



Pathogenesis of Chytridiomycosis, a Cause of Catastrophic Amphibian Declines

Jamie Voyles *et al.*

Science **326**, 582 (2009);

DOI: 10.1126/science.1176765

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of January 19, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/326/5952/582.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2009/10/22/326.5952.582.DC1.html>

<http://www.sciencemag.org/content/suppl/2009/10/22/326.5952.582.DC2.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/326/5952/582.full.html#related>

This article **cites 21 articles**, 7 of which can be accessed free:

<http://www.sciencemag.org/content/326/5952/582.full.html#ref-list-1>

This article has been **cited by** 4 article(s) on the ISI Web of Science

This article has been **cited by** 14 articles hosted by HighWire Press; see:

<http://www.sciencemag.org/content/326/5952/582.full.html#related-urls>

This article appears in the following **subject collections**:

Microbiology

<http://www.sciencemag.org/cgi/collection/microbio>

As habitat range increases with OMZ expansion and intensification, this role will only become more visible and significant. Therefore, the SUP05 metagenome provides a functional template for analysis of gene expression in relation to climatologically relevant biogeochemical transformations within oxygen-deficient oceanic waters. This information should prove useful in the development of monitoring tools to assess microbial community responses to OMZ expansion and intensification.

References and Notes

- R. J. Diaz, R. Rosenberg, *Science* **321**, 926 (2008).
- K. R. Arrigo, *Nature* **437**, 349 (2005).
- A. Paulmier, D. Ruiz-Pino, *Prog. Oceanogr.* **80**, 113 (2008).
- L. Stramma, G. C. Johnson, J. Sprintall, V. Mohrholz, *Science* **320**, 655 (2008).
- F. A. Whitney, H. J. Freeland, M. Robert, *Prog. Oceanogr.* **75**, 179 (2007).
- P. G. Brewer, E. T. Peltzer, *Science* **324**, 347 (2009).
- B. M. Fuchs, D. Woebken, M. V. Zubkov, P. Burkill, R. Amann, *Aquat. Microb. Ecol.* **39**, 145 (2005).
- D. Woebken, B. A. Fuchs, M. A. A. Kuypers, R. Amann, *Appl. Environ. Microbiol.* **73**, 4648 (2007).
- H. Stevens, O. Ulloa, *Environ. Microbiol.* **10**, 1244 (2008).
- G. Lavik et al., *Nature* **457**, 581 (2009).
- E. Zaikova et al., *Environ. Microbiol.* 10.1111/j.1462-2920.2009.02058.x (2009).
- N. Bano, J. T. Hollibaugh, *Appl. Environ. Microbiol.* **68**, 505 (2002).
- M. Sunamura, Y. Higashi, C. Miyako, J. Ishibashi, A. Maruyama, *Appl. Environ. Microbiol.* **70**, 1190 (2004).
- J. J. Anderson, A. H. Devol, *Estuar. Coast. Mar. Sci.* **1**, 1 (1973).
- Materials and methods are available as supporting material on Science Online.
- H. Kuwahara et al., *Curr. Biol.* **17**, 881 (2007).
- I. L. G. Newton et al., *Science* **315**, 998 (2007).
- G. Jost, M. V. Zubkov, E. Yakushev, M. Labrenz, K. Jurgens, *Limnol. Oceanogr.* **53**, 14 (2008).
- C. F. Friedrich, F. Bardischewsky, D. Rother, A. Quentmeier, J. Fischer, *Curr. Opin. Microbiol.* **8**, 253 (2005).
- J. Zopf, T. G. Ferdelman, B. B. Jorgensen, A. Teske, B. Thamdrup, *Mar. Chem.* **74**, 29 (2001).
- R. B. Cardoso et al., *Biotechnol. Bioeng.* **95**, 1148 (2006).
- C. Dahl et al., *J. Bacteriol.* **187**, 1392 (2005).
- R. D. Vetter, *Mar. Biol. (Berlin)* **88**, 33 (1985).
- V. Stewart, Y. Lu, A. J. Darwin, *J. Bacteriol.* **184**, 1314 (2002).
- H. Wang, C. P. Tseng, R. P. Gunsalus, *J. Bacteriol.* **181**, 5303 (1999).
- S. Spiro, J. R. Guest, *FEMS Microbiol. Rev.* **6**, 399 (1990).
- M. Mussmann et al., *PLoS Biol.* **5**, e230 (2007).
- D. P. Pandey, K. Gerdes, *Nucleic Acids Res.* **33**, 966 (2005).
- D. J. Richardson, B. C. Berks, D. A. Russell, S. Spiro, C. J. Taylor, *Cell. Mol. Life Sci.* **58**, 165 (2001).
- S. K. Christensen, M. Mikkelsen, K. Pedersen, K. Gerdes, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 14328 (2001).
- This work was performed under the auspices of the U.S. Department of Energy's Office of Science, Biological, and Environmental Research Program and by the University of California, Lawrence Berkeley National Laboratory, Lawrence Livermore National Laboratory under contract no. DE-AC02-05CH11231, Lawrence Livermore National Laboratory under contract no. DE-AC52-07NA27344, and Los Alamos National Laboratory under contract no. DE-AC02-06NA25396. This work was also supported by grants from the Natural Sciences and Engineering Research Council (NSERC) of Canada 328256-07 and STPSC 356988, Canada Foundation for Innovation (CFI) 17444; Canadian Institute for Advanced Research (CIFAR), and the Center for Bioinorganic Chemistry (CEBIC). D.A.W. was supported by NSERC, Killam Trust, and the Tula Foundation-funded Centre for Microbial Diversity and Evolution (CMDE). We thank M. Robert (Institute of Ocean Sciences, Sidney, BC, Canada), C. Payne, L. Pakhomova, and J. Granger (UBC) for help in sampling and chemical analyses and the captains and crews of the CCGS *John P. Tulley* and *HMS John Strickland* for logistical support. We thank the Joint Genome Institute, including K. Barry, S. Pitluck, and E. Kirtan, for technical assistance and A. Page, K. Mitchell, and S. Lee in the Hallam laboratory for reading the manuscript. This metagenome project has been deposited at the DNA Data Bank of Japan and European Molecular Biology Laboratory, and GenBank, under the project accession ACGS000000000. The version described in this paper is the first version, ACGS01000000. SSU rRNA gene sequences were deposited at GenBank under the accession numbers GQ345343-GQ351265, and fosmid sequences were deposited under the accession numbers GQ351266 to GQ351269 and GQ369726.

Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5952/578/DC1
Materials and Methods
SOM Text
Figs. S1 to S7
Tables S1 to S4
References

22 April 2009; accepted 2 September 2009
10.1126/science.1175309

Pathogenesis of Chytridiomycosis, a Cause of Catastrophic Amphibian Declines

Jamie Voyles,^{1*} Sam Young,¹ Lee Berger,¹ Craig Campbell,² Wyatt F. Voyles,³ Anuwat Dinodom,² David Cook,² Rebecca Webb,¹ Ross A. Alford,⁴ Lee F. Skerratt,¹ Rick Speare¹

The pathogen *Batrachochytrium dendrobatidis* (*Bd*), which causes the skin disease chytridiomycosis, is one of the few highly virulent fungi in vertebrates and has been implicated in worldwide amphibian declines. However, the mechanism by which *Bd* causes death has not been determined. We show that *Bd* infection is associated with pathophysiological changes that lead to mortality in green tree frogs (*Litoria caerulea*). In diseased individuals, electrolyte transport across the epidermis was inhibited by >50%, plasma sodium and potassium concentrations were respectively reduced by ~20% and ~50%, and asystolic cardiac arrest resulted in death. Because the skin is critical in maintaining amphibian homeostasis, disruption to cutaneous function may be the mechanism by which *Bd* produces morbidity and mortality across a wide range of phylogenetically distant amphibian taxa.

Infectious disease can cause population declines (1), and potentially extinctions (2), if multiple variables create favorable conditions for severe outbreaks. A striking example is the global loss of amphibians due to chytridiomycosis (1, 3, 4). Despite an initial reluctance to accept disease as a direct cause of declines (5), *Batrachochytrium dendrobatidis* (*Bd*) is now recognized for its ability to spread rapidly through amphibian populations (6, 7), infect numerous species (1, 6), cause high rates of mortality (6, 8),

and persist even at low host densities (7, 9). These disease characteristics render population recovery from chytridiomycosis especially difficult and provide strong evidence for disease-induced extinctions (2, 8, 10). However, the mechanism by which *Bd* kills amphibians is unknown.

The pathogenesis of chytridiomycosis has been difficult to determine because cutaneous fungal infections are rarely fatal without other predisposing factors (11). Furthermore, *Bd* is in

a phylum of fungi not previously known as pathogens of vertebrates (12), it is confined to the superficial layers of the epidermis (2, 13) with minimal host reaction to infection (13, 14), and no consistent pathological changes in internal organs of diseased amphibians are detectable with light microscopy (3). Differential expression of peptidase genes suggests that *Bd* pathogenicity may have a genetic basis (15), but determining the proximate cause of death has been inherently challenging because multiple physiological systems shut down before death.

Amphibian skin is unique among terrestrial vertebrates because it is actively involved in the exchange of respiratory gases, water, and electrolytes (16–19). Because of the role of amphibian skin in maintaining osmotic balance, other studies have suggested that *Bd* might disrupt cutaneous osmoregulation (3, 20). To test this hypothesis, we tracked the development of *Bd* infections in green tree frogs (*Litoria caerulea*), which are susceptible to chytridiomycosis in

¹School of Public Health, Tropical Medicine and Rehabilitation Sciences, Amphibian Disease Ecology Group, James Cook University, Townsville, QLD 4811, Australia. ²Discipline of Physiology, Bosch Institute, Faculty of Medicine, University of Sydney, Sydney, NSW 2006, Australia. ³School of Medicine, Division of Cardiology, University of New Mexico, Albuquerque, NM 87131, USA. ⁴School of Marine and Tropical Biology, Amphibian Disease Ecology Group, James Cook University, Townsville, QLD 4811, Australia.

*To whom correspondence should be addressed. E-mail: jamie.voyles@gmail.com

laboratory experiments (21), with polymerase chain reaction (PCR) analysis and histopathology on skin biopsies. Clinical signs of disease and mortality occurred in *L. caerulea* individuals with the highest burdens of *Bd* (Fig. 1) and with greatest histopathological changes in the epidermis (fig. S2). We measured epidermal electrolyte

transport in isolated skin preparations, monitored changes in blood and urine biochemical parameters, and monitored cardiac electrical activity with implanted biotransmitters in control, acclimated, and clinically diseased frogs.

To maintain osmotic balance, amphibians must sustain a hyperosmotic internal environment

relative to the external environment (16–19). This is accomplished by tight regulation of electrolyte absorption across the epidermis, involving sodium channels and Na^+K^+ pumps (16–19). Basal electrolyte transport across the skin, measured as equivalent short-circuit current in electrophysiological tests, was lower in skin samples from diseased frogs than in those from control frogs (Fig. 2A) and was accompanied by reduced transepithelial resistance (Fig. 2B). We estimated sodium absorption as the component of the short-circuit current that was blocked by amiloride (Fig. 2E), a specific inhibitor of the sodium channel (22). The residual short-circuit current in the presence of amiloride did not differ between skin samples from diseased and control frogs (Fig. 2F), indicating that *Bd* infection predominantly inhibits sodium absorption. Additionally, responses to carbachol (Fig. 3G), which activates chloride secretion in frog skin (23), and to noradrenaline (Fig. 3H), which stimulates sodium absorption and chloride secretion (24), indicated reduced sodium and chloride channel activity in epidermis from clinically diseased frogs. The biochemical mechanisms of epidermal channel disruption are unknown but could be due

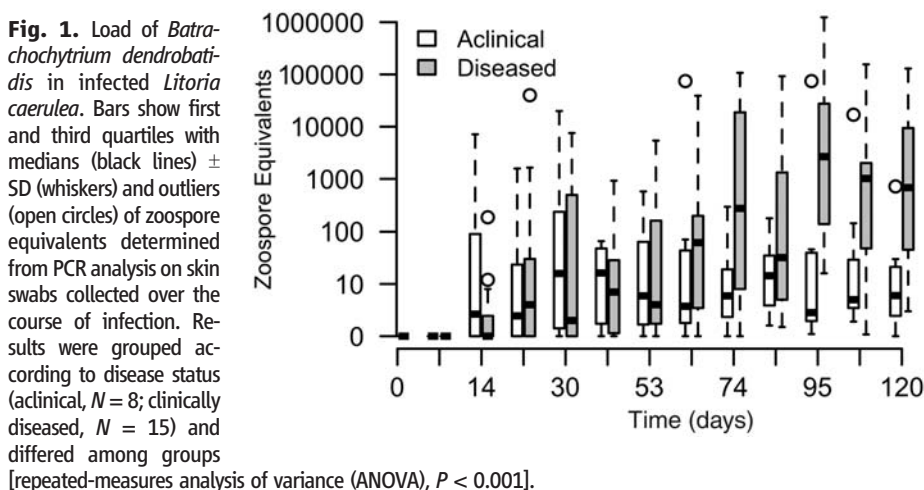


Fig. 1. Load of *Batrachochytrium dendrobatidis* in infected *Litoria caerulea*. Bars show first and third quartiles with medians (black lines) \pm SD (whiskers) and outliers (open circles) of zoospore equivalents determined from PCR analysis on skin swabs collected over the course of infection. Results were grouped according to disease status (acclinal, $N = 8$; clinically diseased, $N = 15$) and differed among groups [repeated-measures analysis of variance (ANOVA), $P < 0.001$].

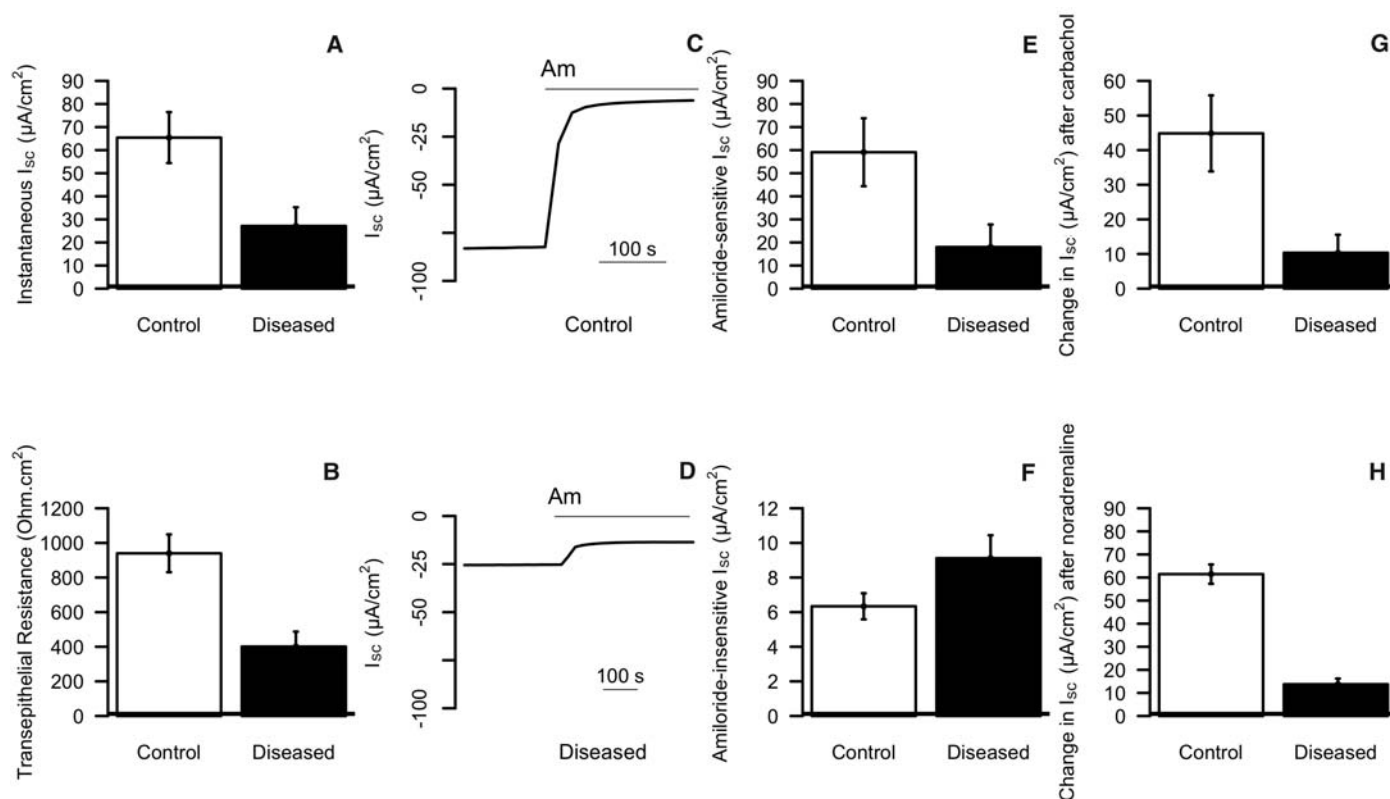


Fig. 2. Electrophysiological measurements of electrolyte transport across ventral skin samples from *L. caerulea* infected with *B. dendrobatidis*. Data in bar graphs show means (\pm SEM) from control ($N = 7$) and clinically diseased ($N = 7$) *L. caerulea*. (A and B) Instantaneous short-circuit current and transepithelial resistance were reduced in skin samples from clinically diseased *L. caerulea* [(A), Student's t test, $P = 0.009$; (B), $P = 0.006$]. (C and D) Original tracings show short-circuit current before and after blocking of sodium absorption with amiloride in skin samples from a control (C) and

clinically diseased *L. caerulea* (D). (E and F) The component of the amiloride-sensitive current (E) differed (Student's t test, $P = 0.038$) between the two groups, but amiloride-insensitive (residual) current (F) did not (Student's t test, $P = 0.79$), indicating inhibition of sodium absorption in clinically diseased *L. caerulea*. (G and H) The change in transepithelial short-circuit current was reduced in clinically diseased *L. caerulea* after treatment with carbachol [(G), Student's t test, $P = 0.015$] or noradrenaline [(H), Student's t test, $P = 0.001$].

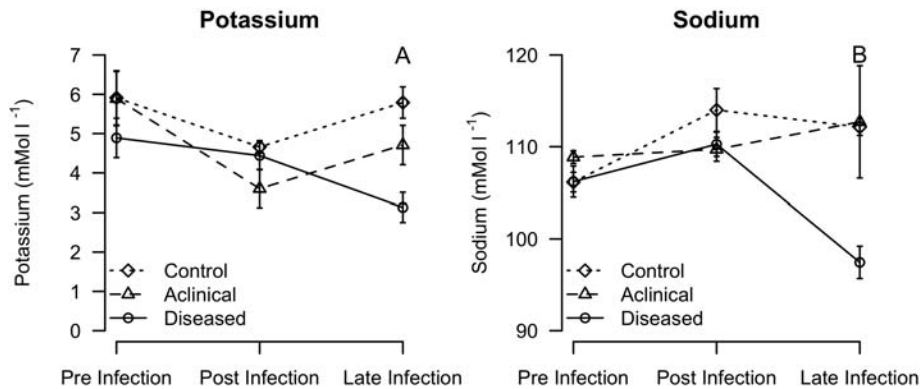


Fig. 3. Blood plasma potassium and sodium concentrations in *L. caerulea*. Blood samples were collected from infected *L. caerulea* (aclinal, $N = 7$; clinically diseased, $N = 11$; uninfected control, $N = 7$) on three sample occasions: 20 days before exposure (Pre Infection), 30 days after exposure (Post Infection), and 60 to 123 days after exposure when clinical signs of disease were obvious (Late Infection). (**A** and **B**) Data show mean (\pm SEM) concentrations of plasma potassium [(A), repeated-measures ANOVA, $P = 0.028$] and plasma sodium [(B), repeated-measures ANOVA, $P = 0.002$].

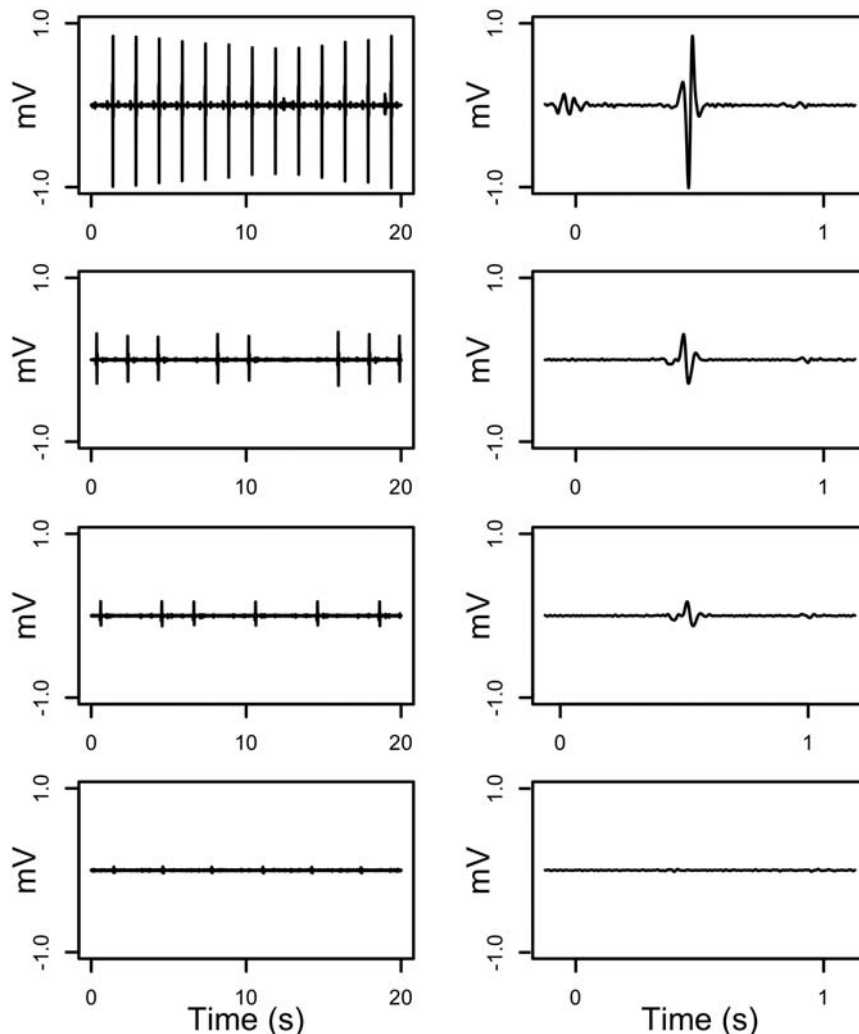


Fig. 4. Original cardiac electrogram tracings from one *L. caerulea* with clinical chytridiomycosis. Heart rate and signal amplitude were determined using 20-s sweeps (left column). Cardiac electrical signal morphology was assessed using 1-s sweeps (right column). Electrograms show cardiac electrical patterns consistent with asystolic cardiac arrest: slowing heart rate, decreasing ventricular depolarization amplitude, and increasing time of ventricular depolarization from (in order from row 1 to row 4) 18, 3, 2, and 0.5 hours before death.

to release of a fungal toxin or direct damage to infected host cells. Nonetheless, these results show that *Bd* compromises electrolyte transport, and thus osmoregulatory function, in the skin of infected *L. caerulea*.

We tested multiple blood and urine parameters as markers of organ function and general health (tables S1 and S2) and observed the greatest and most consistent changes in plasma electrolyte concentrations. Plasma sodium and potassium concentrations were significantly reduced as a result of disease when assessed according to clinical status (Fig. 3). Significant negative correlations existed between intensity of infection (*Bd* load) and the change in plasma sodium, potassium, and calcium concentrations (Pearson correlations: sodium, $r = -0.64$, $P = 0.001$, $N = 23$; potassium, $r = -0.47$, $P = 0.03$, $N = 22$; calcium, $r = -0.44$, $P = 0.04$, $N = 22$). None of the additional parameters changed significantly (tables S1 and S2). We observed no significant decrease in body masses or increases in albumin and total protein concentrations in frogs with chytridiomycosis (table S1), which suggests that there was no change in water volume. Because body mass did not change in diseased frogs, the observed osmotic imbalance most likely resulted from electrolyte loss rather than dilution caused by water uptake.

Several hours before death, the cardiac electrical activity of severely diseased frogs resembled patterns associated with cardiac standstill, also known as asystolic or bradyasystolic cardiac arrest, in other organisms including humans (Fig. 4) (25). Asystolic cardiac arrest occurs when cardiac electrical abnormalities cause contractile failure, reduced blood flow, and ultimately circulatory collapse and death. Although several conditions may initiate this cycle (25), most can be ruled out as factors in this study. Ambient temperatures remained constant, eliminating the possibility of hypothermia. Stable body mass and stable plasma protein and albumin concentrations were evidence against dehydration and hypovolemia (low blood volume). Hypoxia (low blood oxygen) was not completely ruled out, but measurements of peripheral blood oxygen saturation indicated a 20% drop in oxygen only after changes in electrical activity were observed in one individual (26). Furthermore, no changes in blood carbon dioxide were detected in a previous study (20). Thus, shifts in electrolytes and/or acidosis appear to be the most likely cause of cardiac asystolic death.

Although electrolyte imbalance, hypokalemia (low plasma potassium), and hyponatremia (low plasma sodium) could result from depletion via the epidermis or the kidney (17, 18), plasma biochemistry showed no indication of renal damage (table S1). In contrast, the skin, which regulates the bidirectional flux and overall balance of sodium and potassium (17), demonstrated inhibited sodium absorption in the ventral epidermis (Fig. 2), and histology showed degenerative epidermal changes (fig. S2). Thus,

the pathophysiology of chytridiomycosis appears to be disruption to the osmoregulatory functioning of the skin and consequent osmotic imbalance that leads to cardiac standstill.

To test whether treating electrolyte abnormalities would reduce the clinical signs of disease, we administered an oral electrolyte supplement to *L. caerulea* in the terminal stages of infection, when they lost the righting reflex and could no longer correct their body positions (26). Frogs under treatment recovered a normal posture and became more active; one individual recovered sufficiently to climb out of the water onto the container walls, and two individuals were able to jump to avoid capture. These signs of recovery were not observed in any untreated frogs. In addition, treated frogs lived >20 hours longer than untreated frogs [mean time after treatment \pm SEM: treated frogs ($N = 9$), 32 ± 2.8 hours; control frogs ($N = 6$), 10.7 ± 2.2 hours; Student's *t* test, $P < 0.001$]. All treated frogs continued to shed skin and ultimately died from the infection, as expected. It is unlikely that electrolyte treatment could prevent death unless the epidermal damage caused by *Bd* is reversed. Although amphibians can generally tolerate greater electrolyte fluctuations than other terrestrial vertebrates (18), we suggest that depletion of electrolytes, especially potassium, is important in the pathophysiology of chytridiomycosis. Amphibian plasma potassium concentrations are maintained at constant levels across seasons (27), and even moderate hypokalemia is dangerous in humans (28).

Our results support the epidermal dysfunction hypothesis, which suggests that *Bd* disrupts cutaneous osmoregulatory function, leading to electrolyte imbalance and death. The ability of *Bd* to

compromise the epidermis explains how a superficial skin fungus can be fatal to many species of amphibians; their existence depends on the physiological interactions of the skin with the external environment (16–19). Disease outbreaks capable of causing population declines require the alignment of multiple variables, including a life-compromising pathophysiology (1). Resolving the pathogenesis of chytridiomycosis is a key step in understanding this unparalleled pandemic.

References and Notes

1. P. Daszak, A. A. Cunningham, A. D. Hyatt, *Divers. Distrib.* **9**, 141 (2003).
2. F. de Castro, B. Bolker, *Ecol. Lett.* **8**, 117 (2005).
3. L. Berger *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 9031 (1998).
4. D. B. Wake, V. T. Vredenburg, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 11466 (2008).
5. H. McCallum, *Conserv. Biol.* **19**, 1421 (2005).
6. K. R. Lips *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 3165 (2006).
7. L. F. Skerratt *et al.*, *EcoHealth* **4**, 125 (2007).
8. L. M. Schloegel *et al.*, *EcoHealth* **3**, 35 (2006).
9. D. C. Woodhams, R. A. Alford, *Conserv. Biol.* **19**, 1449 (2005).
10. K. M. Mitchell, T. S. Churcher, T. W. J. Garner, M. C. Fisher, *Proc. R. Soc. London Ser. B* **275**, 329 (2008).
11. M. Schaechter, B. I. Eisensteing, G. Medoff, in *Mechanisms of Microbial Disease* (Williams & Wilkins, Baltimore, 1998), pp. 419–439.
12. J. E. Longcore, A. P. Pessier, D. K. Nichols, *Mycologia* **91**, 219 (1999).
13. L. Berger *et al.*, *Dis. Aquat. Organ.* **68**, 65 (2005).
14. D. C. Woodhams *et al.*, *Anim. Conserv.* **10**, 409 (2007).
15. E. B. Rosenblum, J. E. Stajick, N. Maddox, M. B. Eisen, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 17034 (2008).
16. H. Heatwole, in *Amphibian Biology, Vol. 1. The Integument*, H. Heatwole, G. T. Bartholomew, Eds. (Surrey Beatty, Chipping Norton, New South Wales, 1994), pp. 98–168.
17. R. G. Boutilier, D. F. Stiffler, D. P. Toews, in *Environmental Physiology of the Amphibians*, M. E. Feder, W. W. Burggren, Eds. (Univ. of Chicago Press, Chicago, 1992), pp. 81–124.

18. I. J. Deyrup, in *Physiology of the Amphibia*, J. A. Moore, Ed. (Academic Press, New York, 1964), vol. 1, pp. 251–315.
19. K. M. Wright, B. R. Whitaker, in *Amphibian Medicine and Captive Husbandry*, K. M. Wright, B. R. Whitaker, Eds. (Krieger, Malabar, FL, 2001), pp. 318–319.
20. J. Voyles *et al.*, *Dis. Aquat. Organ.* **77**, 113 (2007).
21. L. Berger, G. Marantelli, L. F. Skerratt, R. Speare, *Dis. Aquat. Organ.* **68**, 47 (2005).
22. D. J. Benos, L. J. Mandel, R. S. Balaban, *J. Gen. Physiol.* **73**, 307 (1979).
23. R. H. Alvarado, T. H. Dietz, T. L. Mullen, *Am. J. Physiol.* **229**, 869 (1975).
24. G. A. Castillo, G. G. Orce, *Comp. Biochem. Physiol. A* **118**, 1145 (1997).
25. N. A. Paradis, H. R. Halperin, R. M. Nowak, in *Cardiac Arrest: The Science and Practice of Resuscitation Medicine* (Williams & Wilkins, Baltimore, 1996), pp. 621–623.
26. See supporting material on Science Online.
27. D. R. Robertson, *Comp. Biochem. Physiol. A* **60**, 387 (1978).
28. F. J. Gennari, *N. Engl. J. Med.* **339**, 451 (1998).
29. We thank A. Hyatt and V. Olsen for assistance with PCR and S. Bell, J. Browne, S. Cashins, S. Garland, M. Holdsworth, C. Manicom, L. Owens, R. Puschendorf, K. Rose, E. Rosenblum, D. Rudd, A. Storfer, J. VanDerwal, B. Voyles, and J. Warner for project assistance and editing. Supported by Australian Research Council Discovery Project grant DP0452826, Australian Government Department of Environment and Heritage grant RFT 43/2004, and the Wildlife Preservation Society of Australia. Animals were collected with permission from Queensland Parks and Wildlife Service (scientific permits WISP03866106 and WISP04143907; movement permit WIWM04381507) and New South Wales Parks and Wildlife Service (import license IE0705693).

Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5952/582/DC1
Materials and Methods

SOM Text

Figs. S1 and S2

Tables S1 and S2

References

26 May 2009; accepted 26 August 2009
10.1126/science.1176765

Detection of an Infectious Retrovirus, XMRV, in Blood Cells of Patients with Chronic Fatigue Syndrome

Vincent C. Lombardi,^{1*} Francis W. Ruscetti,^{2*} Jaydip Das Gupta,³ Max A. Pfost,¹ Kathryn S. Hagen,¹ Daniel L. Peterson,¹ Sandra K. Ruscetti,⁴ Rachel K. Bagni,⁵ Cari Petrow-Sadowski,⁶ Bert Gold,² Michael Dean,² Robert H. Silverman,³ Judy A. Mikovits^{1†}

Chronic fatigue syndrome (CFS) is a debilitating disease of unknown etiology that is estimated to affect 17 million people worldwide. Studying peripheral blood mononuclear cells (PBMCs) from CFS patients, we identified DNA from a human gammaretrovirus, xenotropic murine leukemia virus–related virus (XMRV), in 68 of 101 patients (67%) as compared to 8 of 218 (3.7%) healthy controls. Cell culture experiments revealed that patient-derived XMRV is infectious and that both cell-associated and cell-free transmission of the virus are possible. Secondary viral infections were established in uninfected primary lymphocytes and indicator cell lines after their exposure to activated PBMCs, B cells, T cells, or plasma derived from CFS patients. These findings raise the possibility that XMRV may be a contributing factor in the pathogenesis of CFS.

Chronic fatigue syndrome (CFS) is a disorder of unknown etiology that affects multiple organ systems in the body. Patients with CFS display abnormalities in immune sys-

tem function, often including chronic activation of the innate immune system and a deficiency in natural killer cell activity (1, 2). A number of viruses, including ubiquitous herpesviruses and

enteroviruses, have been implicated as possible environmental triggers of CFS (1). Patients with CFS often have active β herpesvirus infections, suggesting an underlying immune deficiency.

The recent discovery of a gammaretrovirus, xenotropic murine leukemia virus–related virus (XMRV), in the tumor tissue of a subset of prostate cancer patients prompted us to test whether XMRV might be associated with CFS. Both of these disorders, XMRV-positive prostate cancer and CFS, have been linked to alterations in the antiviral enzyme RNase L (3–5). Using the Whittemore Peterson Institute's (WPI's) national

¹Whittemore Peterson Institute, Reno, NV 89557, USA.

²Laboratory of Experimental Immunology, National Cancer Institute–Frederick, Frederick, MD 21701, USA. ³Department of Cancer Biology, The Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, OH 44195, USA.

⁴Laboratory of Cancer Prevention, National Cancer Institute–Frederick, Frederick, MD 21701, USA. ⁵Advanced Technology Program, National Cancer Institute–Frederick, Frederick, MD 21701, USA. ⁶Basic Research Program, Scientific Applications International Corporation, National Cancer Institute–Frederick, Frederick, MD 21701, USA.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: judym@wpinstitute.org