

International Amphibian Trade & Global Pathogen Pollution



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Ranavirus is Global in Distribution

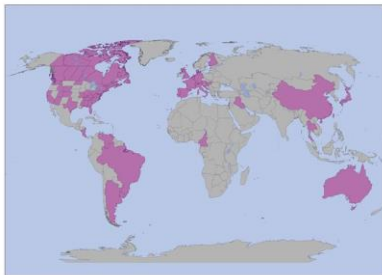
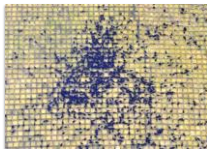


Fig. 1 Global distribution of ranavirus cases

From: Gray, M. J., and V.G. Chinchar, editors. 2015. Ranaviruses: Lethal pathogens of ectothermic vertebrates. Springer International Publishing, New York.

Characteristics of RV Spread

- **Transmission:**
 - Direct skin-skin contact
 - Indirect contact: Infectious particles shed into the environment
- **Environmental persistence:**
 - Days to weeks without a host
- **Survival Limitations:**
 - Complete drying
 - Elevated temperatures (>33C)



Possible Pathways of RV Dispersal

- Trade in live amphibians (e.g. bait trade, food, pets, etc.)
- Transport of contaminated scientific equip., recreational equip., footwear
- Water or mud spread by birds or mammals ('natural' spread?)



RV Spread by International Trade

- Ranaviral disease now listed as a globally notifiable by the World Organization for Animal Health (OIE)
- RV testing & reporting is advised
- Global trade largely continues without pathogen surveillance and regulation



International Wildlife Trade Surveillance How often do we spread RV?

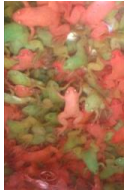


- Easy to test because:
- Frequent shipments
 - Large samples sizes
 - High species diversity
 - Both amphibian and water samples



Increased Risks of Pathogen Pollution?

- May increase pathogen spread & detectability
 - Aquatic amphibians
 - High densities
 - Stress



USA Amphibian Importation 5-year summary (2006 - 2010)

- Species diversity: 300+
- Countries of origin: 80+
- Import volume: 5 million/yr
- Shipments imported: 2,000/yr
- Declared source: wild (27%)/captive (73%)



Case Study #1: Hong Kong

- 4 Shipments sampled
- Exported from HK to USA & sampled upon arrival
- Primarily aquatic amphibians in high densities
- Cloacal swabs for RV detection



First Evidence of Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*) and Ranavirus in Hong Kong Amphibian Trade

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Table 2. Cumulative Bd and Ranavirus detection in amphibians imported from Hong Kong.

Species	Common name	# Bd	Bd+	# RV	RV+	H ₂ O Bd+	Sloughing	Ulcerations	DOA
Bombina orientalis	Oriental fire-bellied frog	56	3	13	10	-	22	0	3
Cynops orientalis	Oriental fire-bellied frog	97	0	78	60	-	7	4	15
Paramesotriton hongkongensis	Hong Kong newt	72	0	54	35	+	8	0	4
Xenopus laevis	African clawed frog	40	28	40	0	+	0	0	1
		265	31	185	105		37	4	23

Number of individuals sampled (n) for either Bd or ranavirus (RV), number of individuals testing positive by PCR (+), and presence of pathogen in water (H₂O Bd+) are expressed. Animal condition recorded upon sampling is provided, including skin sloughing, ulcerations, and the number of sampled specimens that were dead on arrival (DOA). doi:10.1371/journal.pone.0190750.t002

Results

Hong Kong Bd Swabs

Species	Shipment	Date of Import	Exporter	#/Shipment	# Bd	Bd+	# RV	RV+	H ₂ O Bd+
Cynops orientalis	1	05/16/2012	A	500	36	0	35	35	N
Paramesotriton hongkongensis	1	05/16/2012	A	1600	36	0	36	35	Y
Xenopus laevis	2	06/06/2012	B	500	N/A	N/A	N/A	N/A	Y
Cynops orientalis	3	06/06/2012	A	500	36	0	18	0	N
Paramesotriton hongkongensis	3	06/06/2012	A	1600	36	0	18	0	Y
Bombina orientalis	4	09/26/2012	A	1000	56	3 (5.4%)	13	10	N/A
Xenopus laevis	4	09/26/2012	A	1200	40	28 (70%)	40	0	Y
Cynops orientalis	5	09/26/2012	B	200	25	0	25	25	N
TOTAL				7100	265	31 (11.7%)	185	105	

1/4 shipments
2/4 species

Results

Hong Kong RV Swabs

Species	Shipment	Date of Import	Exporter	#/Shipment	# Bd	Bd+	# RV	RV+	H ₂ O Bd+
Cynops orientalis	1	05/16/2012	A	500	36	0	35	35 (97.2%)	N
Paramesotriton hongkongensis	1	05/16/2012	A	1600	36	0	36	35 (100%)	Y
Xenopus laevis	2	06/06/2012	B	500	N/A	N/A	N/A	N/A	Y
Cynops orientalis	3	06/06/2012	A	500	36	0	18	0	N
Paramesotriton hongkongensis	3	06/06/2012	A	1600	36	0	18	0	Y
Bombina orientalis	4	09/26/2012	A	1000	56	3	13	10 (76.9%)	N/A
Xenopus laevis	4	09/26/2012	A	1200	40	28	40	0	Y
Cynops orientalis	5	09/26/2012	B	200	25	0	25	25 (100%)	N
TOTAL				7100	265	31	185	105 (56.8%)	

3/4 shipments
3/4 species

Hong Kong Rapid Response Field Survey

HK Country Parks

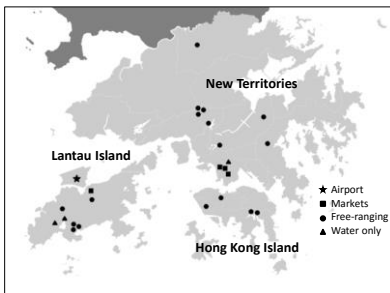
Hong Kong Urban Parks

HK pet markets



Field Sampling Distribution

Samples collected from 3 regions, 25 sites



High Risk of RV Spillover in HK

- Pet Stores: *Xenopus laevis* in containers without lids on street-side, near storm sewer (escape)
- Food Markets: Market bullfrogs suspended over large tub of freshwater fish (contamination)
- Pubic: Exotic reptiles abundant in Hong Kong parks (intentional release)



High Risk of RV Spillover: “Merit Release”

- Religious ceremony where animals are released into the wild
- Frogs typically imported & sold at wet markets
- Group of 21 dead/dying Chinese bullfrogs found in a Country Park
- Most of these tested positive for ranavirus



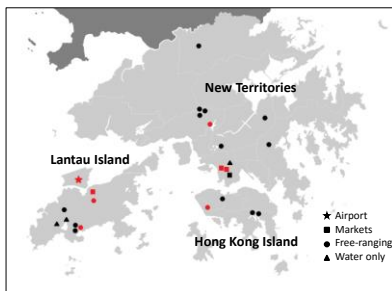
Hong Kong RV Results

409 amphibians sampled:

Source Location	RV Detected?	RV %
Airport	Yes	34.6
Domestic Markets	Yes	37.4 (97.9%*)
Wild	Yes	10.4

*46/47 Chinese bullfrogs from wet markets RV+

Ranavirus Distribution



Hong Kong Conclusions

- RV is widespread in Hong Kong
- No obvious amphibian population declines or mortality events
- Still uncertain whether RV is native or a recent introduction
- Very high-risk of introduction & spread through trade activities
- **Most RV+ amphibians were associated with trade**



Case Study #2: Madagascar



Fig. 1 Global distribution of ranavirus cases

From: Gray, M. J., and V. G. Chinchin, editors. 2015. Ranaviruses: Lethal pathogens of ectothermic vertebrates. Springer International Publishing, New York.

Case Study #2: Madagascar

- 1 shipment: 9 spp; 625 amphibians
- Species targeted for Bd detection
- DOA animals frozen, subset sampled for RV by EcoHealth Alliance



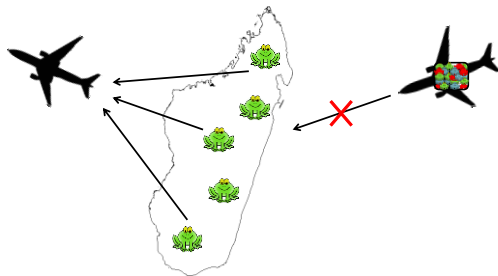
Madagascar Trade RV Results

Species	# Sampled	# RV+
<i>Boophis pyrthus</i>	9	5
<i>Boophis rappiodes</i>	1	0
<i>Boophis microtypanum</i>	6	2
<i>Heterixalus alboguttatus</i>	6	6
<i>Heterixalus betsileo</i>	1	1
<i>Dyscophus guineti</i>	2	2
<i>Scaphiophryne boribory</i>	3	1
<i>Scaphiophryne madagascariensis</i>	1	1
	29	18

•18/29 RV+ dead amphibians (62.1%)
 •8/9 species sampled

But Wait!

RV Presence but no Amphibian Importation?



Madagascar Rapid Response Field Survey



- Sample Sites:
- Twelve regions, 47 sites
 - Elevation range: 10 – 2400m
 - Urban, wilderness, & trade facilities

Survey Species

- Targeted species & life stages associated with water



Sample Types



Results



RESEARCH ARTICLE
 Rapid Response to Evaluate the Presence of Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*) and Ranavirus in Wild Amphibian Populations in Madagascar



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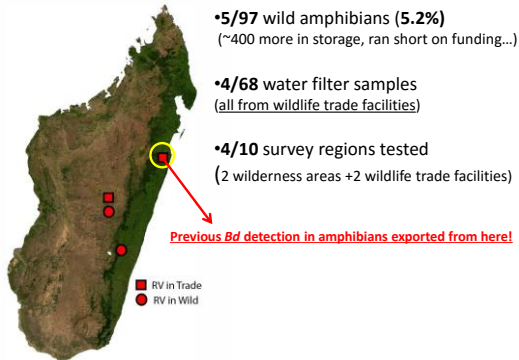
Citation: Koehler JE, Smith KM, Ramirez SD, Rakotonjaniana F, Pearce AP, Brannon JL, et al. (2016) Rapid Response to Evaluate the Presence of Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*) and Ranavirus in Wild Amphibian Populations in Madagascar. PLoS ONE 11(3): e0153335. doi:10.1371/journal.pone.0153335

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Abstract

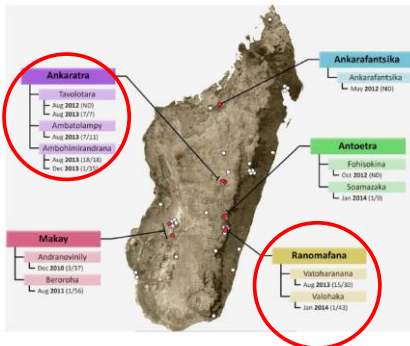
We performed a rapid response investigation to evaluate the presence and distribution of amphibian pathogens in Madagascar following our identification of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, Bd) and ranavirus in commercially exported amphibians.

Ranavirus Detection



Bd & Ranavirus Co-infection?

Bletz et al. 2015 (Bd) + Kolby et al. 2015 (RV)



Amphibian Mortality Event

- Samples tested negative for both Bd & ranavirus
- Cause of death unknown...dont't jump to conclusions!



Potential Pathway of RV Introduction Asian Toad Invasion

- Invasive amphibian species spreading in Madagascar right now
- Possible introduction of RV and Bd?



Asian Toad (*Duttaphrynus melanostictus*) in Toamasina, Madagascar

Madagascar Conclusions

- **Like HK:** RV seems widespread & no obvious disease-associated mortality
- **Unlike HK:** Absence of amphibian trade did NOT prevent RV introduction
- Critically endangered reptiles in Madagascar warrant RV surveillance



Ploughshare tortoises (*Astrochelys yniphora*) at the Durrell Chelonian Captive Breeding Center

Case Study #3: Dominican Republic

- Source of farmed American bullfrogs to USA
- Frogs farmed en masse in outdoor enclosures
- 8% RV previously detected in USA markets (Schloegel et al. 2009)



Schloegel, L. M., A. M. Picco, A. M. Kilpatrick, A. J. Davies, A.D. Hyatt and P. Deszak. 2009. Magnitude of the U.S. trade in amphibians and presence of *Batrachochytrium dendrobatidis* and ranavirus infection in imported North American bullfrogs (*Rana catesbeiana*). *Biological Conservation* 142:1420-1426.

Sampling Methods

- 3 shipments sampled
- 35 animals per shipment
- Bd: skin swabs
- RV: cloacal swabs



Results: Bd & RV Swabs

Shipment#	Origin	Qty/ship	# Bd	# Bd +	# RV	# RV +
1	Dominican Republic	3325	33	21 (63.6%)	35	29 (82.9%)
2	Dominican Republic	4470	34	31 (91.2%)	35	33 (94.3%)
3	Dominican Republic	2280	35	8 (22.9%)	35	35 (100%)
TOTAL		12075	102	60 (58.8%)	105	97 (92.4%)

High prevalence of both Bd & RV in bullfrog shipments
 58.8% vs 62% Bd prevalence in bullfrog markets in the USA
 &
 92.4% vs 8% RV prevalence (Schloegel et al. 2009)

Bullfrog Escape & Pathogen Spillover



- Holes in bags allowed frogs to escape into boxes
- 1 damaged box allowed frogs to escape outside when unloaded from plane
- Release or escape likely to spread both RV & Bd

Overall Conclusions

- Globalization drives international pathogen pollution
- RV+ amphibians are commonly traded (both in domestic & international markets)
- Absence of wildlife trade does NOT prevent RV spread
- Biosecurity is necessary to control spread of Rv+ animals AND of RV-contaminated materials (water, soil, amphibian hitch-hikers, etc.)
- Translocation of RV strains & species continues to threaten global biodiversity

Acknowledgements

- United States Fish & Wildlife Service
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- National Geographic Society
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- World Wide Fund for Nature- Hong Kong
- Madagascar's Chytrid Emergency Cell
- IUCN SSC Amphibian Specialist Group-MG
- Durrell Wildlife Conservation Trust-MG
- Malagasy Direction Generale Des Forets and to Madagascar National Parks
- Amphibian Disease Lab- San Diego Zoo Institute for Conservation Research
- Washington State University



"It seems to me that if you wait until the frogs and toads have croaked their last to take some action, you've missed the point."
 ~Kermit the Frog

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