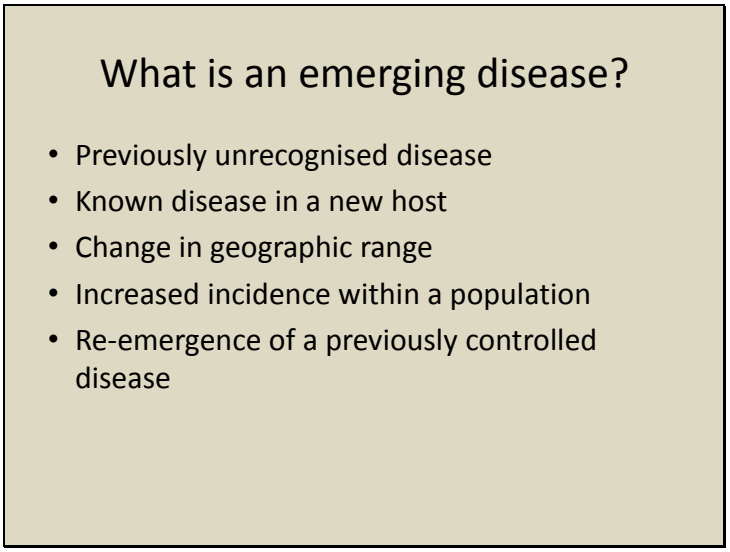
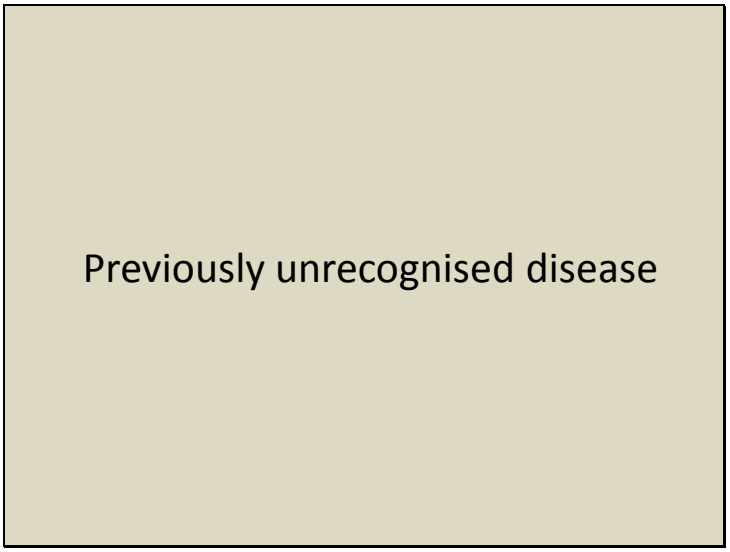
Slide 1



Some emerging diseases fulfill more than one of these criteria

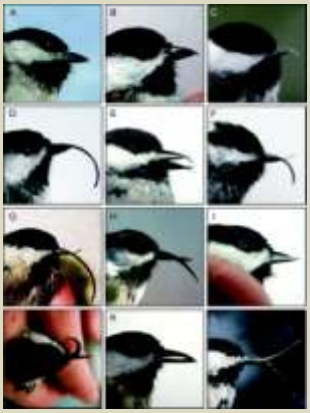
Slide 2



Slide 3

Avian keratin disorder

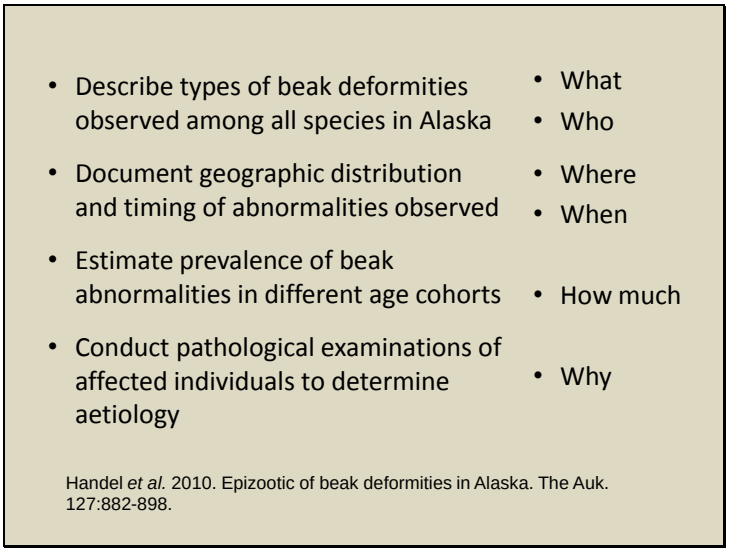
Slide 4



- 1998
- Reports of beak deformities in chickadees at bird feeders
- Two distinct areas of Alaska

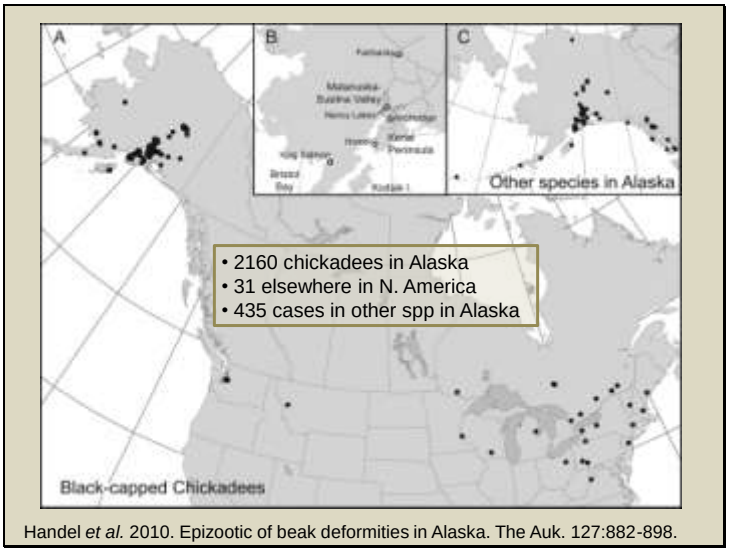
Handel *et al.* 2010. Epizootic of beak deformities in Alaska. The Auk. 127:882-898.

Slide 5



Study planned involving USGS Alaska Science Centre; Dept of Biology and Wildlife, University of Alaska; USGS National Wildlife Health Center and US Fish and Wildlife Service

Slide 6



Handel *et al.* 2010. Epizootic of beak deformities in Alaska. The Auk. 127:882-898.

Who and where (Handel *et al.* 2010): The researchers conducted retrospective literature searches and solicited reports of affected birds from wildlife professionals as well as the public through the National Audubon Society, Cornell Ornithology Lab Feederwatch program and list serves used by banders and birders. Results cover the years 1986 to 2009.

Affected chickadees in Alaska were concentrated around Anchorage and the Matanuska-Susitna Valley, however this is likely biased by concentration of human population and accessibility by road. Reports do cover most of the bird's range in Alaska.

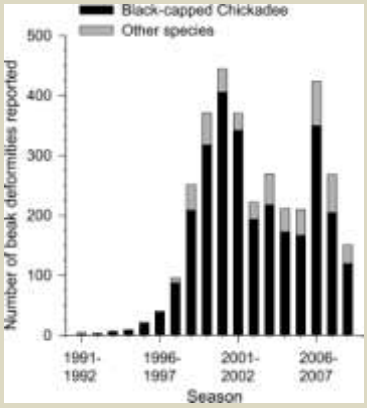
Slide 7

Van Hemert & Handel. 2010. Beak deformities in Northwestern crows: Evidence of a multispecies epizootic. The Auk. 127:746-751

Who and where (Van Hemert and Handel, 2010):
In a two year study focusing on Northwestern crows, 19/186 crows caught in Alaska demonstrated beak deformities, which is a prevalence of 19%. Retrospective and prospective collection of case reports found a total of 148 affected Northwestern crows in Alaska and a further 64 crows in BC and Washington, but it is unknown if these are Northwestern or American crows.

FIG. 2. Map showing distribution of all documented beak deformities (black dots) of Northwestern Crows in Alaska, and of what could be Northwestern or American crows where their distributions overlap in southern British Columbia and northern Washington. Numeric subtotals adjacent to open circles show minimum numbers of individuals with beak deformities for areas where there were multiple observations. Inset map shows study sites (stars) in Alaska where we estimated the prevalence of deformities in Northwestern Crows during winter 2007 and 2008. Most reports from southern British Columbia and northwestern Washington (dashed circle) occurred within a known area of overlap between ranges of Northwestern and American crows.

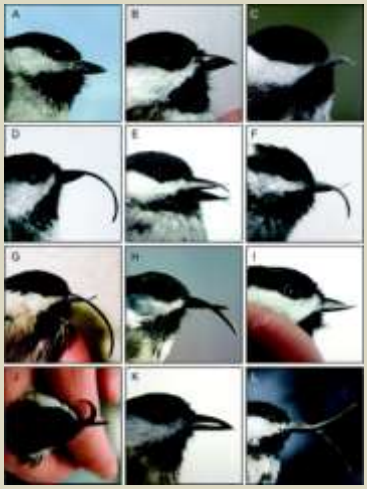
Slide 8



Handel *et al.* 2010. Epizootic of beak deformities in Alaska. The Auk. 127:882-898.

When: Massive increase in numbers in late 90's, however this coincides with greater media coverage, so this could be a biased result (Handel *et al.* 2010).

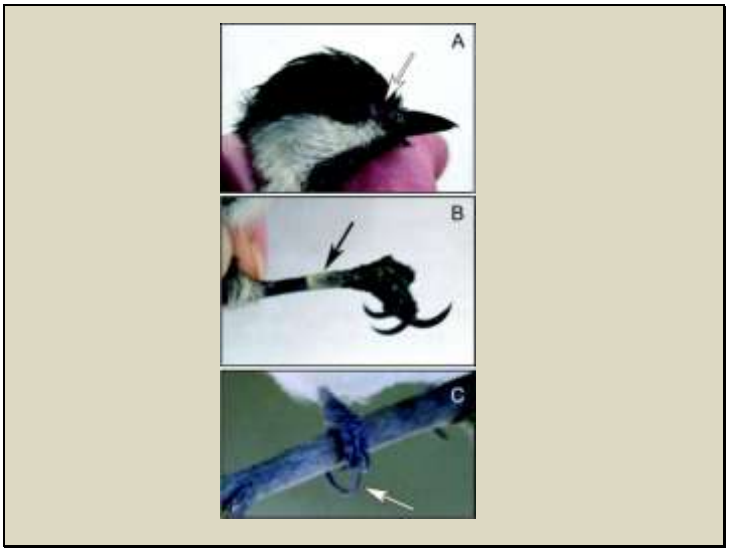
Slide 9



Handel *et al.* 2010. Epizootic of beak deformities in Alaska. The Auk. 127:882-898.

What (Handel *et al*. 2010):
In the case of the chickadees, most individuals with gross beak abnormalities had elongation of the upper beak, often with a pronounced decurvature. In some cases, the lower beak was also elongated and often upcurved, resulting in a crossed presentation. It was relatively rare to have only the lower beak overgrown and one case of lateral curvature of the upper beak. The surface of the beak was typically rough and irregular with raised lateral ridges and pallor.

Slide 10



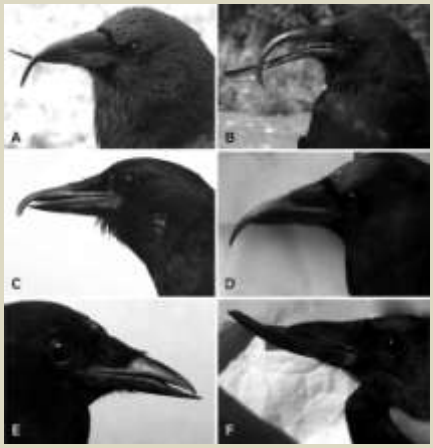
Not uncommonly in affected chickadees, claws were overgrown and there was alopecia, scaling and crusting of the skin.

Other lesions found in Black-capped Chickadees in Alaska from 1999 to 2009: (A) dry, reddened skin in loral region; (B) dry, sloughing scales on tarsometatarsus; (C) overgrown claw on middle toe. (Photograph C by D. Kuhle; others by C. M. Handel.) (Handel *et al.* 2010)

Slide 11

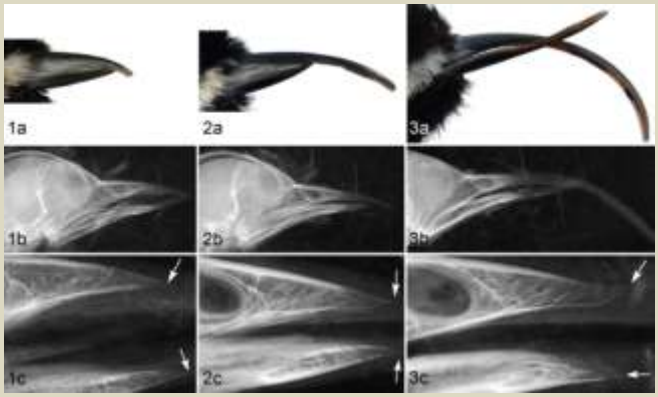
FIG. 5. Examples of other species in Alaska with beak deformities that ranged in the extent of crossing and elongation: (A) Boreal Chickadee (L. Quakenbush, Fairbanks, 1994); (B) Red-breasted Nuthatch (D. Henderson, Anchorage, 2007); (C) Hairy Woodpecker (R. Van Dusseldorp, Kenai, 2009); (D) Black-billed Magpie (J. Tileston, Anchorage, 2004); (E) Northwestern Crow (H. Cline, Seward, 2007); and (F) Yellow-rumped Warbler (C. Erwin, Kantishna, 1998). (Handel *et al.* 2010)

Slide 12



Van Hemert & Handel. 2010. Beak deformities in northwestern crows: Evidence of a multispecies epizootic. The Auk. 127:746-751

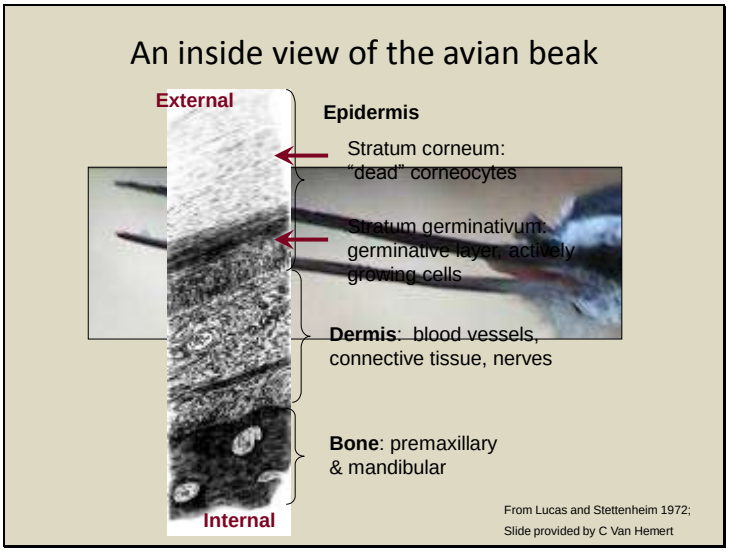
Slide 13



Published in: C. Van Hemert; A. G. Armién; J. E. Blake; C. M. Handel; T. M. O'Hara;
Veterinary Pathology 50, 500-513.

Mild cases: no bony remodelling; Moderate and Severe cases: growth of the beak extends well beyond the bone and there is significant remodelling of the premaxillary and mandibular bones. (Van Hemert *et al.* 2012)

Slide 14



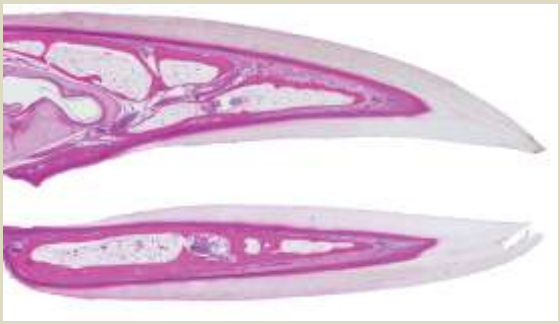
Before getting to the histopathology, let's start with a review of the normal beak.

Stratum corneum, as a whole is called the rhamphotheca. Just like finger nails, the rhamphotheca is continuously growing. The tip and cutting edges (tomia) are worn down with use. Stratum corneum is also called the cornified plate.

Slide 15

Histology of keratinized tissues

Normal beak



Slide provided by C Van Hemert

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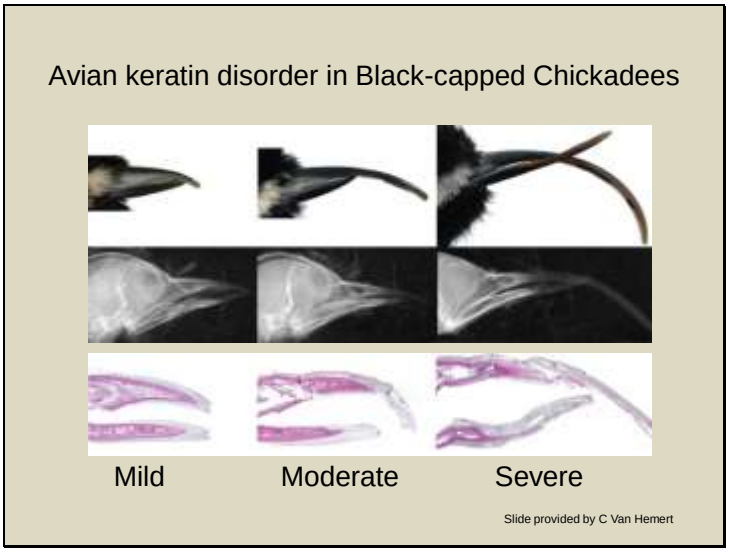


The most obvious and consistent change seen is hyperkeratosis of the stratum corneum, varying from slight thickening to thickening and elongation to more than triple the bird's normal beak size.

Slide 17

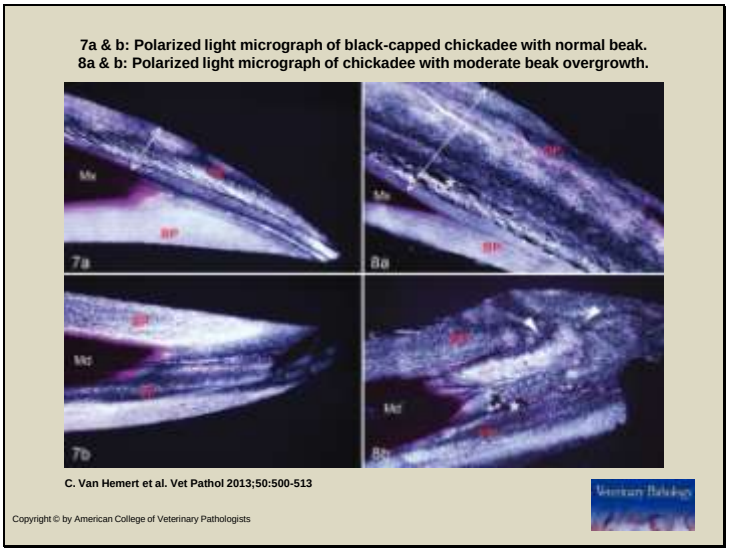
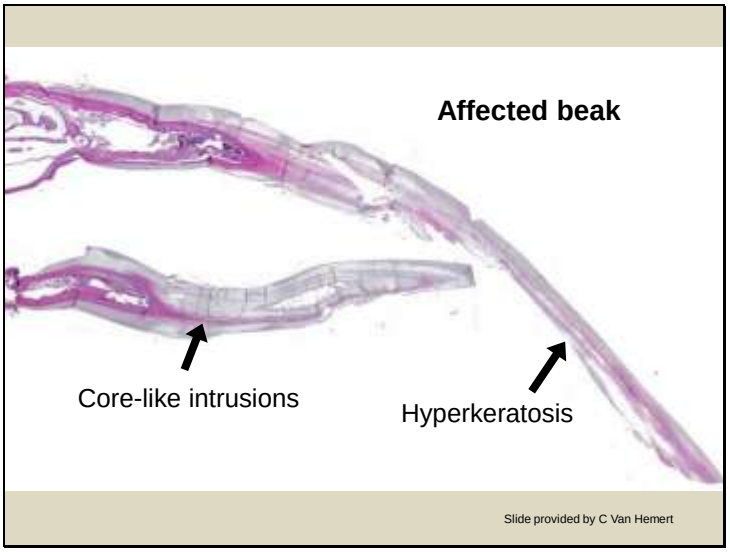


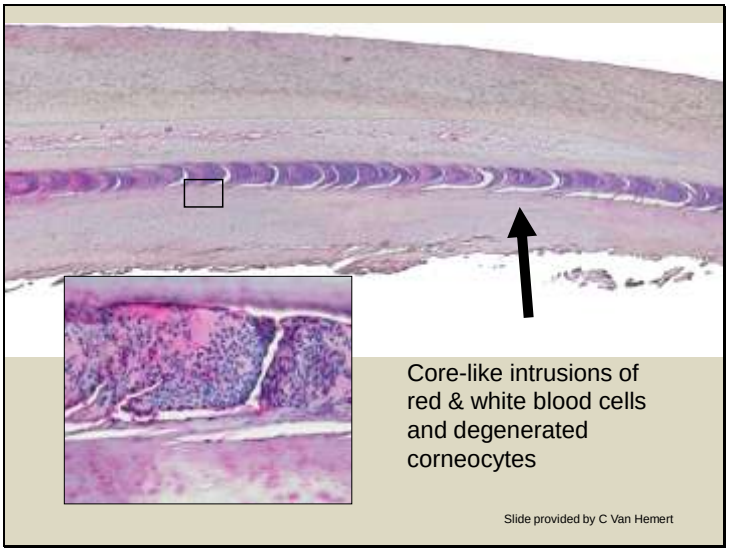
Figure 7.(a,b) Polarized light micrograph of beak; black-capped chickadee with normal beak. Dorsal (DP) and buccal (BP) plates cover the premaxillary bones (Mx) of the upper beak. BP and ventral (VP) plates cover the mandibular bones (Md) of the lower beak. **Note the linear organization of the stratum corneum** and the proportional thickness of the dorsal plate (double-headed arrow). Figure 8. (a) Polarized light micrograph of upper beak; black-capped chickadee with moderate beak overgrowth. **Note disorganized appearance of the stratum corneum, indicated by attenuation and irregular patterns of birefringence and disarray and separation (asterisk), between the DP and BP that cover the Mx.** The proportional thickness (double-headed arrow) of the dorsal plate is significantly increased relative to the normal appearance shown in Fig. 7a. HE. (b) Polarized light micrograph of lower beak; black-capped chickadee with moderate beak overgrowth. **Note disorganized appearance of the stratum corneum, indicated by attenuation and irregular, swirling, and scalloped patterns of birefringence (arrowheads) and disarray and separation (asterisk), between the BP and VP that cover the Md** (Van Hemert *et al.* 2012).

Slide 18



In more severely affected beaks, in addition to hyperkeratosis, we can core-like intrusions extending from the viable tissues into the rhamphotheca. These intrusions occur along the juncture of the external and buccal plates and often persisting to the tip of the beak.

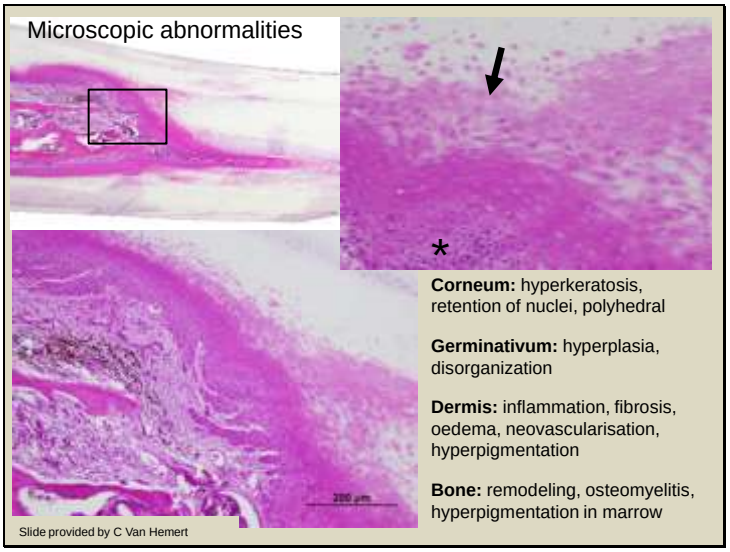
Slide 19



Core-like intrusions of red & white blood cells and degenerated corneocytes

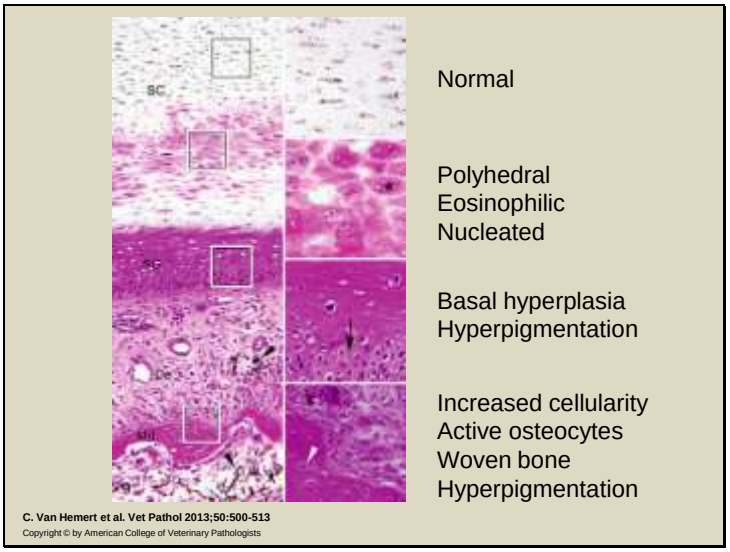
Slide provided by C Van Hemert

Slide 20



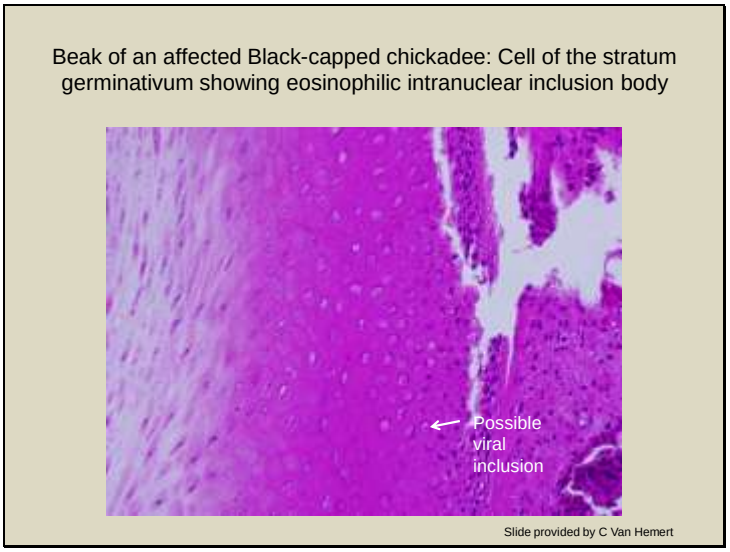
Particularly in moderate to severely affected birds, further changes are regularly recognized, including retention of nucleated cells within the stratum corneum, which exhibit increased eosinophilia and are polyhedral rather than flattened. There is also hyperplasia and disorganization of the germinativum, demonstrated by the asterisk, as well as inflammation, fibrosis, oedema, neovascularisation and hyperpigmentation in the dermis and remodeling of the bone, as mentioned before, osteomyelitis and hyperpigmentation in the marrow. Changes were more common near the tip of the beak.

Slide 21



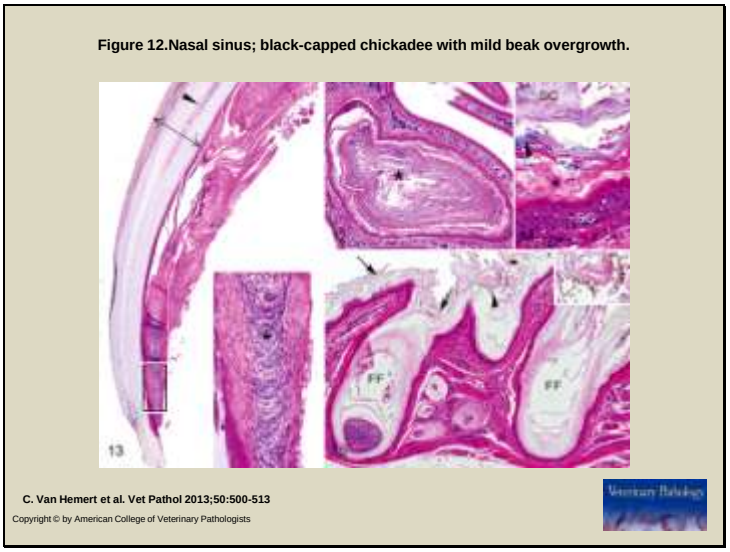
Lower beak; black-capped chickadee with severe beak overgrowth. Insets on the right correspond to layers shown in boxes, from top to bottom: a region of the stratum corneum **(SC) showing corneocytes with normal appearance**, a region of the stratum corneum with incompletely differentiated and eosinophilic corneocytes, the stratum germinativum (SG), and the interface between the dermis (De) and mandibular bone (Md). **Note increased eosinophilia, polyhedral (rather than flattened) morphology, and incomplete differentiation of corneocytes (asterisk) in focal areas of the stratum corneum. Hyperplasia and disorganization of basal cells (black arrow) are evident in the stratum germinativum. Osseous changes include increased cellularity and active osteocytes (white arrowhead) and production of woven bone. Hyperpigmentation is evident in the dermis and marrow (black arrowheads).** Hematoxylin and eosin. (Van Hemert *et al.* 2012)

Slide 22



28% of affected birds (n=30) and none of control birds (n=22) exhibited eosinophilic intranuclear and sometimes intracytoplasmic inclusions in the stratum germinativum. Material was unsuitable for EM. (Van Hemert *et al.* 2012)

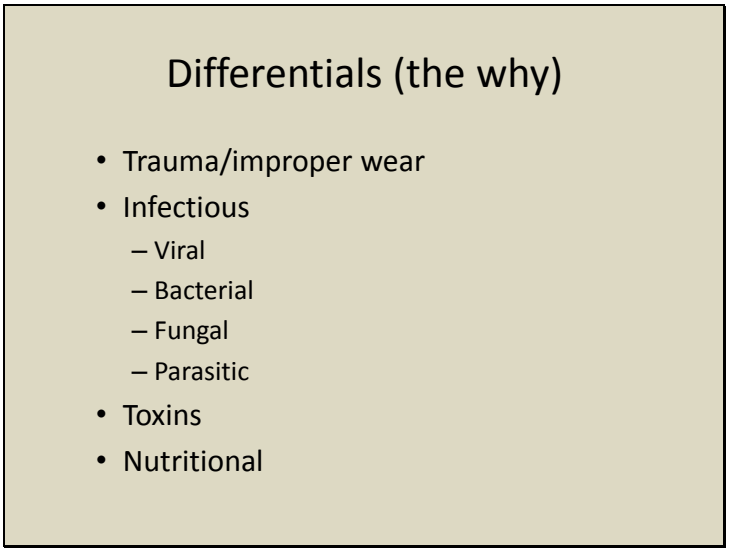
Slide 23



Lesions also occurred in other tissues. Demonstrated here is severe hyperkeratosis and bacterial infection found in the sinus cavity. Other changes included hyperkeratosis in the claws of most affected birds with similar core-like intrusions of nuclear remnants and amorphous debris as seen in the beaks, and hyperkeratosis of the skin with lymphoplasmacytic dermatitis and oedema.

Figure 12. Nasal sinus; black-capped chickadee with mild beak overgrowth. There is severe hyperkeratosis and bacterial infection (asterisk). Hematoxylin and eosin (HE). **Figure 13.** Claw; black-capped chickadee with moderate beak overgrowth. There is mild hyperkeratosis of the dorsal plate (double-headed arrow) with clusters of eosinophilic corneocytes (arrowhead) in the stratum corneum. Inset corresponds to the box in the main figure and shows a core-like intrusion of highly nucleated material along the juncture between the dorsal and ventral plates (asterisk). This material is composed of red and white blood cells and degenerating corneocytes. HE. **Figure 14.** Skin of the head; black-capped chickadee with severe beak overgrowth. Hyperkeratosis in the stratum corneum (SC) and hyperplasia in the stratum germinativum (SG) are associated with staphylococcal bacteria (arrowhead). HE. **Figure 15.** Skin of the head; black-capped chickadee with moderate beak overgrowth. Hyperkeratosis (arrows) of the skin and feather follicles (FF) is associated with yeast-like microorganisms (arrowhead; inset). HE. (Van Hemert *et al.* 2012)

Slide 24

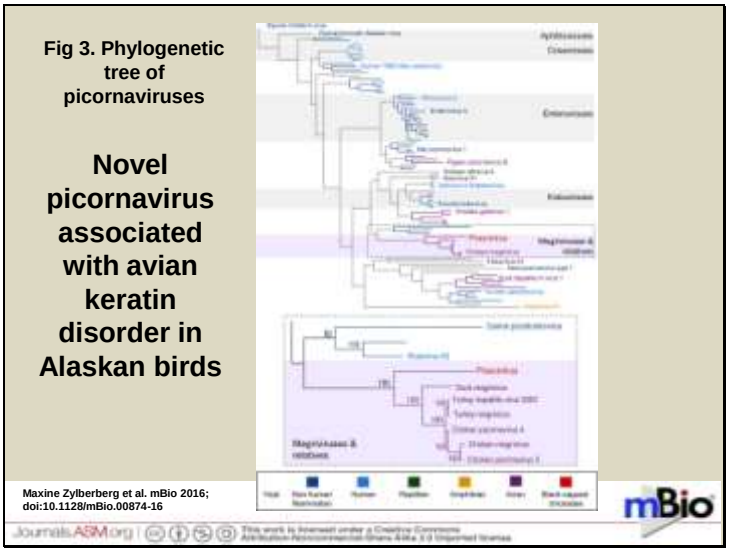


Why:
Infectious – PBFDV, poxvirus, polyomavirus; beak deformity secondary to bacterial sinusitis in an Antipodes Island Parakeet (B. Gartrell); fungal rhinosinusitis in an Amazon parrot (Mans and Guzman); beak malformation in a Darwin's ground finch caused by nares fly (Galligan and Kleindorfer), knemidocoptes

Toxins – Aflatoxin, selenium, PCBs

Metabolic – vitamins A and D, pantothenic acid, biotin, folic acid, calcium; vitamin D related embryonic beak deformities in turkeys (Stevens); nutritional secondary hyperparathyroidism in fledgling crows (Tangredi)

Slide 25



In July 2016, Zylberberg *et al* published results indicating a novel picornavirus may be the causative agent.

Authors used unbiased, metagenomic next-generation sequencing to search for candidate pathogens and identified and sequenced the complete coding region of a novel picornavirus, which they are calling poecivirus

19/19 affected chickadees positive

2/9 control chickadees positive

2 northwestern crows, 2 red-breasted nuthatches with AKD also positive

Phylogenetic tree of picornaviruses. (Top) Relationship between picornavirus polyproteins. Color indicates host taxa, with poecivirus highlighted in light red. TMEV, Theiler's murine encephalomyelitis virus. (Bottom) Detail of boxed portion of top tree showing the relationship between poecivirus and its closest relatives; numbers indicate the percentage of bootstrap support for a given branch.

Slide 26

Significance for Australia

- Is this truly a new pathogen?
- Migratory birds?
- Appears to affect many species
- Do we need to watch for this?
- Could this be DDx for PBFD?

Slide 27

References

- Handel *et al.* 2010. Epizootic beak deformities among wild birds in Alaska: An emerging disease in North America? The Auk. 127:882-898.
- Van Hemert *et al.* 2013. Macroscopic, histologic and ultrastructural lesions associated with avian keratin disorder in black-capped chickadees (*Poecile atricapillus*). Vet Path. 50:500-513.
- Zylberberg *et al.* 2016. Novel picornavirus associated with avian keratin disorder in Alaskan birds. mBio. 7:1-10.

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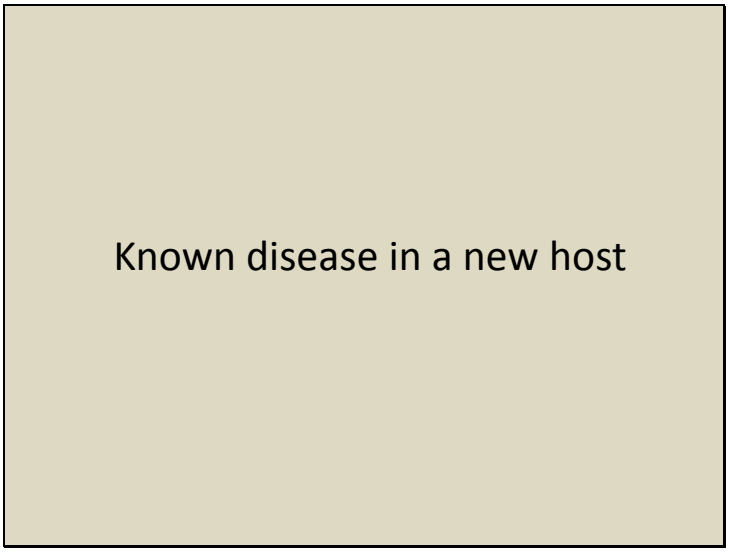
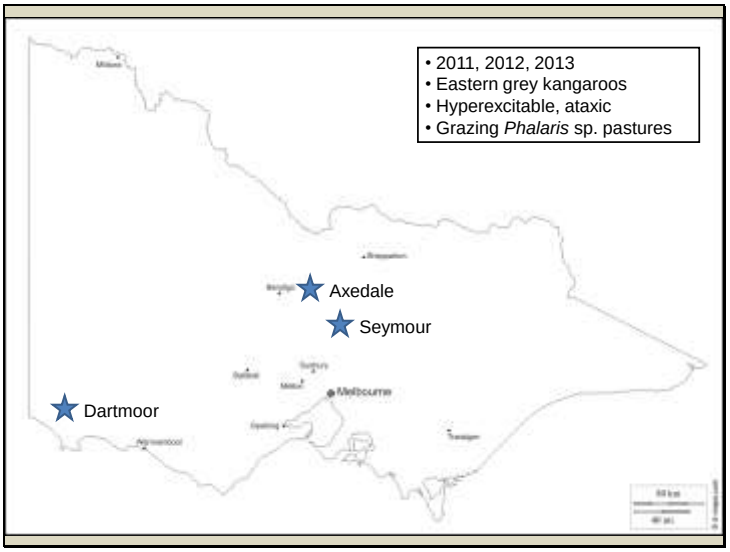


Slide 29

Phalaris staggers

Slide 30

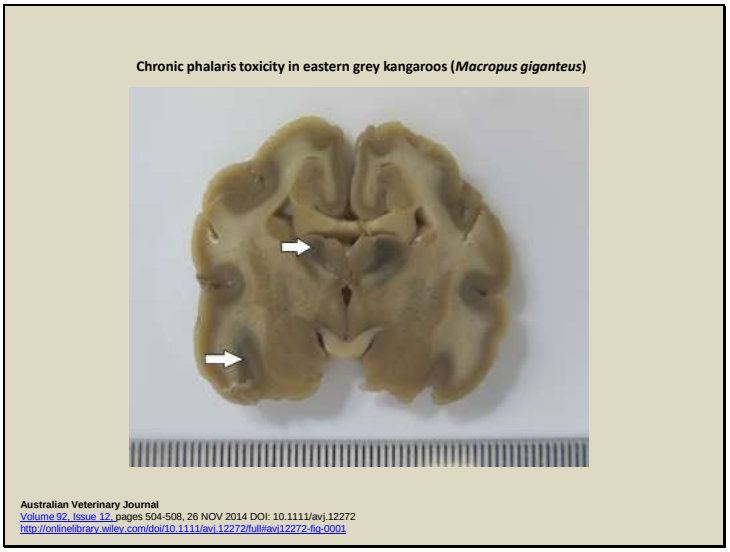

• 2011, 2012, 2013
• Eastern grey kangaroos
• Hyperexcitable, ataxic
• Grazing *Phalaris* sp. pastures
Axedale
Seymour
Melbourne
Dartmoor

Slide 31

Video: Pam Whiteley & Barbara Bacci, Axedale Vic

All the kangaroos exhibited neurological signs characterised by ataxia, wide-based stance, hypermetric gait and generalised muscle tremors. Tail balancing was exaggerated and erratic. Clinical signs were exacerbated if affected kangaroos were disturbed, at which time hyperexcitability was more obvious. Affected kangaroos would also collapse before rising, often after several attempts (Bacci *et al*. 2014).

Slide 32



Necropsy was performed on seven animals over the 3 years
Cerebral cortex and thalamus
Case N.3: transverse section of the brain of an eastern grey kangaroo with incoordination and ataxia shows prominent greenish discolouration of the grey matter. Arrows indicate areas of pigmentation.(Bacci *et al*. 2014)

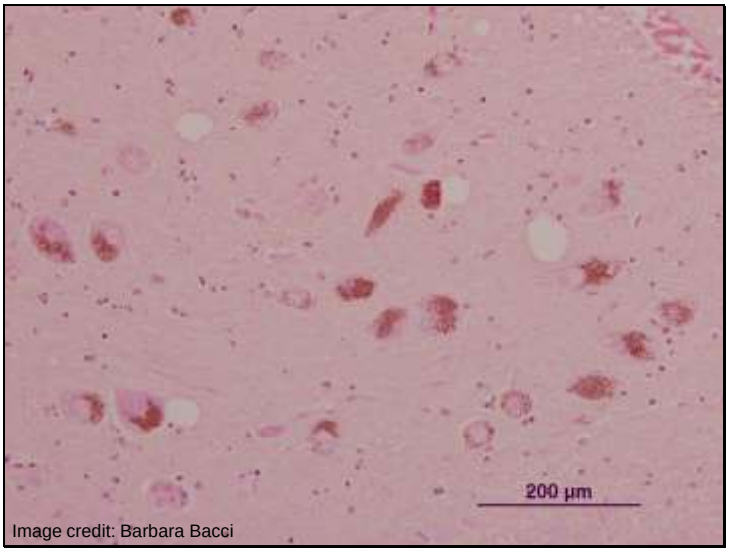
Slide 33

Hippocampus and midbrain
Grossly, all brains showed prominent greenish discolouration of the grey matter, which was most pronounced in the thalamus, brainstem and cortex

Slide 34

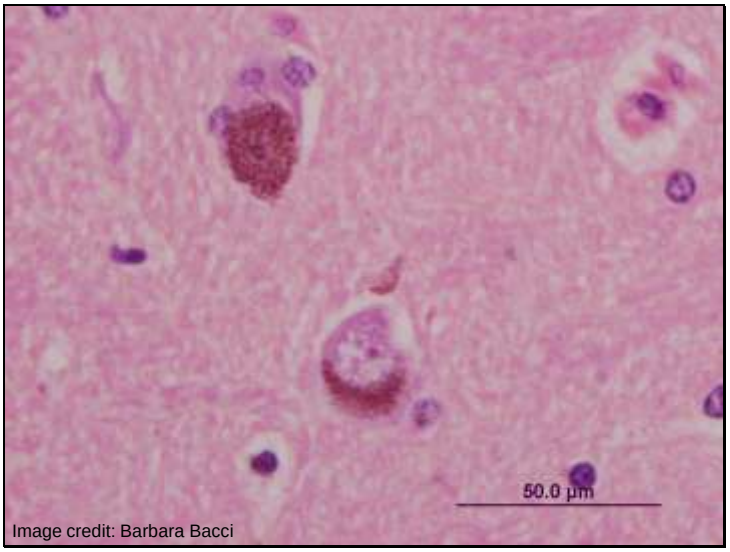
Brainstem

Slide 35



Microscopic examination of sections of the brains from all seven cases showed brown pigmentation of neurons, particularly large motor neurons, appearing as an accumulation of discrete granules in the cytoplasm, sometimes displacing the nucleus at the periphery (Bacci *et al,* 2014)

Slide 36



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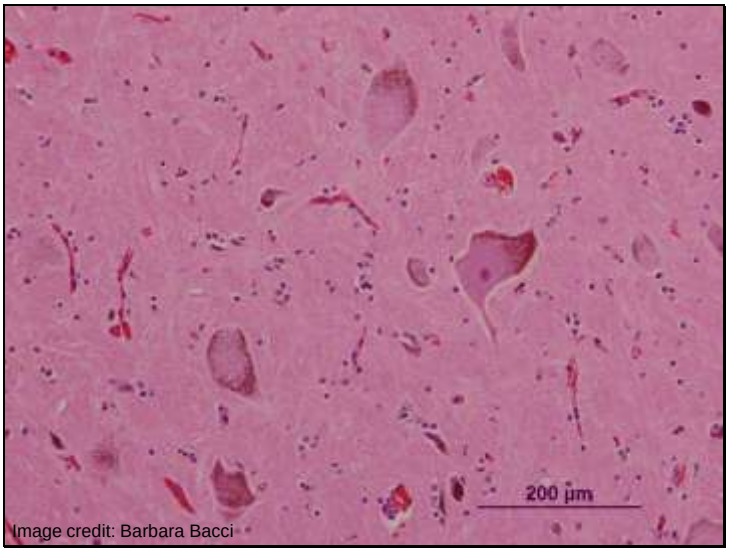
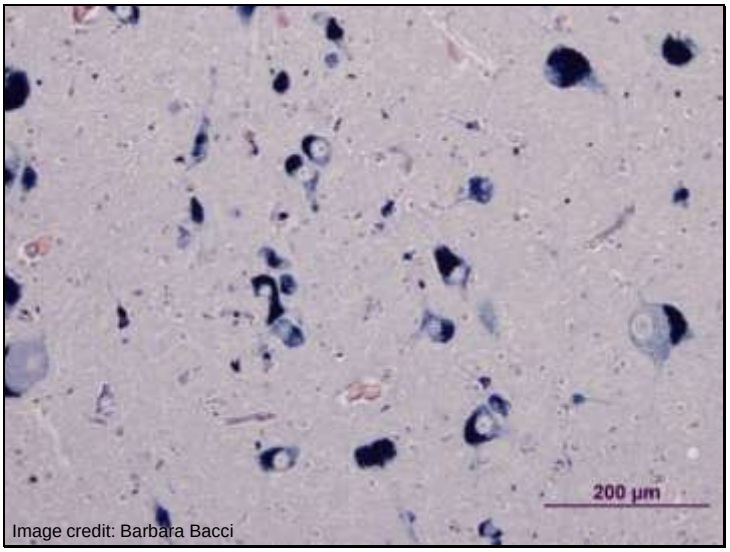


In all cases, perivascular macrophages and astrocytes contained a lighter, green-brown pigment (Bacci *et al*, 2014).

Slide 38



Fontana-Masson, periodic acid-Schiff, Perl's Prussian blue, toluidine blue and Schmorl's reaction special stains were applied to the tissues. The granular pigment stained black with Fontana-Masson and blue with Schmorl’s reaction.

Slide 39

Granular pigmentation

- Large motor neurons of thalamic nuclei and brainstem and spinal ventral motor neurons
- Less in cerebellar Purkinje cells and neurons of the cortex, pyramidal hippocampus and dorsal horns of spinal cord
- Most consistently affected areas of brainstem:
  - red nucleus, lateral and medial geniculate bodies, reticular, pontine, vestibular, olivary and oculomotor nuclei
- Renal tubular cells and retinal ganglion cells

The proximal and distal tubular epithelium of the kidney's cortex and medulla contained pigment that stained positively with Fontana-Masson. There was no evidence of pigmentation within glomerular tufts.
Three of the retinas examined showed moderate amounts of brown granular pigmentation of the ganglion cells, visible with HE as brown and with Fontana-Masson as black pigment (Bacci *et al.* 2014)

Phalaris spp. toxicity diagnosis

- Clinical signs and history of *Phalaris* exposure
- Gross and histo pigmentary changes in brain are highly suggestive
- Confirmation of melanin-like pigment using Fontana-Masson and Schmorl's stains
- Grass samples identified by agronomist (University of Melbourne)

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Phalaris spp. toxicity

- Group of Mediterranean plants
- Usually seen when rapid new growth is grazed in response to rain following prolonged dry periods
- Toxic compounds are indole-derived tryptamine alkaloids, structurally similar to serotonin
- Competitively inhibit serotonin breakdown → accumulation of serotonin and other catecholamines → excitation
- Compounds produce green and brown pigment when metabolised.

Well known disease of sheep and other livestock. In sheep there are 3 manifestations, two of which are per-acute sudden death (cardiac and polioencephalomalacic manifestations) and a chronic manifestation known as phalaris staggers with muscle tremors over the head, trunk and limbs, incoordination, lateral recumbancy and seizures.

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Summary of epidemiological findings of kangaroos found to have green discoloration of the grey matter at post mortem examination

Year	Month	Location	Animals	Clinical signs	Observer
1984	April–November	Kangaroo Island, SA	13 WGK, 8 TW	Neurological abnormalities	Dr Peter Phillips
1998	June, July	Seymour, VIC	EGK	Jumping erratically, circling, crashing into things and falling over	Dr John Dalziel, Dr Malcom Lancaster
2001	July	Seymour	EGK	Hyperexcitability, ataxia	Dr John Dalziel, Dr Malcom Lancaster
2011	September	Seymour	EGK	Hyperexcitability, ataxia	Present study
2012	January	Dartmoor, VIC	EGK	Hyperexcitability, ataxia	Present study
2013	June	Axedale, VIC	9 EGK	Hyperexcitability, ataxia	Present study

WGK, western grey kangaroos; EGK, eastern grey kangaroos; TW, Tamar wallabies.

Australian Veterinary Journal
Volume 92, Issue 12, pages 504-508, 26 NOV 2014 DOI: 10.1111/avj.12272

Does a disease exist if it's not in the literature? Diseases also emerge into our knowledge base

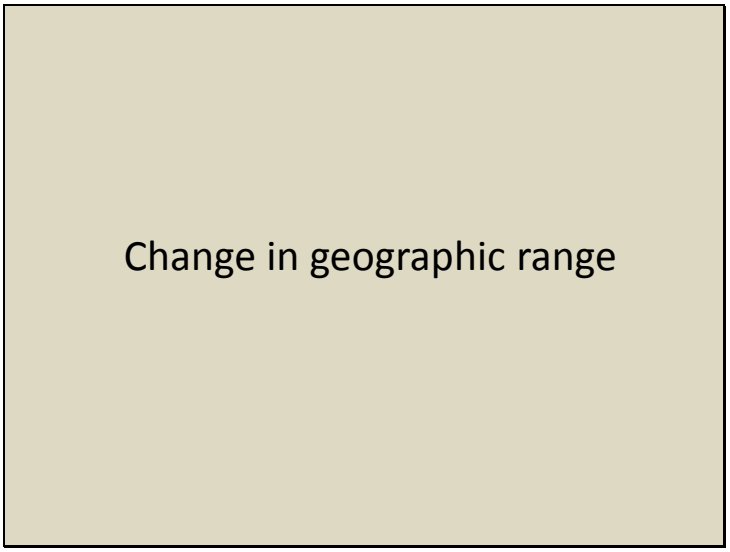
Is this an emerging disease?

- *Phalaris* spp. are introduced plants
- With changing climate, more events may be occurring
- Recognition of the disease is emerging
  - Is a disease really a disease until it is published?

References

- Bacci, Whiteley, Barrow, Phillips, Dalziel and El-Hage. 2014. Chronic phalaris toxicity in eastern grey kangaroos (*Macropus giganteus*). AVJ 92:504-508.

Slide 45



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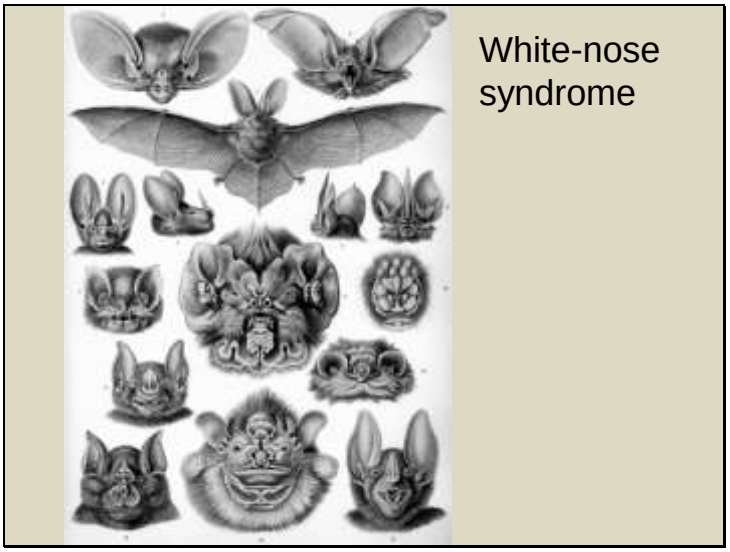


A great example of introduction of a pathogen to a naïve population is white-nose syndrome in bats. White-nose syndrome appears to have been present in Europe for a very long time, where it occasionally causes fatal disease, but typically is not a big issue for the bat populations there.

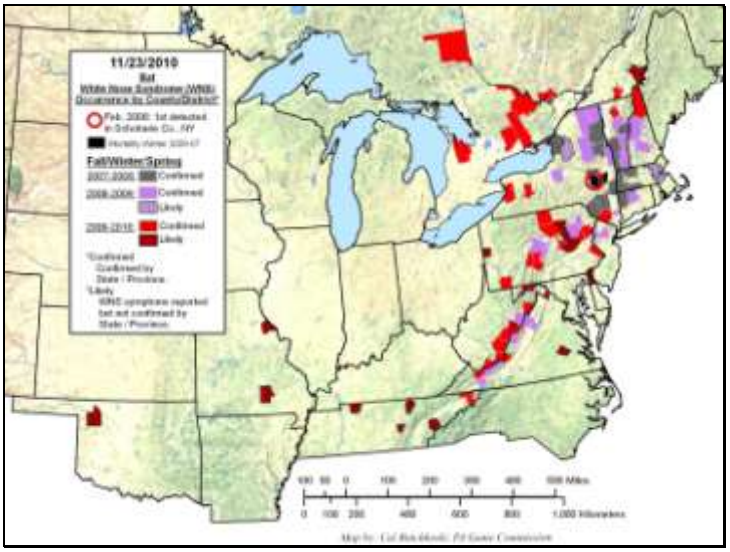
Slide 47

Sometime prior to February 2006 it is believed the causative agent of white-nose syndrome, *Pseudogymnoascus destructans,* was introduced to a bat cave near Albany NY, possibly on contaminated equipment of cavers.

Slide 48

The result of this introduction has been devastating with estimates of number of bats dying from this disease reaching 6 million. The fungus proliferates on the skin of hibernating bats, causing dehydration, electrolyte imbalances and disruption of their torpor. The hibernating bats awake repeatedly during the winter, burning up limited fat reserves. They often leave hibernation sites in late winter, dehydrated and in search of food, ultimately dying.

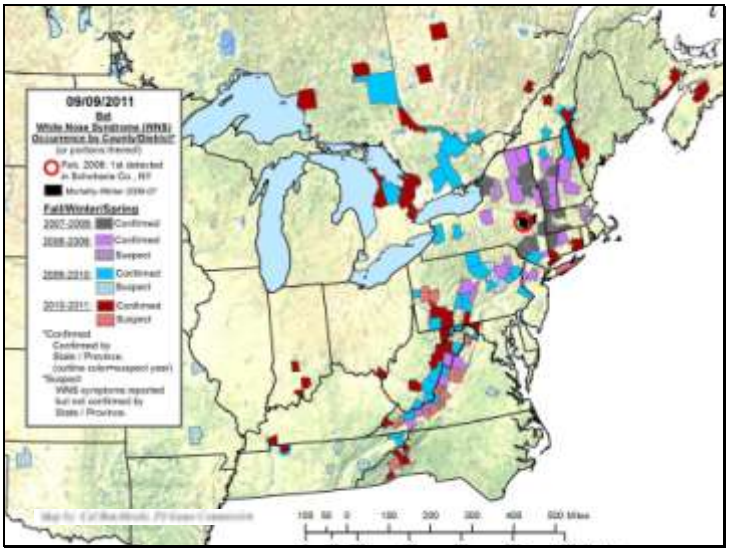
Slide 49



White nose syndrome has been very closely monitored since its first detection and therefore is a great example of an emerging disease resulting from the introduction of pathogen to a new geographic region.

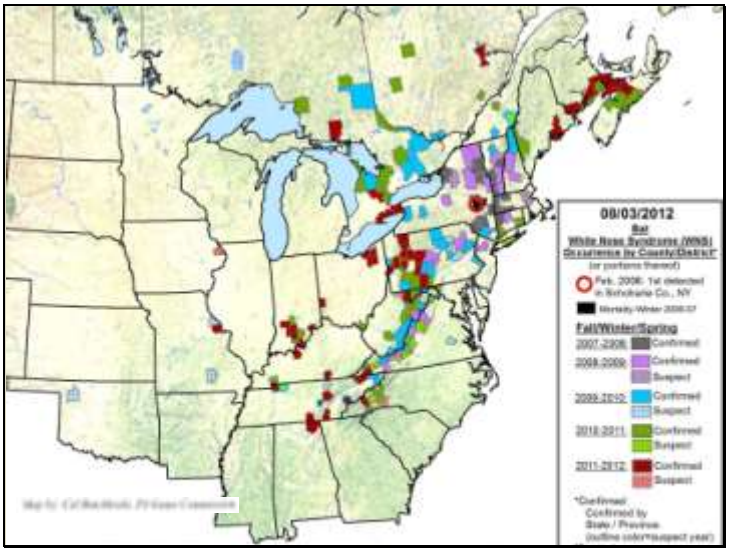
In this series of maps, the newest detected sites of infection are always shown in red. Here we see the index case near Albany NY circled in red. The mortality the following winter of 2006-2007 was restricted to an area quite close to that site. From there the spread of the fungus was initially concentric in 2007-2008 and then followed a north-south line in 2008-2009, likely a result of some restriction to westward spread by the Appalachian mountains. However, but 2009-2010, it had breached that barrier and had also reached Canada.

Slide 50

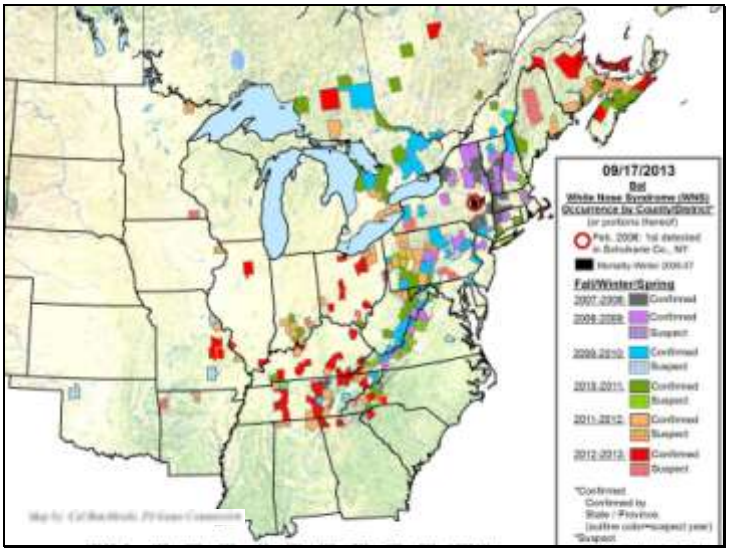


Remember, the most recent detections are always shown in red. From 2010 to 2015, the spread continued westward and north as was generally expected, leading to detections in 5 eastern Canadian provinces and 28 states in that time.

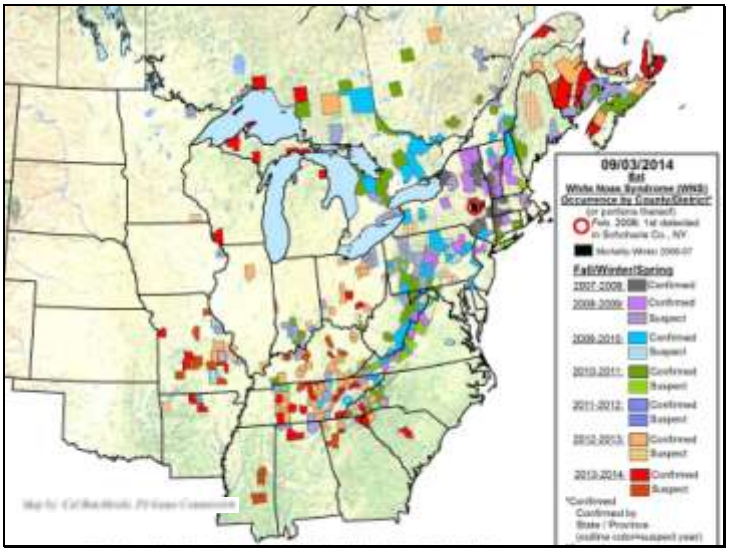
Slide 51


08/03/2012
Fall/Winter/Spring
2007-2008
2008-2009
2009-2010
2010-2011
2011-2012
Confirmed
Suspect

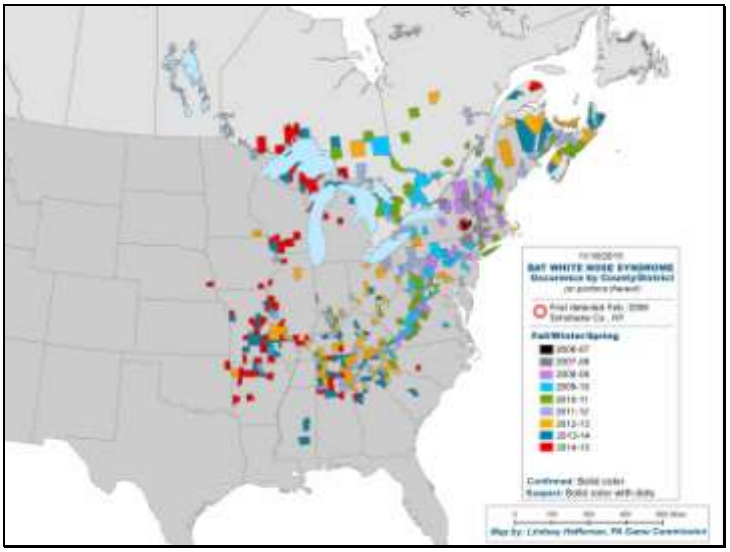
Slide 52


09/17/2013
Bat
White Nose Syndrome (WNS)
Occurrence by County/District
(or portions thereof)
Feb. 2008: 1st detected in Schoharie Co., NY
Fall/Winter/Spring
2007-2008 Confirmed
2008-2009 Confirmed
Suspect
2009-2010 Confirmed
Suspect
2010-2011 Confirmed
Suspect
2011-2012 Confirmed
Suspect
2012-2013 Confirmed
Suspect
*Confirmed
Confirmed by
State / Province
(outline color=suspect year)
*Suspect

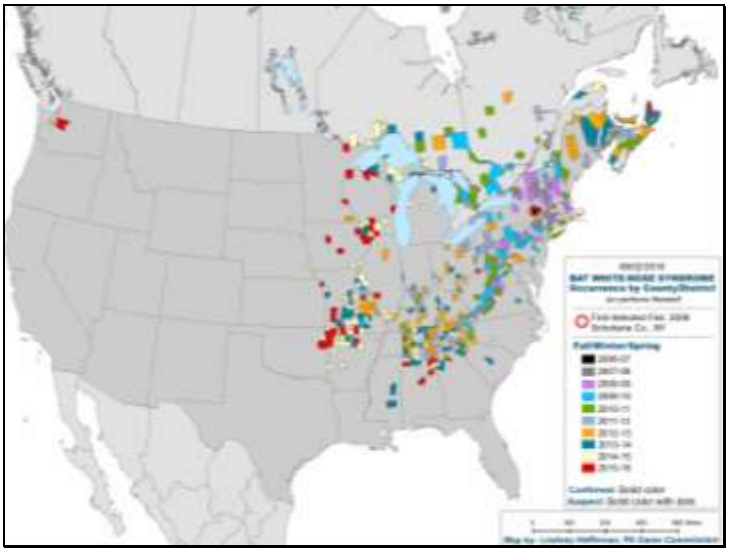
Slide 53


09/03/2014
Bd
White Nose Syndrome (WNS)
Occurrence by County/District
Fall/Winter/Spring
2007-2008
2008-2009
2009-2010
2010-2011
2011-2012
2012-2013
2013-2014

Slide 54


BAT WHITE NOSE SYNDROME
Occurrence by County/District
Fall/Winter/Spring
Confirmed: Solid color

Slide 55



However, last year the pattern of spread changed with infection being confirmed in Washington state. This is too far to expect the spread to have occurred by bat migration and it is suspected that the fungus has again been transplanted by human movement, thus essentially initiating a second emergence. It will be interesting to see if it will now spread westward from this point.

Slide 56

Gross lesions

- Powdery white mold on muzzle
- Mold also seen on patagium, phalanges, etc.
- No consistent internal lesions

Slide credit: S. McBurney, CWHC

Powdery mold is generally not seen in laboratory submissions, as it is lost during the process of collection and submission.

Slide 57

Slide 58

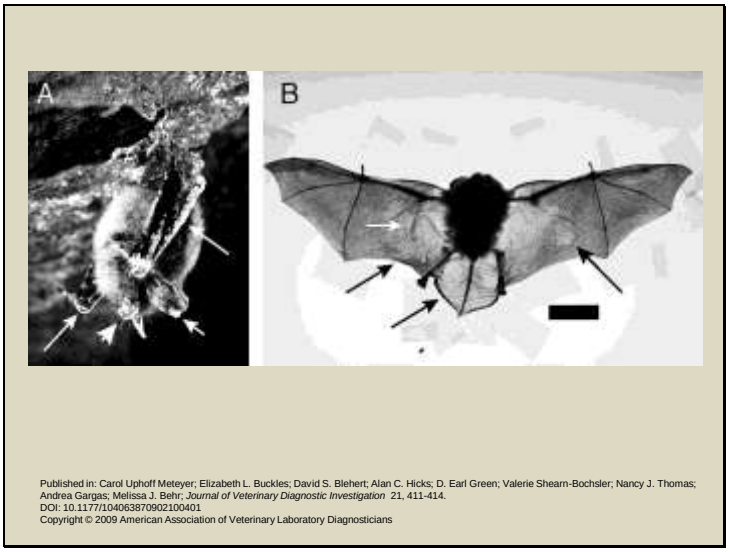
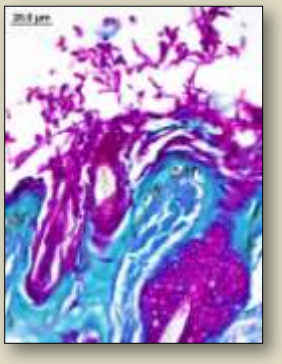


Figure 1. *Myotis lucifugus* with white-nose syndrome, New York. A, little brown bat with white foci of fungus on the face (arrowhead) and an irregular pattern of white fungus on the wing (long arrows) and ear (short arrow). B, little brown bat submitted to the National Wildlife Health Center, Madison, Wisconsin. The image illustrates the taping method used to extended wings, which are backlit on a light box. Damage to wing with contraction of wing membrane (white arrow) and loss of pigmentation (black arrows). Bar = 2.5 cm. (Meteyer *et al.* 2009)

Slide 59

Histopathology

- Skin of muzzle & patagia
- Proliferation of fungus
  - Most abundant superficially
  - Invasion of hair follicles and associated glands
  - Curved conidia (spores)
- ± superficial bacteria

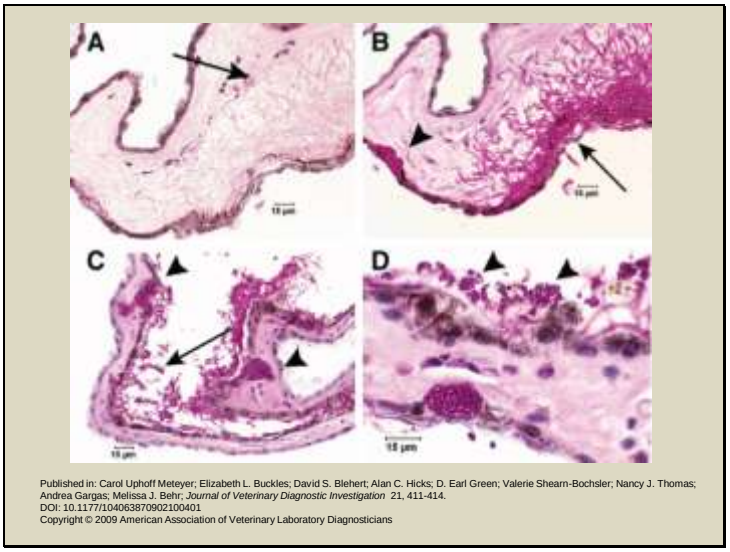


Slide credit: S. McBurney, CWHC

Carol Uphoff Meteyer described rolling wing membranes around a stick to fix them, so had a more substantial tissue for embedding and sectioning

Preferred tissue samples to diagnose this fungal infection are rostral muzzle with nose and wing membrane fixed in 10% neutral buffered formalin. To optimize detection, the muzzle is trimmed longitudinally, the wing membrane is rolled, and multiple cross sections are embedded to increase the surface area examined. Periodic acid–Schiff stain is essential to discriminate the nonpigmented fungal hyphae and conidia.
Fungal hyphae form cup-like epidermal erosions and ulcers in the wing membrane and pinna with involvement of underlying connective tissue. In addition, fungal hyphae are present in hair follicles and in sebaceous and apocrine glands of the muzzle with invasion of tissue surrounding adnexa. Fungal hyphae in tissues are branching and septate, but the diameter and shape of the hyphae may vary from parallel walls measuring 2 mm in diameter to irregular walls measuring 3–5 mm in diameter. When present on short aerial hyphae, curved conidia are approximately 2.5 mm wide and 7.5 mm in curved length. Conidia have a more deeply basophilic center, and one or both ends are usually blunt (Meteyer *et al*, 2009).

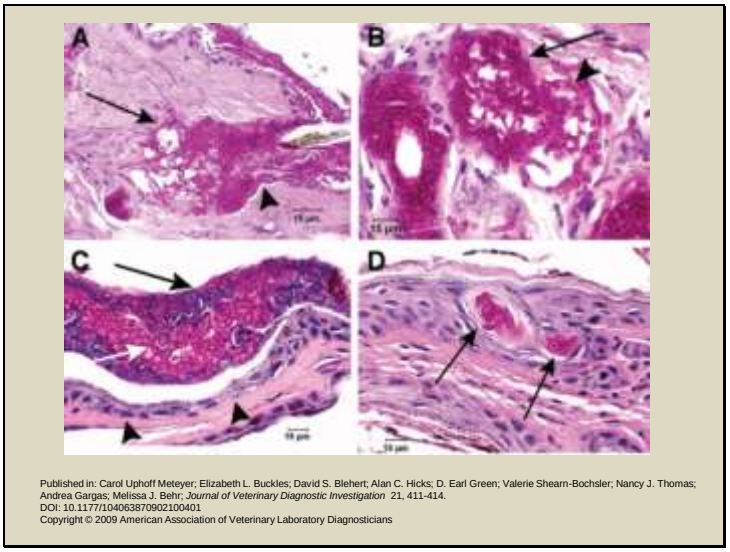
Slide 60



Published in: Carol Uphoff Meteyer; Elizabeth L. Buckles; David S. Blehert; Alan C. Hicks; D. Earl Green; Valerie Shearn-Bochsler; Nancy J. Thomas; Andrea Gargas; Melissa J. Behr; *Journal of Veterinary Diagnostic Investigation* 21, 411-414.
DOI: 10.1177/104063870902100401

Figure 2. Wing membranes of *Myotis lucifugus* bats infected with white-nose syndrome (WNS). A, histologic section of wing membrane from the same bat as in Figure 1B. Invasive fungus (arrow) stains poorly with hematoxylin and eosin stain, and inflammatory infiltrates are not present. Bar = 15 µm. B, periodic acid-Schiff (PAS) stain of serial section from same tissue as in panel A. Fungal hyphae stain bright magenta. Hyphae are associated with cup-shaped epidermal erosions (arrowhead) and ulcers (arrow) with invasion of the underlying connective tissue. Bar = 15 µm. C, section of wing membrane, collected while inside the cave, from a little brown bat immediately after euthanasia. Exuberant fungal growth is present on the surface of the skin (arrow) and penetrates the wing membrane (arrowheads) without associated inflammation. PAS stain. Bar = 15 µm. D, conidia on the surface of the wing membrane of a cave-dwelling little brown bat fixed immediately after euthanasia in the cave. The characteristic curved conidia measure approximately 2.5 µm in diameter and 7.5 µm in curved length, have one or two blunt ends, and have a deeply basophilic central region (arrowheads). These conidia are identical to those of *Geomyces* sp. fungus isolated from bats with WNS.1 A focal cluster of fungal hyphae is present within the epithelium on opposite wing margin. PAS stain. Bar = 15 µm. (Meteyer *et al*, 2009)

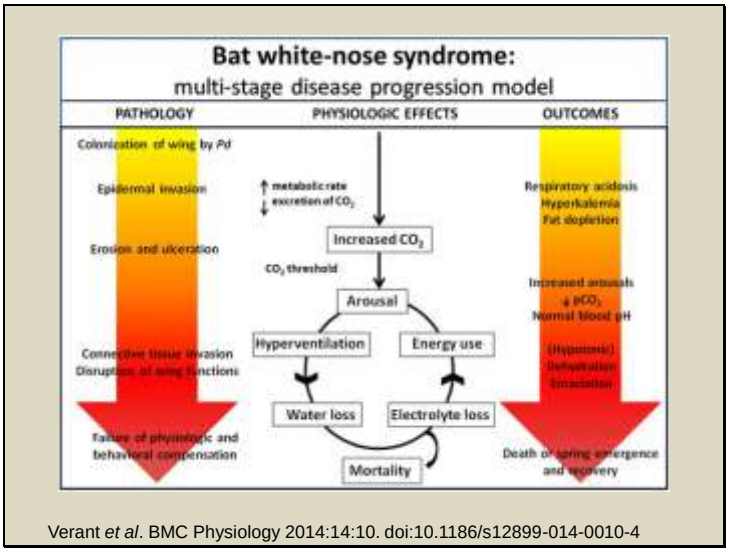
Slide 61



Published in: Carol Uphoff Meteyer; Elizabeth L. Buckles; David S. Blehert; Alan C. Hicks; D. Earl Green; Valerie Shearn-Bochsler; Nancy J. Thomas; Andrea Gargas; Melissa J. Behr; *Journal of Veterinary Diagnostic Investigation* 21, 411-414.
DOI: 10.1177/104063870902100401

Figure 3. *Myotis lucifugus* with white-nose syndrome (WNS) submitted to the National Wildlife Health Center, Madison, Wisconsin, from Connecticut and Vermont. A, histologic section of bat muzzle with fungal hyphae filling the hair follicle (arrowhead), obliterating the epidermal sheath, and invading the regional connective tissue (arrow). No inflammatory response is present and bacteria colonize the surface. Periodic acid-Schiff (PAS) stain. Bar = 15 µm. B, fungal hyphae obscure the follicular epithelium (white arrow) and associated sebaceous gland (long arrow) of a bat's muzzle. The fungal hyphae are branching, septate (arrowhead), and of variable morphology ranging from parallel walls measuring 2 µm diameter to bulging or globose walls measuring 3–5 µm in diameter. There is no associated inflammation. PAS stain. Bar = 15 µm. C, wing membrane from a bat collected in May after emergence from hibernation but unable to fly. Inflammatory cells (long arrow) surround fungal hyphae (white arrow) forming a cellular crust overlying intact epidermis (arrowheads). PAS stain. Bar = 15 µm. D, different bat with similar history to that in panel C. Quiescent nests of fungus are surrounded by a thin layer of amorphous material within the epidermis of the wing (arrows). PAS stain. Bar = 15 µm. (Meteyer *et al*, 2009)

Slide 62



Verant *et al*. BMC Physiology 2014:14:10. doi:10.1186/s12899-014-0010-4

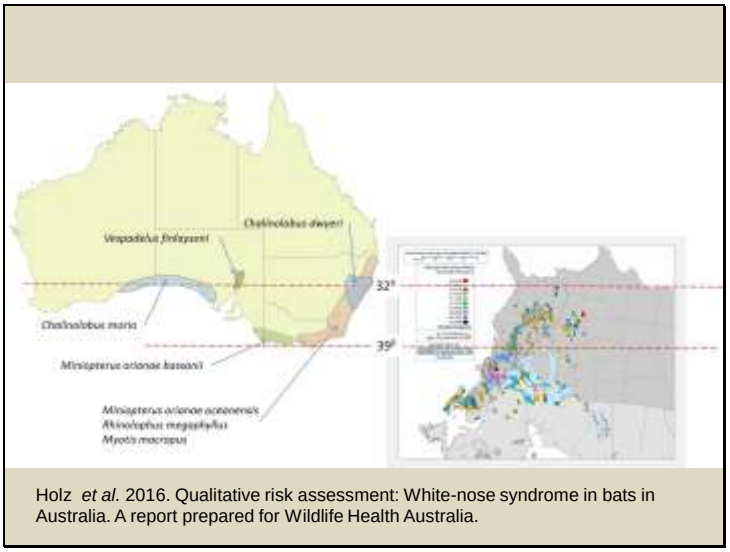
Disease progression model for bat white-nose syndrome (WNS). We propose a mechanistic multi-stage disease model for WNS in a hibernating bat that encompasses current knowledge on the progression of fungal-induced wing pathology and physiologic sequelae leading to mortality from disease. Initial colonization and invasion of the wing epidermis by *Pseudogymnoascus destructans* (*Pd*) results in increased energy expenditure, chronic respiratory acidosis (elevated blood pCO_2 and bicarbonate), and hyperkalemia (elevated blood potassium). Erosion and ulceration of the epidermis stimulate increased frequencies of arousal from torpor, which remove excess CO_2 and normalize blood pH, but contribute to dehydration and depletion of fat reserves. As wing pathology becomes more extensive and severe, these effects are exacerbated by water and electrolyte loss across the epidermis (hypotonic dehydration), which stimulate more frequent arousals and create a positive feedback loop that ultimately leads to mortality when energy reserves and compensatory mechanisms become exhausted. (Verant *et al,* 2014).

Slide 63

Currently Australia is believed to be free from the pathogen responsible for WNS, but according to a risk assessment commissioned by WHA, it is likely to enter at some point in the future with cavers identified as the most likely mechanism of arrival. That assessment has identified these seven cave-dwelling species from southern Australia as the most at risk (Holz *et al.* 2016).

"In summary, based on the available information, for large scale mortalities to occur several criteria probably apply: a) Bats need to roost and hibernate in caves that maintain a year round temperature below 20 o C. b) Bats need to be susceptible to infection with P. destructans and the development of WNS, and c) Bats need to cluster together in large numbers in regions with severe winters, which limit free water and prey availability, leading to dehydration and starvation as a result of repeated arousals from hibernation" (Holz *et al*. 2016).

Slide 64



Holz *et al.* 2016. Qualitative risk assessment: White-nose syndrome in bats in Australia. A report prepared for Wildlife Health Australia.

Figure 4. Australian latitudes, which correlate with the southern US latitudes where *P. destructans has been found, and the Australian cave-dwelling bats that are found within* these latitudes. The individual species distributions marked on the Australian map do not show the full extent of their distribution beyond the key latitude band (Holz *et al*. 2016).

There are no cave dwelling bats in Tasmania.

Slide 65

References

- Holz *et al.* 2016. Qualitative risk assessment: White-nose syndrome in bats in Australia. A report prepared for Wildlife Health Australia. (downloaded http://wildlifehealthaustralia.com.au on 6.2.2017)
- Meteyer *et al.* 2009. Histopathologic criteria to confirm white-nose syndrome in bats. JVDI 21:411-414.
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- www.whitenosesyndrome.org (accessed 30.1.2017)

Slide 66

Increased incidence in a population

Slide 67

During summer migrations the saigas' nose helps filter out dust kicked up by the herd and cools the animal's blood. In the winter it heats up the frigid air before it is taken to the lungs.

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The saiga has had a tumultuous history. During the last glacial ice age it ranged from the British Isles through Central Asia and across the Bering land bridge to Alaska and the Yukon and Northwest Territories of Canada. In modern history, its range has been confined to the steppes of Central Asia. It was hunted heavily in the late 19th century, reaching near extinction in the 1920's, but had recovered remarkably by the 50's when 2 million were thought to roam the USSR. However, collapse of the USSR led to increased hunting for food and for horn which was sought after in the Chinese medicine market. At one point, the WWF was encouraging hunting of the species for horn in an attempt to reduce pressure on rhino. The saiga was listed as critically endangered on the IUCN's red list in 2001 when the population was thought to be as low as 50,000. Again the population has been able to bounce back to a degree, reaching around 300,000 by 2015.

Slide 69

Currently the largest population of saiga is present in the middle of Kazakhstan, with smaller populations around the border, within Russia and a pocket of a different subspecies in Mongolia.

Slide 70

Every year in May, pregnant females gather together in vast herds and simultaneously give birth in an attempt to overwhelm predators with supply, giving each calf a greater chance of survival.

In May of 2015, the BBC Planet Earth II film crew was in Kazakstan to document this amazing event along with vets and biologists who have been working on the recovery of the species.

Slide 71

Credit: Royal Veterinary College, Alexa Wolfs
Pri. November 17, 2015. "**What's killing the endangered Saiga antelope of Central Asia?"**

However, what they witnessed instead was this.

Slide 72

Washington Post . Online May 29, 2015. Dead Saiga antelopes lie on a field in the Zholoba area of the Kostanay region, Kazakhstan. (Reuters/Kazakhstan's Ministry of Agriculture)

In the course of 10 days, approximately 200,000 saiga, primarily females and newborns, died. A recent census indicates 80% of the population was lost.

Slide 73

Dr. Richard Rock of the Royal Veterinary College was on hand. By his report, the death rate among animals that became sick was 100% and they died within hours of initial signs. He also explained that epidemiologically the speed of the outbreak was not consistent with an infectious disease which should take time to pass through a herd.

Slide 74

32 post mortem examinations were performed. The culprit: haemorrhagic septicaemia due to Pasteurella multocida, a commensal organism that is known for causing occasional cases of septicaemia and pneumonia in grazing animals, but the extent of this outbreak is unprecedented. It is true that all the females were within days of parturition, causing some increased stress, but it appears as though there must be some other factor resulting in mass immunosuppression. Dr. Kock is speculating that an extreme weather event may have been the trigger. Days before the event started, the temperature spiked to 37˚C during the day before plummeting to -5˚C overnight. Particularly the high temperatures never used to occur at that time of year and he suggests that climate change is causing greater temperature fluctuations, which in this case had disastrous results.

Slide 75

Why is this an emerging disease?

- Pasteurella multocida is not new in this population and deaths have likely occurred previously, but this represents a massive increase in incidence.

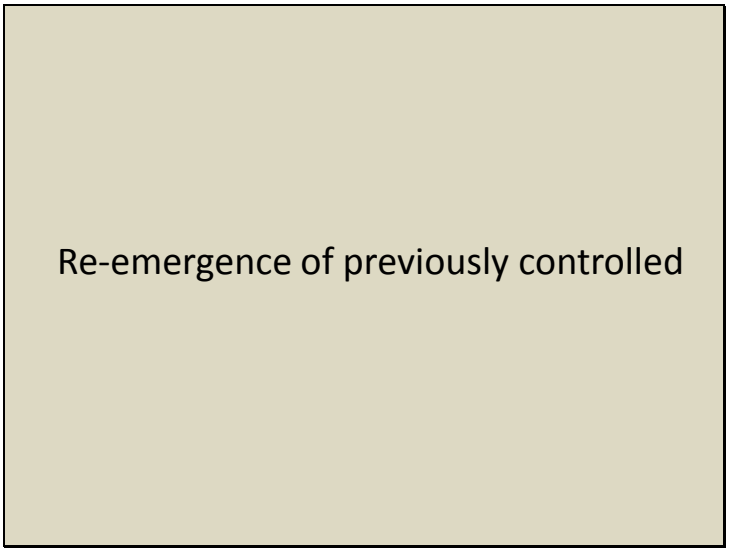
Slide 76

References

- Publication of gross, histopathological and epidemiological findings are pending
- EJ Milner-Gulland, Morgan E, Kock R. Dec 4, 2016. *Planet Earth II: why more than 200,000 saiga antelopes died in just days.* The Conversation.
- Wernick, A. Nov 17, 2015. *What's killing the endangered Saiga antelope of Central Asia?* Public Radio International.

Unfortunately, the main researchers involved in the outbreak are off to another outbreak. Starting in December, members of the Mongolian subspecies began dying in high numbers. This time the culprit appears to be pest-de-petit ruminants, which had been recently circulating in livestock.

Slide 77



Slide 78

Slide 79

Key deer

- Subspecies of WTD
- Became isolated on the keys 13,000 years ago
- Population low in 1950's of around 24
- Population as of mid-2016 was 1000

The Key deer is a subspecies of white-tailed deer which migrated to the Florida Keys from the mainland over a land bridge during the Wisconsin glaciation.

Key deer were hunted as a food supply by native tribes, passing sailors, and early settlers. Hunting them was banned in 1939, but widespread poaching and habitat destruction caused the subspecies to plummet to near-extinction by the 1950s.

Slide 80

The range of the Key deer originally encompassed all of the lower Florida Keys (where standing water pools exist), but is now limited to a stretch of the Florida Keys from about Sugarloaf Key to Bahia Honda Key.

On September 29, 2016, fly larvae were collected from a Key deer on Big Pine Key. These were subsequently confirmed as New World screwworm.

Slide 81

New World Screwworm fly

- *Cochliomyia hominivorax*
- Larvae feed on LIVE vertebrate tissue
- Was eliminated from USA in 1982 (sterile insect release)

In contrast to other blow flies in the same genus, the larvae of this fly feed on LIVE warm-blooded vertebrate flesh

Was eliminated from the USA in 1982, primarily through the use of sterile insect release programs which lead to frustrated breeding attempts. Has since been eliminated from much of Central America, but still exists throughout much of South America and 5 Caribbean countries (University of Florida Featured Creatures http://entnemdept.ufl.edu/creatures/livestock/secondary_screwworm.htm)

Slide 82

Image credit: WDA Newsletter Jan 2017

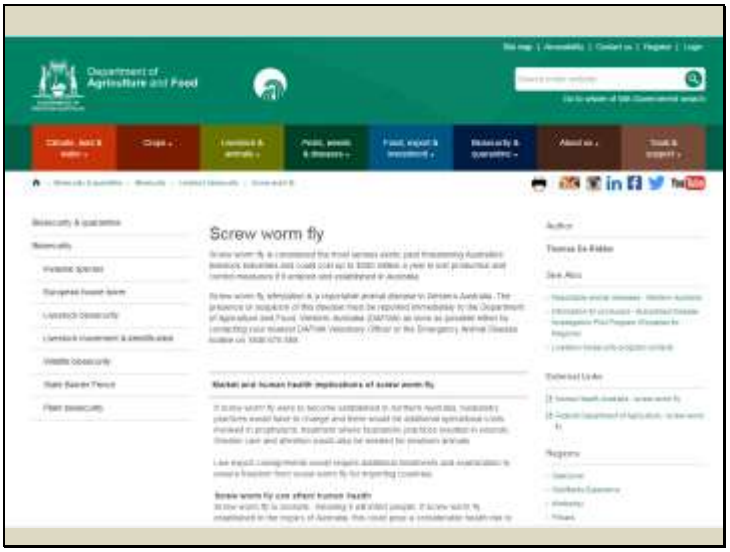
Larvae of the screwworm enter an animal through an open wound or, in the case of newborns, the navel. Consumption of flesh leads to the creation of massive wounds, and can be fatal.

In the case of Key deer, most infestations in this outbreak have occurred in males with cranial wounds as a result of the fighting during the rut.

As of Jan 7, 135 deer had been found dead or euthanased as a result of screwworm infestation (USFWS National Key Deer Refuge https://www.fws.gov/refuge/National_Key_Deer_Refuge/News_Releases.html). This is >10% of the pre-outbreak population, but fortunately as a quirk of the circumstances, a larger proportion of males has been affected, which should allow for more rapid recovery if the outbreak can be controlled. Unfortunately, fawning season will begin soon and females will be more vulnerable then as will the new fawns which can be infested via the umbilicus.

Other species confirmed with infections include dogs, cats, a pig and a raccoon (Florida Dept of Agriculture and Consumer Services http://www.freshfromflorida.com/Divisions-Offices/Animal-Industry/Consumer-Resources/Reportable-Animal-Diseases/New-World-Screwworm/New-World-Screwworm-Detection-Information)

Slide 83



Screwworm is identified as a invasive pathogen risk in Australia.

Slide 84

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- USFWS National Key Deer Refuge website (accessed Jan 24, 2017)
  https://www.fws.gov/refuge/National_Key_Deer_Refuge/News_Releases.html
- Florida Dept of Agriculture and Consumer Services website (accessed Jan 24, 2017)
  http://www.freshfromflorida.com/Divisions-Offices/Animal-Industry/Consumer-Resources/Reportable-Animal-Diseases/New-World-Screwworm/New-World-Screwworm-Detection-Information
- University of Florida featured creatures website (accessed Jan 24, 2017)
  http://entnemdept.ufl.edu/creatures/livestock/secondary_screwworm.htm