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An Acute Mortality Event Associated with Novel *Macrorhabdus ornithogaster* Infection and Underlying Factors in a Newly-Established Captive Group of American White Ibis (*Eudocimus albus*) Nestlings

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ABSTRACT: Twenty-four American white ibis (*Eudocimus albus*) nestlings were collected in Florida (USA) on 17 April 2017 to establish a captive flock. On 7 May 2017, three birds died suddenly, following severe lethargy, hemorrhaging from the mouth and nares, anorexia, and production of bright-green colored feces. An additional ibis with delayed growth and pathological fractures was euthanized 18 May 2017. Severe ventriculitis associated with *Macrorhabdus ornithogaster* was noted in all four birds, bacterial sepsis was confirmed in one bird by culture and histologic examination, and bacterial endotoxemia was suspected in two birds based on gross and histologic examination, but no bacteria were isolated from these birds. Birds also had vitamin E liver levels consistent with coagulopathy previously described in pelicans. We sampled feces from 91 adult, free-living, healthy ibis in Florida in July 2017 and found 71% were shedding organisms with morphologic characteristics consistent with *Macrorhabdus* sp. Molecular characterization of the ibis-origin *M. ornithogaster* showed it was phylogenetically related to numerous *M. ornithogaster* sequences. It is unknown if *M. ornithogaster* infection resulted in clinical disease as a result of dietary or stress-related dysbiosis, or other factors. *Macrorhabdus*-associated disease has not previously been confirmed in wading birds. We discuss potential associations of gastric *M. ornithogaster* infection with morbidity and mortality in these cases and highlight the need for additional studies on this pathogen in free-living birds.

Key words: *Eudocimus albus*, *Macrorhabdus ornithogaster*, ventriculitis, white ibis.

American white ibis (*Eudocimus albus*) are increasingly found in urban areas (e.g., parks, golf courses, landfills) in South Florida, in response to decreasing wetland availability (Hernandez et al. 2016) and resources provided by urban habitats (Murray et al. 2018). As part of a study on the effects of urbanization on avian ecology and health, we collected 24 ibis nestlings (range 7–14 d old) on 17 April 2017 from a rookery in Broward County, Florida, USA (26° 07′27.4″N 80°32′29.3″W), to establish a captive flock and conduct a study on the impact of diet on pathogen dynamics. All procedures were reviewed by the University of Georgia's Institutional Animal Care and Use Committee (IACUC no. A2016 11-019) and under Florida Wildlife Conservation Commission (LSSC-11-00119F) and US Fish and Wildlife Service (MB779238-0) permits. Nestlings initially received oral electrolytes and were then transitioned to a dilute avian recovery formula (Emeraid, Piscivore diet, Cornell, Illinois, USA). The following day, nestlings were transported to the University of Georgia, Athens (UGA, Athens, Georgia, USA). Two equal groups were housed in separate pens. Ibis nestlings grow rapidly; thus husbandry, nutrition, and environmental enrichment provided were adjusted over time (see Table 1 for diet composition and delivery methods relevant to this study; for additional husbandry details see Supplementary Materials).

From the time of collection, ibis 73 (pen 1) exhibited regurgitation, slow weight gain, and persistent leukocytosis. It was hospitalized on 24 April 2017 at the UGA College of Veterinary Medicine and treated with subcutaneous and oral fluids, antibiotics (trimethoprim-sulfamethoxazole, 48 mg/kg every 12 h,

TABLE 1. Diet components and delivery methods for white ibis (*Eudocimus albus*) nestlings collected from the wild to establish a captive colony for experimental purposes. The diet fed was designed in collaboration with a nutritionist (L. Hoopes) and was modified from diets used at other captive facilities to rear ibis and closely related wading birds. When a new diet was introduced, it was done in a stepwise fashion by replacing no more than 25% of the diet with the new diet at each feeding.

Time from collection	Diet components	Diet delivery
1–3 d	Emeraid® piscivore diet	Gavage feeding four times per day
3–7 d	Mazuri® flamingo breeder pellet,[a] egg yolk, water, salmon oil, vionate, calcium carbonate, Thiamine-E® paste[b] and smelt	Syringe feeding four times per day
After 7 d	Mazuri piscivore pellet, egg yolk, water, salmon oil, vionate, calcium carbonate, Vitamin E/thiamine paste[c] and smelt	Food consumed off of platters four times per day

[a] Mazuri pellet, Mazuri Exotic Animal Nutrition, St. Louis, Missouri, USA.
[b] Thiamine-E paste, Mazuri Exotic Animal Nutrition, St. Louis, Missouri, USA.
[c] The commercially available vitamin E and thiamine paste became unavailable for a few days and a homemade replacement was made with the same ingredients at the same proportions; however, it inadvertently resulted in a total dose 2.5× higher per bird than the commercial paste.

orally, HiTech Pharmacal, Amityville, New York, USA), a histamine H_2 receptor antagonist (famotidine, 0.5 mg/kg, orally every 24 h, Pepcid Complete, Johnson & Johnson, New Brunswick, New Jersey, USA) and probiotics (PetAg Bene-Bac Plus, 1 g every 24 h, orally, PetAg, Hampshire, Illinois, USA) for a presumed infection and gastrointestinal ulceration. It was reintegrated into the colony a week later but had additional health problems, including two folding fractures treated with calcium glubionate (50 mg/kg orally every 24 h, Neo-Calglucon, Rugby Labs, Duluth, Georgia, USA), exposure to natural sunlight, meloxicam (2 mg/kg orally every 24 h, Metacam, Boehringer Ingelheim, Duluth, Georgia, USA), and movement restriction.

An acute mortality event occurred in pen 1 on 7 May: one bird (60) was found dead in the morning without evidence of external trauma, and a second bird (55) was very lethargic, isolated, prostrate, passing blood from the nares and oral cavity, and died during handling. A third bird (63) was found lethargic that afternoon and died during supportive care. An additional bird (74) became lethargic that evening; received subcutaneous fluids, vitamin K (2.5 mg/kg intramuscularly every 24 h, Aquamephyton, Teligent Pharma, Buena, New Jersey, USA) and milk thistle extract (100 mg/kg orally every 12 h, Pet Well-Being, Crestwood, Kentucky, USA) and made a full recovery. At that point, birds in both pens were 33–37 d old, in good body condition, and growing as expected (excluding 73). We found bright green feces in pen 1 but not in pen 2. Given the hemorrhagic presentation of one bird and based on previous reports of acute hypervitaminosis E in other aquatic birds (Nichols et al. 1989), we provided vitamin K (2.5 mg/kg orally twice daily for 2 d then once daily for 5 d) to the remaining birds in pen 1.

Necropsies conducted on the three birds that died revealed similar lesions, principally copious amounts of dark green to dark red mucoid material in the lumen of the ventriculus, proventriculus and intestines, and multifocally and irregularly reddened serosa of the small and large intestines. From each bird, brain, tongue, esophagus, trachea, lung, air sac, heart, spleen, liver, kidney, adrenal, gonads, tongue, esophagus, proventriculus, gizzard, pancreas, small intestine, large intestine, skin, skeletal muscle, and bursa of Fabricius were examined histologically. Histopathology findings and case diagnostic interpretations are summarized in Table 2 and were consistent with widespread and severe

Macrorhabdus infection and associated ventricular damage (Fig. 1), characterized by fragmentation of koilin, separation, disruption, and necrosis of epithelium, heterophilic inflammation, and ventricular hemorrhage with disruption of the koilin layer of the ventriculus by large numbers of long yeast-like periodic acid Schiff stain (PAS)-positive organisms that ranged from 20 to 60 μm long and 1 to 5 μm wide. Liver and spleen were submitted for bacterial isolation based on histopathology lesions and the clinical history. Ibis 60 had a secondary bacterial septicemia from which *Vibrio* sp. and *Salmonella enterica* Group D were cultured, and 55 and 63 had a suspected bacterial infection or endotoxemia, based on gross and histopathologic examination (inflammation in 55 and necrosis in 63), but culture of the liver (55, 63) and spleen (63) yielded no growth. On 18 May, bird 73 suffered another pathologic fracture and was euthanized and necropsied. On histology it also had severe, multifocally extensive ventriculitis with intralesional *Mo*, which may have caused chronic regurgitation, poor body condition, and poor growth, and contributed to its osteopenia (Table 2).

In the three birds that died acutely, *Mo*-associated mucosal disruption probably precipitated sepsis and toxemia. As part of our study, we monitored *Salmonella* sp. shedding weekly in all birds. Its prevalence peaked at 86% (Day 3 postcollection), and averaged 67% until Week 5, but no birds ever exhibited clinical signs of salmonellosis before or after this event. Our work with *Salmonella* spp. in free-living nestlings demonstrates that shedding birds, even nestlings, do not exhibit clinical disease (Christie 2021). Although we did not observe gross or histopathologic lesions consistent with hypervitaminosis E, liver vitamin E levels in the birds that died were higher (252–455 μg/g dry weight liver, Michigan State University Veterinary Diagnostic Laboratory (MSU VDL, Lansing, Michigan, USA) than those reported in chickens (45–120 μg/g, MSU VDL) and similar to liver levels of pink-backed pelicans (*Pelecanus rufescens*) that died of acute vitamin E toxicosis (244–500 μg/g; Nichols et al. 1989; Sünder et al. 1999). Serum vitamin E levels for the surviving 20 ibis nestlings appeared elevated (15.34–37.13 μg/mL), yet they remained healthy, which may mean that accepted normal liver and serum vitamin E levels of other species may not be appropriate for ibis.

To confirm the identity of the histologically documented yeast, we tested scrolls of formalin-fixed paraffin-embedded proventriculus from the three birds that died for *Mo* using next-generation sequencing (see Supplementary Materials). The contigs from the three ibis contained four sequences from the ribosomal RNA gene regions. Partial 18S, 5.8S, and 26S rRNA gene and internal transcribed spacer region 1 (ITS1) sequences were 97.2–100% similar to *Mo* sequences from several hosts (Supplemental File 2). Phylogenetic analysis of a 600–base pair (bp) 18S rRNA sequence fragment confirmed the close relationship of *Mo* from the ibis with *Mo* recovered from other birds (Fig. 2). We submitted sequences derived during this study to GenBank (accession numbers OP481209, OP481210, and OP497965).

Macrorhabdus ornithogaster (*Mo*) is an anamorphic Ascomycetes yeast that grows at the proventricular–ventricular junction in numerous bird species (Tomaszewski et al. 2003). The yeast is typically reported in psittacines but also causes clinical disease in passerines and galliformes (Phalen 2014). Manifestations range from no clinical signs to sudden death or vomiting, diarrhea, chronic weight loss, and signs of gastric ulceration (blood loss and anemia). In budgerigars, the typical syndrome is characterized by chronic weight loss, vomiting, and diarrhea, but an acute hemorrhagic event has been reported in parrotlets (Phalen 2014). Presumptive diagnosis is through history, clinical signs, and microscopic examination of feces with confirmation based on histologic examination or polymerase chain reaction (PCR).

Following the mortality event, we examined fresh feces from birds in the affected pen, and all birds were shedding organisms with morphologic characteristics described for *Mo*. Thus, we treated all birds in both pens with amphotericin B at 25 mg/kg (Diamondback

TABLE 2. Principal pathologic findings of wild-caught white ibis (*Eudocimus albus*) nestlings that died acutely or were euthanized and were diagnosed with *Macrorhabdus ornithogaster* ventriculitis and sepsis. Immunohistochemical staining of tissues for Eastern equine encephalitis and West Nile virus were negative.

ID No.	Age (d)	Sex	Weight (g)	Manner of death	Gross pathology	Histopathology	Culture results
60	33 d	F	538	Found dead; no clinical signs	Diffuse pulmonary congestion, multifocal myocardial reddening, mild hepatomegaly and liver mottling, mild renomegaly, splenic pallor, dark green to dark red mucoid material in the lumen of the ventriculus, proventriculus, and intestines, mild intestinal trematodiasis	1. Hepatitis and splenitis, acute, multifocal, severe, necrotizing, with intralesional Gram-negative rods. 2. Ventriculitis, acute, multifocally extensive, severe, necrotizing, hemorrhagic, and heterophilic with *Macrorhabdus ornithogaster*–associated koilin degeneration 3. Enteritis, subacute, multifocal, lymphoplasmacytic, with intralesional Gram-negative bacterial infection and mild trematodiasis	Spleen: *Vibrio cholera, Salmonella enterica* Group D
55	33	F	678	Died during handling; lethargic	Multifocal subcutaneous hemorrhage, multifocal epicardial hemorrhage, diffuse pulmonary congestion, mild diffuse hepatomegaly and hepatic congestion, diffuse splenic pallor, mild diffuse renomegaly with friable kidneys, moderate amounts of mucoid hemorrhagic material in the oral cavity and copious dark red to green mucoid to watery material throughout the gastrointestinal tract, diffuse intestinal serosal reddening, mild intestinal trematodiasis	1. Hepatitis and splenitis, acute, multifocal, severe 2. Ventriculitis, acute, multifocally extensive, severe, necrotizing, heterophilic, with *Macrorhabdus ornithogaster*–associated koilin degeneration and Gram-negative bacteria 3. Enteritis, subacute, diffuse, moderate, with intralesional Gram-negative bacteria and mild trematodiasis	Liver: no significant growth

TABLE 2. Continued.

ID No.	Age (d)	Sex	Weight (g)	Manner of death	Gross pathology	Histopathology	Culture results
63	33	M	738	Died after acute illness	Diffuse pulmonary congestion, mild renomegaly with friable kidneys, copious dark red to green mucoid material in the lumen of the proventriculus, ventriculus, esophagus, and small intestines, diffuse reddening of the proventricular serosa, mild intestinal trematodiasis	1. Hepatitis and splenitis, acute, multifocal, acute, severe, necrotizing 2. Ventriculitis, acute, multifocally extensive, severe, necrotizing and heterophilic with *Macrorhabdus ornithogaster*–associated koilin degeneration and Gram-negative bacteria colonization. 3. Enteritis, chronic, diffuse, severe, lymphoplasmacytic, and mild trematodiasis	Spleen, liver: no growth
73	43	F	NA	Euthanized: delayed growth and pathological fractures	Left tibiotarsus midshaft closed fracture, diffuse skeletal muscle pallor, diffuse hepatic and renal pallor	1. Ventriculitis, acute, multifocally extensive, severe, with *Macrorhabdus ornithogaster*–associated koilin degeneration and hemorrhage 2. Hepatic lipidosis, diffuse, mild 3. Enteritis of Meckel's diverticulum chronic, diffuse, moderate, lymphohistiocytic, 4. Left and right humerus, osteopenia with healed fractures, diffuse, severe; left tibiotarsal bone, osteopenia, diffuse, severe with a healed fracture and an acute fracture with hemorrhage	NA

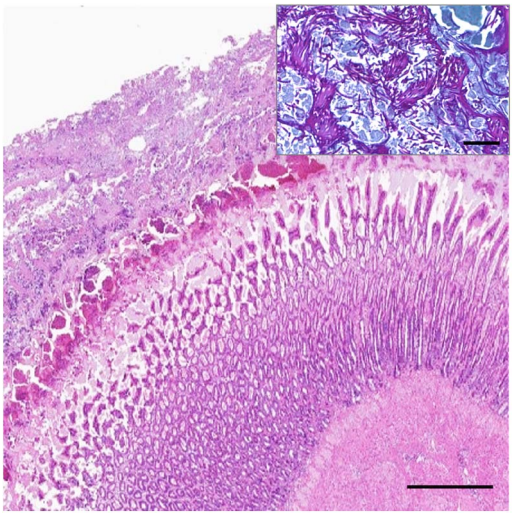

FIGURE 1. Section of ventriculus (koilin layer and epithelium) 2× (scale bar 500 μm) with koilin disruption, necrosis, inflammation, and hemorrhage, with invasion of *Macrorhabdus ornithogaster*; inset at 40× (scale bar 25 μm) showing a more detailed view of the *Macrorhabdus ornithogaster* (periodic acid–Schiff stain) disrupting the koilin.

Drugs, Scottsdale, Arizona, USA) orally twice daily for 2 d, then once daily for 30 d. No further morbidity or mortality occurred. On 20 November 2017, captive experimental work was completed, and the remaining 20 ibis were euthanized. To understand the progression of ventriculitis, we performed histologic examination of the proventriculus and ventriculus from seven birds (representing both pens), which revealed mild to moderate lymphoplasmacytic proventriculitis that may be consistent with previous *Mo* infection, but *Mo* were no longer visualized with Gram stain. All birds were in excellent body condition, had grown according to expectations, and were clinically healthy at euthanasia.

To understand the source and significance of *Mo* for free-living ibis better, we collected fecal samples in July 2017 from 91 apparently healthy, adult, free-living ibis in the region where our captive nestlings were collected. We microscopically observed organisms consistent with *Mo* morphology in 65 (71%) ibis. This is consistent with reports that document a high prevalence of infected adult birds without clinical disease, yet species-specific differences have been reported (e.g., disease has been described in young ostriches; Phalen 2010; Piasecki et al. 2012).

Although it is currently accepted that the detection of *Mo* without associated pathologic lesions may be clinically irrelevant, both species-specific susceptibility and predisposing factors are probably important and have not been fully investigated (Phalen 2010). In this mortality event, we hypothesize that an overgrowth of *Mo*, possibly because of stress or another immunosuppressive event, caused disruption of the ventricular koilin and direct damage to the ventricular epithelium (visible histologically). Because the ventriculus functions as a critical barrier to prevent entry of pathogenic bacteria (Rodrigues and Choct 2018), *Mo*-induced disruption of the ventricular mucosa probably facilitated systemic bacterial infection. Additional factors including captivity stress, hypervitaminosis E, dysbiosis as influenced by diet, or other unknown factors may have initiated the development of disease. Because of a temporary lack of commercially available vitamin E and thiamine paste (Thiamin-E paste; Mazuri Exotic Animal Nutrition, St. Louis, Missouri, USA), birds had received a homemade supplement that provided 2.5 times the recommended dose of vitamin E daily for 7 d prior to the mortality event. Although this did not result in clinical or histopathologic signs of hypervitaminosis E, it may have played a role in this event.

This mortality illustrates that that *Mo* colonizes young white ibis and, at least under captive conditions, can be a factor in morbidity and mortality in ibis. Future studies to identify *Mo* from young birds may elucidate further details about its importance to free-living ibis health, although, because of rookery inaccessibility, the causes of ibis nestling mortalities are often difficult to document. Finally, *Mo* should be considered in free-living birds when sudden death, and bright green feces is noted, especially in young birds, or in captive birds with a clinical history of enteric disease or poor growth.

We thank Dr. David Phalen for his technical assistance at the onset of this investigation.

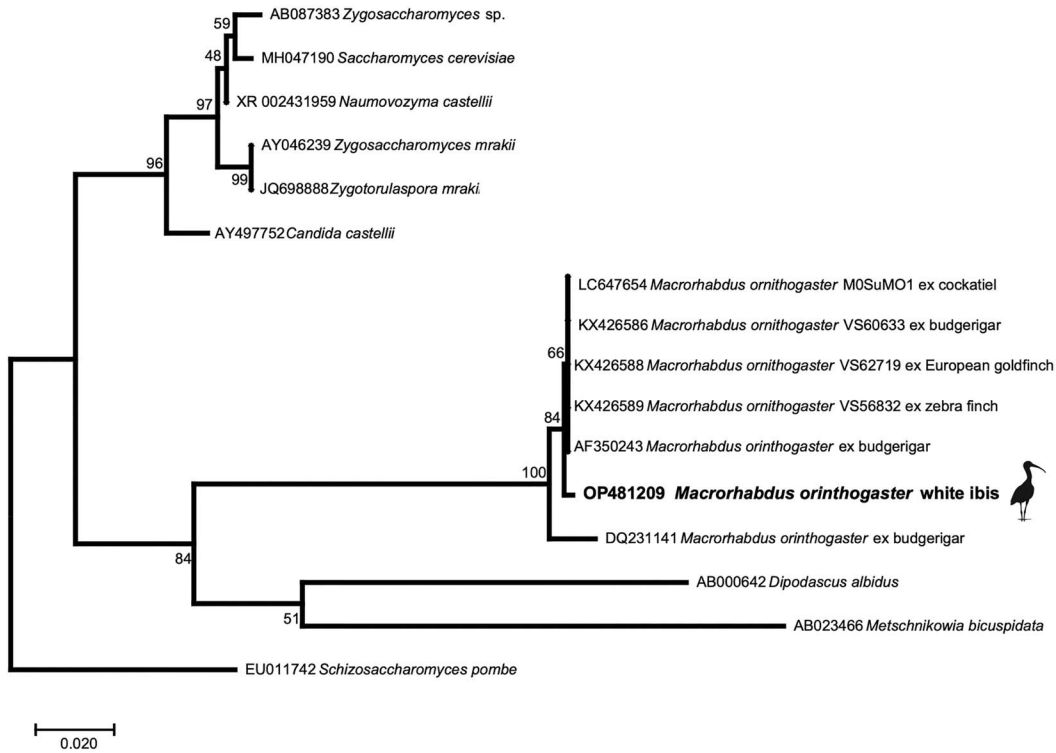

FIGURE 2. Maximum-likelihood tree showing the evolutionary relationships of the ibis *Macrorhabdus ornithogaster* 18S rRNA gene sequence from the ibis from this study with published sequences. The evolutionary history was inferred by using the maximum-likelihood method based on the Hasegawa-Kishino-Yano model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There was a total of 616 positions in the final data set. Evolutionary analyses were conducted in MEGA11 (Kumar et al. 2016).

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-22-00141.

LITERATURE CITED

Christie K. 2021. *Avian influenza virus and salmonella dynamics of nestling American white ibis (Eudocimus albus)*. MSc Thesis. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, Georgia, 120 pp.

Hernandez SM, Welch CN, Peters VE, Lipp EK, Curry S, Yabsley MJ, Sanchez S, Presotto A, Gerner-Smidt P, et al. 2016. Urbanized white ibises (*Eudocimus albus*) as carriers of *Salmonella enterica* of significance to public health and wildlife. *PLoS One* 11:0164402.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874.

Murray MH, Kidd AD, Curry SE, Hepinstall-Cymerman J, Yabsley MJ, Adams HC, Ellison T, Welch CN, Hernandez SM. 2018. From wetland specialist to hand-fed generalist: Shifts in diet and condition with provisioning for a recently urbanized wading bird. *Philos Trans R Soc B* 373:20170100.

Nichols DK, Wolff MJ, Phillips LG Jr, Montali RJ. 1989. Coagulopathy in pink-backed pelicans (*Pelecanus rufescens*) associated with hypervitaminosis E. *J Zoo Wildl Med* 20:57–61.

Phalen DN. 2010. Implications of *Macrorhabdus* in clinical disorders. In: *Clinical avian medicine*, Vol. 2, Harrison GJ, Lightfoot TL, editors. https://www.ivis.org/library/clinical-avian-medicine/implications-of-macrorhabdus-clinical-disorders. Accessed March 2021.

Phalen DN. 2014. Update on the diagnosis and management of *Macrorhabdus ornithogaster* (formerly megabacteria) in avian patients. *Vet Clin North Am Exot Anim Pract* 17:203–210.

Piasecki T, Prochowska S, Celmer Z, Sochacka A, Bednarski M. 2012. Occurrence of *Macrorhabdus ornithogaster* in exotic and wild birds in Poland. *Medycyna Weterynaryjna* 68:245–249. [Summary in English.]

Rodrigues I, Choct M. 2018. The foregut and its manipulation via feeding practices in the chicken. *Poultry Sci* 97:3188–3206.

Sünder A, Halle I, Flachowsky G. 1999. Vitamin E hypervitaminosis in laying hens. *Arch Anim Nutr* 52:185–194.

Tomaszewski EK, Loga KS, Snowden KF, Kurtzman CP, Phalen DN. 2003. Phylogenetic analysis identifies the 'megabacterium' of birds as a novel anamorphic ascomycetous yeast, *Macrorhabdus ornithogaster* gen. nov., sp. nov. *Int J Sys Evol Microbiol* 53:1201–1205.

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