod-borne viruses were detected via virus isolation.

Toxicology

A pooled sample of the liver from birds W22-006 A-D was submitted to the California Animal Health and Food Safety Laboratory (CAHFS) at UC-Davis for gas-chromatography-mass spectrometry (GC/MS) and liquid-chromatography-mass spectrometry (LC/MS) testing. The screens are designed to potentially detect a large number of organic compounds belonging to diverse chemical classes (pesticides, environmental contaminants, drugs and natural products). Caffeine and theobromine were identified in the submitted pooled sample by LC/MS. This combination is commonly found in chocolate.