Report to the Western Association of Fish and Wildlife Agencies
From the USGS National Wildlife Health Center
July 24, 2016

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The USGS National Wildlife Health Center provides national leadership to safeguard wildlife and ecosystem health through dynamic partnerships and exceptional science.

The following information is of a topical nature for wildlife management agencies and entities; many partners and collaborators are involved in gathering and researching this information.

***Batrachochytrium salamandrivorans*: An Emerging Disease of Salamanders**

A recently described fungal pathogen, *Batrachochytrium salamandrivorans* (Bsal), has been associated with the near extinction of fire salamanders (*Salamandra salamandra*) in the Netherlands and Belgium. Initial infection trials suggest that this fungus is highly pathogenic for some salamander and newt species that occur in North America. International trade of amphibians is thought to be a primary route of spread for Bsal, similar to the closely related chytrid fungus, *Batrachochytrium dendrobatidis* (Bd). Indeed, Bsal was most recently detected in commercially traded salamanders in the United Kingdom.

The NWHC has adapted a previously developed molecular assay to detect Bd and Bsal in North American salamanders. The NWHC maintains a large collection of archived amphibian tissues and is in the process of screening these samples to determine if Bsal is already present in the U.S. In addition to the molecular assay, the NWHC is collaborating with amphibian diagnosticians throughout North America and has published a case definition of criteria for confirming Bsal in diagnostic specimens (citation below).

NWHC scientists conducted a risk assessment for Bsal to determine where introductions of Bsal are likely to occur and, if introduced, what species would be expected to decline. Results were published in Royal Society Open Science and indicate that the overall risk of Bsal to North American salamanders is high with high potential for introduction and subsequent severe biological consequences. A USGS press release helped to publicize the findings of the risk assessment.

The USGS risk assessment informed a U.S. Fish and Wildlife Service interim rule prohibiting the importation and interstate transport of certain salamander species. For more information, please see this Federal Register link to the interim rule and listed species:
https://www.federalregister.gov/articles/2016/01/13/2016-00452/injurious-wildlife-species-listing-salamanders-due-to-risk-of-salamander-chytrid-fungus

For more information, please see this Wildlife Health Bulletin on Bsal.

White *et al.* 2016. Amphibian: a case definition and diagnostic criteria for *Batrachochytrium salamandrivorans* Chytridiomycosis. Herpetological Review 47(2):207-209. (**PDF**)

National Surveillance for Bsal
In support of ongoing efforts for early detection of introduction of Bsal to North America, the NWHC is partnering with the USGS Amphibian Monitoring and Research Initiative (ARMI) to implement national surveillance for Bsal. The NWHC is performing the diagnostics for Bsal and helping guide a risk-based surveillance design that follows the results of the recently published risk assessments by Richgels et al. and Yap et al. The NWHC and ARMI plan to test up to 10,000 salamanders for Bsal in 2016. In pilot work with USGS ARMI, the NWHC tested over 500 salamander swabs from 37 sites on the West Coast, Gulf Coast, and Mid-Atlantic region in 2015 and no Bsal was detected. The NWHC also continues to provide epidemiologic investigation and diagnostic services for wildlife mortality and morbidity events, including salamander species listed in the USFWS interim rule. **Contact:** NWHC epidemiology team for guidance on collecting and submitting salamanders: 608-270-2480; nwhc-epi@usgs.gov. **Contact:** LeAnn White, 608-270-2491, clwhite@usgs.gov for Bsal surveillance or diagnostic questions.

White-Nose Syndrome Update, 2015/2016 Surveillance Season
Ongoing national surveillance efforts confirm that the geographic range of bat white-nose syndrome (WNS) and the causative fungus, *Pseudogymnoascus destructans (Pd)*, have expanded during 2015/2016. Specifically, *Pd* continued to spread among bats at new hibernacula in eastern Nebraska, eastern Oklahoma, and central Iowa, and WNS was confirmed for the first time in Minnesota where *Pd* has been present since 2012. Although not unexpected because WNS has been long-present in New England, WNS was also confirmed for the first time in Rhode Island. As previously reported in a Wildlife Health Bulletin from April 2016, scientists at the National Wildlife Health Center (NWHC) confirmed WNS in a western subspecies of little brown bat *(Myotis lucifugus alascensis*) from King County, Washington. The area where this bat was found is approximately 1,300 miles from the previous westernmost detection of *Pd*.

Following this initial detection of *Pd* and WNS in Washington state, scientists from the NWHC, the Washington Department of Fish and Wildlife, and other state and federal agencies have been working to identify strategies suitable for facilitating additional disease surveillance in the western U.S. to better define prevalence and geographic range of *Pd*. These strategies included testing nonlethal samples collected from hibernating and post-emergent bats, sediment and environmental swab samples collected from bat hibernacula, guano samples collected from post-emergent bats, and screening of bat carcasses that were previously submitted to State Health Department Laboratories for rabies testing. Despite the known presence of *Pd* in Washington, all samples collected from live bats and from environmental and guano samples were negative for *Pd*. However, a single silver-haired bat (*Lasionycteris noctivagans*) submitted to the Washington Department of Health (WDOH) for rabies testing and subsequently transferred to the NWHC was positive for *Pd* by molecular testing (real-time PCR). This bat was originally collected during the same month and in the same county as the Washington bat with previously confirmed WNS. Histological analysis indicated that this second *Pd*-positive bat from Washington lacked lesions indicative of WNS. However, detection of *Pd* on this silver-haired bat by PCR is consistent with previous reports for this species in the eastern U.S. and suggests that silver-haired bats may be a carrier of the fungal pathogen. Overall, 34 bats submitted to the WDOH between November 2015 and June 2016 were received by the NWHC, and one bat from this group (the silver-haired bat described above) tested positive for *Pd*. An additional 65 bats submitted to the WDOH during winter 2014/2015 were also tested and all were negative for *Pd*.

The arrival of WNS to western North America represents a significant change in the geographic distribution of this disease and in the previously established pattern of fungal spread in North America. It also presents new challenges for conducting WNS surveillance. For example, it can be difficult and impractical in many areas of the western U.S. to collect non-lethal samples during winter and spring from bat species and hibernacula most likely to harbor *Pd*. In such areas, screening bat carcasses opportunistically found on the landscape during winter and spring, including those submitted to State

Health Department Laboratories for rabies testing, for presence of *Pd* is recommended. Where feasible, continued non-lethal surveillance when *Pd* prevalence is highest (*i.e.*, during winter and spring) is highly encouraged in states at the edge of known *Pd* distribution and in all other western states. Active surveillance for *Pd* on bats during other times of the year (*i.e.*, during summer and fall) is discouraged because of low likelihood for detection.

Of note, *Pd* has been detected in the hibernaculum environment and on bats from multiple sites across China (Hoyt et al. 2016. Emerging Infectious Diseases).

The NWHC provides diagnostic and epidemiological assistance to investigate unusual bat mortality events throughout the year. Recent detection of WNS in Washington illustrates the ongoing importance of investigating wildlife mortality events as part of a comprehensive wildlife disease surveillance strategy, and we encourage wildlife managers to report unusual bat mortality or bats displaying clinical signs suggestive of WNS to the NWHC for further investigation. We can also answer questions about designing WNS surveillance and response plans relevant to your state, and with testing samples collected as part of opportunistic or targeted surveillance efforts in accordance with the national *Pd* surveillance strategy. Tribal, state, and federal agencies who have questions about ongoing surveillance efforts or who may wish to participate should contact Anne Ballmann (608-270-2445, aballmann@usgs.gov).

Please visit www.whitenosesyndrome.org for more information about the national multi-agency WNS response effort. A recently completed WNS poster and handout are available for use as needed at https://www.whitenosesyndrome.org/resource/white-nose-syndrome-poster-available-your-use

WNS Multi-State Surveillance Project

The USGS National Wildlife Health Center (NWHC) continues to lead a 3-year surveillance project designed to assist state and federal wildlife agencies nationwide with early detection of *Pd* in new areas, and to address specific research priorities identified by partners in conjunction with the White-Nose Syndrome National Plan. During the first two years of the project swabs from more than 2,100 bats, representing 16 North American species, and 580 environmental substrates from 110 hibernacula in 23 states were returned to the NWHC for analysis. This project has resulted in the detection of *Pd* at 16 hibernacula of previously unknown *Pd* status in six states, including nine sites where there was no physical or behavioral evidence of WNS observed in the bat population. Nearly all detections of *Pd* originated from swabs collected from bats rather than from environmental substrates collected inside of hibernacula. Information on biotic and abiotic factors continues to be collected at all hibernacula surveyed to assess the potential importance of various parameters in contributing to presence of *Pd*.

Partners are reminded that the NWHC continues to provide diagnostic and epidemiological assistance to investigate unusual bat mortality events. Federal, state, or tribal agencies wishing to participate in the expanded national *Pd* surveillance strategy should contact Dr. Anne Ballmann at the NWHC (608-270-2445, aballmann@usgs.gov).

Highly Pathogenic Avian Influenza in North America - 2015 Third Quarter Update

The USGS National Wildlife Health Center (NWHC) continues to play an active role in surveillance and research into the emergence of highly pathogenic avian influenza (HPAI) viruses in North America. In the third quarter of 2015 (July - September), the NWHC tested 391 bird carcasses submitted from mortality and morbidity investigations nationwide. The NWHC also tested 4,758 swab samples from live or hunter-harvested waterfowl, primarily from the Mississippi and Atlantic Flyways, as part of the Interagency Surveillance Plan for Highly Pathogenic Avian Influenza in Waterfowl in the United States.

Nationally, there were no detections of HPAI virus in the third quarter of 2015 through the NWHC's mortality testing or through national surveillance samples collected from live or hunter-harvested birds. Samples from two mallards (*Anas platyrhynchos*) tested PCR positive for the Eurasian lineage

HPAI H5 gene, which was first detected in North America in late 2014; however, virus was not isolated or sequenced from either sample. One sample was from a live mallard in Davis County, Utah in July and the second sample was from a hunter-harvested mallard in Morrow County, Oregon in November.

The NWHC is continuing to accept mortality and morbidity event submissions for HPAI testing under our expanded submission criteria. To date, no humans or other mammals have shown signs of disease from these particular viruses but field personnel handling live or dead wild birds should take appropriate precautions.

For more information, see the USGS Role and Response to Highly Pathogenic Avian Influenza fact sheet.

Note: The Interagency Steering Committee for Surveillance for Highly Pathogenic Avian Influenza in Wild Birds recently developed FAQs on HPAI and North American Wild Birds. These are available online here. (http://www.nwhc.usgs.gov/disease_information/avian_influenza/2016%2006%20AI%20_FAQs_Final.pdf)

Predation on Nesting Laysan Albatross (Midway Atoll)
The NWHC Honolulu Field Station (HFS) is assisting the Midway Atoll National Wildlife Refuge in dealing with the loss of adult Laysan albatross (*Phoebastria immutabilis*) to aggression by non-native mice. The mice chew on the birds while they are nesting, causing wounds and infection, which has led to a noticeable loss of adult breeding birds. The refuge is currently implementing mouse control efforts. HFS is involved with diagnosing cause of death and other measures. **Contact**: Thierry Work, 808-792-9520, thierry_work@usgs.gov.

Determining Causes of Marianas Crow Population Decline (Island of Rota)
The NWHC Honolulu Field Station (HFS) is assisting the USFWS to determine causes of declines in critically endangered Marianas crows on the island of Rota. Recent evidence suggests that infectious disease (the cause of which is yet to be identified) might be playing a role. HFS is collaborating with various institutions including Centers for Disease Control and U.S. Department of Agriculture in attempts to determine what is killing these crows. **Contact**: Thierry Work, 808-792-9520, thierry_work@usgs.gov.

Seabird Mortality Along Southern Coastal Alaska
The NWHC has been working closely with the U.S. Fish and Wildlife Service Region 7 Migratory Bird Management office, field offices, and Refuges, as well as the Alaska Department of Fish and Game since March 2015, responding to and monitoring large-scale seabird mortality primarily impacting common murres (*Uria aalge*) throughout the Gulf of Alaska, Bering Sea, and inland lakes and rivers. Avian mortalities documented from May to September were also concurrent with whale, pinniped, sea otter (*Enhydra lutris*), and fish mortality. Estimated total numbers of birds involved are in the hundreds of thousands.

Between March 2015 and February 2016, 150 avian carcasses were collected by partners and shipped to the NWHC for comprehensive diagnostic necropsies. The common finding for both juvenile and adult specimens was emaciation and starvation. All carcasses have tested negative for highly pathogenic avian influenza virus. Additional laboratory testing has included bacteriological, parasitological, and virological analyses; heavy metal panels; and algal toxin analyses. Histopathologic examinations have been conducted on suitable specimens. Overall, no consistent indications of infectious disease or toxins have been found.

All agencies involved are working to understand complex ecosystem-wide drivers that may be impacting seabirds in the northern Gulf of Alaska. In particular, unusually warm ocean waters first recorded in 2013 have persisted through this winter and represent a significant deviation from normal. These conditions

may be causing changes in prey distribution or abundance, resulting in starvation of seabirds. Additionally, potential impacts of harmful algal blooms continue to be investigated.

The NWHC encourages wildlife biologists and resource managers along the west coast of the lower 48 states to be aware that mortalities might become more widespread during migration and as environmental conditions change in spring, and we encourage the reporting of marine bird and mammal mortality events to help determine the overall magnitude and scope of these events and potential impacts to species involved. **Contact**: NWHC Field Epidemiology Team, 608-270-2480, NWHC-epi@usgs.gov.

Brevetoxin Detected in Green Tree Frogs (Texas)

In September 2015, a mortality event involving American green tree frogs (*Hyla cinerea*) at Padre Island National Seashore, Texas, was reported by the National Park Service. Personnel estimated 30-40 adult green tree frogs died; many showed signs of neurologic impairment including tremors, weakness, and abnormal movements and postures. Two days previously, a red tide event (harmful algal bloom) began along the coastline of the park, roughly 1 km from where the frogs were found, and a storm accompanied by winds, surf, and high tides had occurred the morning of the observed mortality. There was no further green tree frog mortality, but increases in coyote (*Canis latrans*) and ground squirrel deaths at Padre Island National Seashore were noted in the following weeks.

Five green tree frog carcasses were submitted to and necropsied at the NHWC. All had adequate body fat reserves and food in their stomach, suggesting an acute death. Testing for ranavirus was negative. Due to the lack of necropsy findings, the clinical history of neurologic abnormalities, and the concurrent presence of the red tide, brevetoxin was considered as a possible cause of death. Samples sent to the Florida Fish and Wildlife Conservation Commission Fish and Wildlife Research Institute (St. Petersburg) for ELISA testing were preliminarily positive for brevetoxin. Testing performed by Texas A & M University on an additional six frogs and a ground squirrel from this mortality event were also positive for brevetoxin. Testing is on-going to further characterize this finding.

Brevetoxin is produced by *Karenia brevis*, the algal organism responsible for red tides. When present at high enough levels, this toxin can cause large fish die-offs. Mammals that ingest toxin can develop signs of neurological impairment followed by rapid death. The NWHC believes this to be the first documented incident of a harmful algal bloom associated with amphibian mortality. The route of exposure in this terrestrial, freshwater frog species whose diet consists of flies, mosquitos, and other small insects is unclear, but exposure may have resulted from absorption of the toxin through the skin. **Contact**: NWHC Field Epidemiology Team, 608-270-2480, NWHC-epi@usgs.gov.

Additional Disease Research

Sylvatic Plague Vaccine for Prairie Dogs

Laboratory studies have demonstrated that oral vaccination of prairie dogs against plague using raccoon pox-vectored vaccine is feasible, resulting in significant protection against challenge with *Yersinia pestis*, the bacterium that causes sylvatic plague. The Sylvatic Plague Vaccine (SPV) Subcommittee, under the direction of the Executive Committee of the Black-footed Ferret Recovery Implementation Team, is continuing its work to complete development and delivery of the sylvatic plague vaccine as a management tool to combat plague in prairie dogs and promote the recovery of the black-footed ferret. Field trials completed by Colorado Parks and Wildlife and USGS National Wildlife Health Center (NWHC) in 2012 confirmed the safety of the vaccine in wild prairie dogs and non-target animals. NWHC and numerous federal, state, and tribal partners have completed the third year of field trials to assess vaccine efficacy in free-ranging prairie dogs. Vaccine and placebo baits have been distributed in select prairie dog colonies in seven western states: Arizona, Colorado, Montana, South Dakota, Texas, Utah, and Wyoming. Final analysis of data and samples are currently underway to evaluate vaccine efficacy.

Additional field trials to determine optimal use of SPV began in 2016 in western states. **Contact**: Tonie Rocke, 608-270-2451, trocke@usgs.gov.

Investigating the Genetic Diversity of Prion Protein Gene among Subspecies of Bighorn Sheep
The range of bighorn sheep (*Ovis canadensis*) overlaps many of the areas where chronic wasting disease (CWD) is endemic in free-ranging deer, as well as areas where scrapie in domestic sheep has been observed. Given the potential contact that bighorn sheep have with transmissible spongiform encephalopathies (TSEs), risk assessments of the susceptibility of bighorn sheep to both CWD and scrapie are warranted. Determining the sequence of the prion protein gene (Prnp) is one method to assess the potential susceptibility of a host to TSEs. Recent work at the NWHC showed a small number of Rocky Mountain bighorn sheep (*O. canadensis canadensis*) from two herds in Washington all possessed a genotype identical to a scrapie-susceptible domestic sheep ($A^{136}R^{154}Q^{171}$). Using an *in vitro* prion protein conversion assay, we also showed that the species barrier of bighorn sheep to elk (*Cervus canadensis*) or white-tailed deer (*Odocoileus virginianus*) CWD or domestic sheep scrapie was low, indicating risk for TSE infection of bighorn sheep. Limitations of this previous study were the small number of bighorn sheep sampled, their origin from only two herds, and testing only one subspecies of bighorn sheep. The purpose of this study is to characterize the diversity of Prnp among various subspecies of bighorn sheep, including the endangered Sierra Nevada bighorn sheep (*O. canadensis sierra*), to assess the risk that TSEs could pose to these populations. **Contact:** Daniel Walsh, 608-270-2481, dwalsh@usgs.gov

Cross-species Transmission of Chronic Wasting Disease to Native North American Voles Reveals Prion Strain Diversity
Recent findings at the NWHC have established that meadow voles (*Microtus pennsylvanicus*), native North American rodent species, are susceptible to chronic wasting disease (CWD) via environmentally relevant exposure routes. This finding demonstrates that natural infection of these scavenging rodents that overlap in range with CWD epizootics is possible and implicates a role for meadow voles as possible reservoir, vector, or bridge species for CWD on the landscape. Interspecies transmission of CWD to meadow voles also revealed the existence of a mixture of CWD strains in white-tailed deer (*Odocoileus virginianus*) isolates from wild populations, suggesting that CWD strain diversity is larger than previously recognized, and may be influenced by the genetic make-up of the cervid host. This finding is important because novel CWD strains may pose unique disease risks and outcomes to cervids, other wildlife, domestic animals and humans. To address this concern, studies are currently underway in collaboration with the U.S. Food and Drug Administration and the U.S. Department of Agriculture to test the susceptibility of a variety of domestic and wildlife species, as well as humanized transgenic mice, to the novel CWD strains identified in this research. Future work will focus on characterizing CWD strain diversity from geographically disparate outbreaks and various affected cervid species toward revealing the currently unknown distribution and prevalence of CWD strains on the landscape. This focus will provide insight into the adaptation and evolution of the CWD agent in regions of interest. This study advances the current understanding of CWD strain diversity and interspecies transmission and has implications for wildlife management as well as environmental, animal, and public health.
Contact: Christina Carlson, 608-270-2442, cmcarlson@usgs.gov

Identifying Priority Chronic Wasting Disease Surveillance Areas for Mule Deer in Montana
Wildlife biologists at Montana Fish, Wildlife and Parks were interested in developing a targeted cost-effective surveillance program for chronic wasting disease (CWD). Though no cases of CWD have been documented as of June 2016 in wild populations in the state of Montana, cases have been found less than 50 miles away in Saskatchewan and Wyoming. We identified areas of “high risk” for CWD introduction by overlapping maps of mule deer (*Odocoileus hemionus*) density and estimating the distance to the nearest infected herd. We estimated mule deer density across the state of Montana using aerial survey data to estimate deer numbers, and resource selection functions to distribute the deer across a habitat suitability gradient. We overlapped maps of estimated deer density and estimated distance to the nearest

known infected herd to identify areas where deer densities were predicted to be high and distances to infected herds were minimal. These areas were labeled as high risk areas for potential CWD introduction. This information is being used by the state of Montana to conduct limited surveillance for CWD in mule deer. **Contact:** Robin Russell, 608-270-2474, rerussell@usgs.gov

Russell, R.E., J.A. Gude, N. J . Anderson, J. M. Ramsey. 2015. Identifying priority chronic wasting disease surveillance areas for mule deer in Montana. Journal of Wildlife Management. 79:989-997.

New chapters: 2015 Field Manual of Wildlife Diseases
New and updated chapters of the USGS Field Manual of Wildlife Diseases are now available online. Chapters will be added online as they are completed.
This manual provides information that will enhance the ecological understanding of wildlife diseases by nonspecialists so various aspects of wildlife disease can be more effectively managed. Other readers, from students to science professionals, may also find the information presented to be of interest and value.

The introduction provides background information regarding the convergence of wildlife disease with wildlife management as a wildlife conservation concern. Section B focuses on concepts associated with disease surveillance and response to outbreaks. Section C deals with specific techniques for disease surveillance and investigation. Section D, “Diseases of Wild Birds,” and others that follow will address specific diseases of concern in various taxonomic groups. Electronic links facilitate timely access to a wide variety of supplemental information.

The 1999 Field Manual of Wildlife Diseases – General Field Procedures and Diseases of Birds remains available online.

New USGS Circular
"*Baylisascaris* larva migrans" by Kevin Kazacos is now available online. This publication is about the common raccoon roundworm, *Baylisascaris procyonis*, an intestinal parasite, which is the most commonly recognized cause of clinical larva migrans in animals. Baylisascariasis is a zoonotic disease, meaning it is transmissible from animals to humans. This circular is part of a series of publications on zoonotic diseases available from the NWHC and the USGS Publications Warehouse. A printed version of this publication will be available in the near future.

Disease Investigation Services
To request diagnostic services or report wildlife mortality, please contact the NWHC at 608-270-2480 or by email at NWHC-epi@usgs.gov, and a field epidemiologist will be available to discuss the case. To report wildlife mortality events in Hawaii or Pacific Island territories, please contact the Honolulu Field Station at 808-792-9520 or email Thierry Work at thierry_work@usgs.gov. Further information can be found at http://www.nwhc.usgs.gov/services/.

Wildlife Mortality Reporting and Diagnostic Services Request Worksheet

To view, search, and download historic and ongoing wildlife morbidity and mortality event records nationwide visit the Wildlife Health Information Sharing Partnership event reporting system (WHISPers) online database at http://www.nwhc.usgs.gov/whispers/

To be added to the NWHC list to receive Wildlife Health Bulletins, please contact Gail Moede Rogall at gmrogall@usgs.gov

USGS Wildlife Health Information on Twitter
Follow us at http: twitter.com/USGSWILDLIFE

Request for Wildlife Mortality and Morbidity Event Reporting
The NWHC Quarterly Wildlife Mortality Report, published in the Wildlife Disease Association's newsletter and on the NWHC Web site, is intended to inform wildlife professionals of wildlife events of interest. We kindly request the help of wildlife professionals in submitting investigations of recent die-offs of mammals, birds, amphibians, and reptiles for inclusion in this report. Credit will be given to appropriate diagnostic laboratories and wildlife management agencies. The report can be found online at http://www.nwhc.usgs.gov/mortality_events/ongoing.jsp.

To view NWHC Quarterly Wildlife Mortality Reports, please visit:
http://www.nwhc.usgs.gov/publications/quarterly_reports/index.jsp

THANK YOU

The NWHC thanks all the state, federal and tribal agencies who worked with us the past year. We are at your service to provide technical support, field investigation assistance, diagnostic capabilities, and collaborative research projects.