

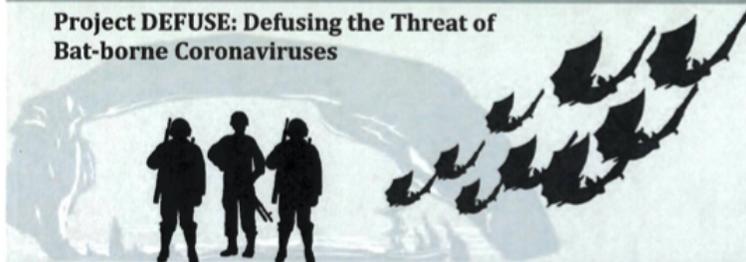
# DRASTIC RESEARCH

## Project DEFUSE

DARPA - PREEMPT (HR001118S0017)

**PROPOSAL: VOLUME I**  
DARPA - PREEMPT (HR001118S0017)  
LEAD ORGANIZATION: EcoHealth Alliance (Other Nonprofit)  
OTHER TEAM MEMBERS:  
Duke NUS Medical School (Other Educational)  
University of North Carolina (Other Educational)  
Wuhan Institute of Virology (Other Educational)  
USGS National Wildlife Health Center (Other Nonprofit)  
Palo Alto Research Center (Large Business)

**Project DEFUSE: Defusing the Threat of  
Bat-borne Coronaviruses**



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Identifying Number: HR001118S0017-PREEMPT-PA-001  
Award Instrument Requested: Grant  
Places and Periods of Performance: 12/1/18 - 5/31/22; Palo Alto, CA; Kunming and  
Wuhan, China; Chapel Hill, NC; New York, NY; Singapore; Madison, WI  
Total funds requested: \$14,209,245  
Proposal validity period: 6 months  
Date proposal submitted: 3/27/18

Documents made available by an anonymous source

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## CONTEXT & SUMMARY

These leaked documents describing bat research proposed by EcoHealth Alliance should be considered in light of the following context:

On August 27<sup>th</sup>, 2021, the US intelligence community issued a 502-word summary of the conclusions drawn up by the joint investigation ordered by President Biden in late May. Conspicuously absent from the brief statement were any indications that the evidence presented in testimony to Congress had been part of the intelligence community analysis - at least not in the unclassified version that was released.

The lifting of the gain-of-function (GOF) moratorium in late 2017, via the Potential Pandemic Pathogen Care and Oversight framework (P3CO), has allowed GOF research with SARS-like coronaviruses to resume with very few practical limits. In particular the absence of clear definitions of GoF, creative interpretations of the guidelines, and rather discretionary decisions to refer research projects or not, all contributed to reducing the effectiveness of the P3CO framework - despite the fact that other agencies of the US federal government actively maintained the GOF standards.

DRASTIC recently became aware of documents which show that EcoHealth Alliance (EHA) in concert with the WIV were looking towards implementing an advanced human pathogenicity BatCoV research project that clearly qualifies as GoF, in a grant proposal submitted to a funding proposal call by the Defense Advanced Research Projects Agency (DARPA) in the spring of 2018. The EHA / WIV proposal (named 'DEFUSE') was ultimately rejected for full funding (but leaving open the door for partial funding), in part because it skirted the GOF guidelines.

In other words, a branch of the federal government had already judged aspects of EHA's research, and the corresponding shared research plan with the WIV, as falling under the definition of GOF, only for HHS to approve similar work **without P3CO review** in 2018 and 2019. In particular, the P3CO framework was designed to allow greater flexibility for vaccine development, and in June of 2018 the NIH's Vaccine Research Center (VRC) expanded its existing partnership with Moderna to include full-scale research into a pan-coronavirus (CoV) vaccine platform. EcoHealth Alliance repeatedly took advantage of this flexibility to continue their work with the Wuhan Institute of Virology

DRASTIC has reviewed the contents of these documents. They detail past achievements and planned experiments in collaboration with researchers from the Wuhan Institute of Virology (WIV), East China Normal University (ECNU), UNC-Chapel Hill, Duke-National University in Singapore, the USGS National Wildlife Health Center (NWHC) and Palo Alto Research Center (PARC).

The grant proposal includes some elements of research that are already public via scientific papers, as well as other elements that have never been made public; these include vaccinating wild bats using aerosolized viruses and further work on published and unpublished CoV strains that could fill the extant gaps in our understanding of the origins of SARS-CoV-2.

These grant proposal documents also show a staggeringly deep level of involvement of EHA with the WIV, on matters of national interest (such as DURC), for instance by proposing that the DARPA grant pays a good chunk of key WIV researchers salaries, or that some of these WIV researchers should be invited to DARPA headquarters in Arlington. All the while without proper risk assessment and considerations for ethical and social issues and with an incorrect evaluation of what constitutes

## KEY DOCUMENTS

### DARPA PREEMPT program (HR00111880017):

- Brief introduction': <https://www.darpa.mil/program/preventing-emerging-pathogenic-threats>
- Press release: <https://www.darpa.mil/news-events/2018-01-04> ([archived version](#))
- Grant Opportunity: <https://www.grants.gov/web/grants/view-opportunity.html?oppld=300198>
- Copies of some of the main Grant Opportunity files: <https://bit.ly/39yeFNj>
- Description of the Selected teams: <https://www.darpa.mil/news-events/2019-02-19>  
The teams are led by (1) Autonomous Therapeutics, Inc., (2) Institut Pasteur, (3) Montana State University, (4) The Pirbright Institute and (5) the University of California, Davis.  
Most of the teams are made of US, UK or Australian partners, plus one partner in Estonia (Tartu) and the Institut Pasteur network in Asia. None of the teams include Chinese partners.
- Examples of funded PREEMPT projects:
  - o \$9.37mIn [award for UCDavis](#) team PREEMPT project
  - o Montana State University team PREEMPT project.
- 

### EHA “DEFUSE” proposal to DARPA PREEMPT:

- **D1:** 75 page 'PROPOSAL: Volume I'
- **D2:** 8-page budget
- **D3:** Summary of Rejection Letter (DARPA): <https://bit.ly/3Eyv82f>
- **D4:** Executive Slides

**Note:** page numbers are given as the n<sup>th</sup> page in the corresponding PDF document. Hence (D1, p.10) means 10<sup>th</sup> PDF page of document D1.

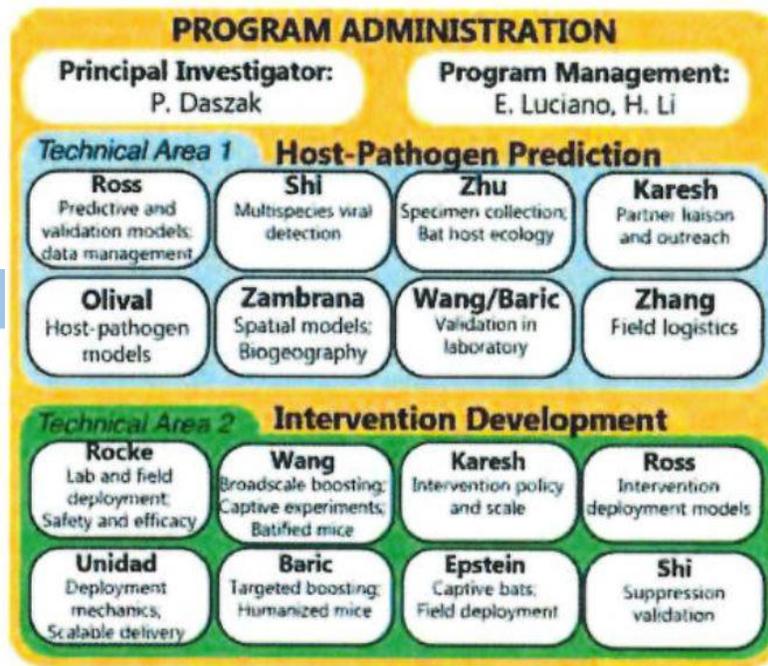
## TIMELINE

- 2014 May 27 Award to EHA of [NIH R01AI110964 grant](#) (“Understanding the Risk of Bat Coronavirus Emergence”) for 5 years (June 2014 - May 2019) - with subgrants to WIV, East China Normal University (Shanghai) and (starting year 3) Wuhan University.<sup>1</sup>
- WIV: ~\$695,000  
East China N. Uni: ~\$259,000  
Wuhan University: ~\$442,000  
(all direct costs over 5 years, see pages 5 and 189 of [grant](#))
- Early 2017: China sets up its [own version of DARPA](#), under the Central Military Commission.
- 2018 Jan 4 [Launch](#) of DARPA Preventing Emerging Pathogenic Threats (PREEMPT) program.
- 2018 Jan 19 [Notice](#) (BAA) for proposals for PREEMPT Program HR001118S0017, with a span of 3.5 years (Dec 18 - May 22).
- 2018 Jan 19 [Cables](#) from U.S. Embassy health and science officials about lack of trained personnel as the WIV P4 and the potential danger of newly found BatCoVs that can directly infect humans.
- 2018 Mar 27 EHA fills a proposal for DARPA PREEMPT project under HR001118S0017 by stated deadline: Project DEFUSE (Defusing the Threat of Bat-Borne Coronaviruses) for a requested amount of \$14,209,245 (over 3.5 years).
- 2018 ?? **Rejection** of EHA project DEFUSE due to important concerns
- 2018 Nov Application for renewal of NIH R01AI110964 grant “Understanding the Risk of Bat Coronavirus Emergence’ ([p. 316 of grant](#)) - with subgrants to WIV (inc. Wuhan University work), Institute of Pathogen Biology (IPB, Beijing), East China Normal University (Shanghai)
- WIV: ~\$353,000  
IPB: ~\$350,000  
East China N. Uni: ~\$368,000  
(all direct costs over 5 years - in particular see p. 420 for East China Normal University via the consultancy of Dr Guangjian Zhu)
- 2019 Jul 24 [Fast renewal](#) of NIH R01AI110964 grant grant for 5 years (June 2019 - May 2024)
- 2020 Apr 24 Suspension of NIH R01AI110964 grant
- 2020 Jul 8 Reinstantiation of NIH R01AI110964 grant - but all activities are suspended until [NIH conditions](#) are satisfied (see also p 320 of [grant](#)).
- 2020 Aug 20 New 5-year grant for ‘Understanding Risk of Zoonotic Virus Emergence in EID Hotspots of Southeast Asia’ (excluding China)

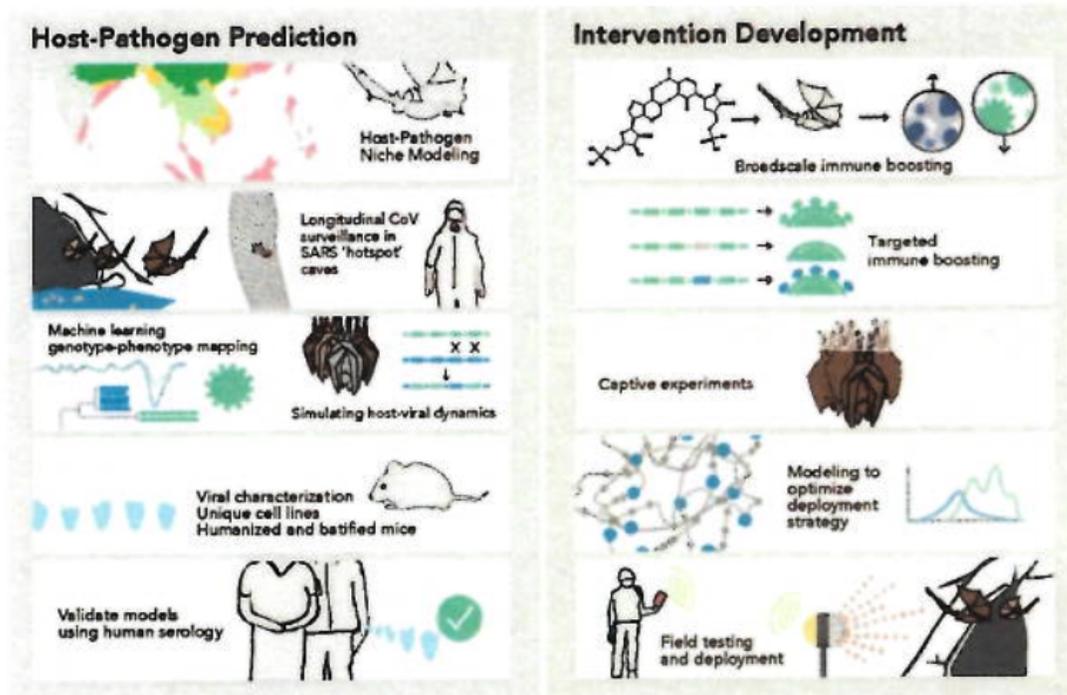
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<sup>1</sup> As noted in the [Reinstantiation](#) letter sub-wards were not (and are likely still not) correctly reported by EHA in the Federal Subaward Reporting System. The numbers above are compiled from a careful reading of the grant documents [recently disclosed](#) via an FOI request.

# 'DEFUSE' PROPOSAL OVERVIEW



## CONCEPT



# FINDINGS

## 1. ECOHEALTH ALLIANCE (EHA) TRIED TO BYPASS THE P3CO/DURC FRAMEWORKS

EHA confidently assessed in its proposal that the work to be carried was neither subject to P3CO (GoF) nor DURC (Dual Use Research of Concern) restrictions:

*“These QSo strain viral spike glycoproteins will be synthesized, and those binding to human cell receptor ACE2 will be inserted into SARSr-CoV backbones (non-DURC, non-GoF).” (D1, p.6)*

The paragraph above actually contains the only mentions of GoF and DURC in the whole DEFUSE project proposal - and it dismisses them.

Nevertheless the DARPA review of the DEFUSE project concluded that the project potentially involved GoF. This was part of the reasons for the rejection of the project as such, and of a qualification for any partial funding:

*“Given the team's approach does potentially involve GoF/DURC research (they aim to synthesize spike glycoproteins that may bind to human cell receptors and insert them into SARSr-CoV backbones to assess capacity to cause SARS-like disease), if selected for funding an appropriate DURC risk mitigation plan should be incorporated into contracting language that includes a responsible communications plan”*

Effectively EHA unsuccessfully proposed the use of bat-SARSr-CoV backbones and not the human evolved SARS-CoV in what looks like a deliberate attempt at circumnavigating the restrictions of the [P3CO framework and related DURC restrictions](#).

*“An enhanced PPP is a PPP resulting from the enhancement of a pathogen's transmissibility and/or virulence. Wild-type pathogens that are circulating in or have January 9, 2017 2 been recovered from nature are not enhanced PPPs, regardless of their pandemic potential.*

Source: <https://www.phe.gov/s3/dualuse/Documents/P3CO-FinalGuidanceStatement.pdf>

We also know from emails FOI'd by USRTK that Jonathan Epstein asked on the 23<sup>rd</sup> March for help from Ralph Baric for some wording for a section in the proposal that would address ‘*communicating dual-use information*’:

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**To:** Baric, Ralph S[rbaric@email.unc.edu]  
**From:** Jon Epstein[epstein@ecohealthalliance.org]  
**Sent:** Fri 3/23/2018 6:54:18 PM (UTC-04:00)  
**Subject:** dual use safety language

Hi Ralph,  
DARPA wants a written section on communicating dual-use information. Do you have some written text you could send me:

A communication plan that addresses content, timing, and the extent of distribution of potentially sensitive dual-use information. The plan must also address how input from DARPA, other government, and community stakeholders will be taken into account in decisions regarding communication and publication of potentially sensitive dual-use information.

Cheers,  
Jon

That email request for help was sent 4 days before the deadline for submitting the proposals (27<sup>th</sup> Mar 2018), which suggests that little attention was being paid to this question. Not surprisingly, the review of the proposal effectively concluded that no satisfying DURC language had been included:

*“.. if selected for funding an appropriate DURC risk mitigation plan should be incorporated into contracting language that includes a responsible communications plan”*

## 2. EHA WOULD HAVE USED US TAXPAYER MONEY TO PAY SHI ZHENGLI AT HALF TIME AND PENG ZHOU AND BEN HU AT QUARTER TIME

EHA DEFUSE proposed to have Shi Zheng Li work half time (8h/day, 22d/month) for the two first years on the project, with Peng Zhou and Ben Hu as 1/4<sup>th</sup> of their time, all drawing salaries through the DARPA grant (D2, p.8).

We note that, by contrast, the previous grant NIH R01AI110964 did not provide for a salary to Shi Zheng Li. See p. 71 of the previous [grant](#): “Dr. Shi will not take salary on this grant and is funded by discretionary sources at her Institute.”

## 3. EHA DEFUSE WOULD HAVE INVITED SHI ZHENG LI TO A PROJECT KICKOFF AT DARPA HEADQUARTERS

As per D2, top of page 4, the budget of EHA DEFUSE contains an entry for an invitation of Shi Zheng Li and one key WIV personnel (likely Peng Zhou or Ben Hu) to a kickoff meeting at the DARPA headquarters in Arlington, TX.

## 4. EHA ‘HAD’ 3 KEY CAVE SITES IN YUNNAN FOR SARS-R COV COLLECTION

Three caves in Yunnan Province are specified as of particular importance:

*“Our strategy begins by a complete inventory of bats and their SARSr-CoVs at **our intervention test site cave complex in Yunnan, China** that harbours bats with high-risk SARSr-CoVs. We will collect data from three caves in that system (one is our intervention test site and two control sites) on: monthly bat abundance and diversity, viral prevalence and diversity, individual bat viral load and host physiological markers; and genomic characterization of low- and high-risk SARSr-CoV strains among bat species, sexes, and age classes; satellite telemetry and mark-recapture data on bat home range and inter-cave movement; and monitoring of daily, weekly and seasonal changes in bat populations.”* (D1, p.5)

*“However, **our test cave site in Yunnan Province**, harbours a quasispecies (QS) population assemblage that contains all the genetic components of epidemic SARS-CoV<sup>34</sup>, We have isolated three strains there (WIV1, WIV16 and SHCO14) that unlike other SARSr-CoVs, do not contain two deletions in the receptor-binding domain (RBD) of the spike, have far higher sequence identity to SARS-CoV (**Fig. 1**), use human ACE2 receptor for cell entry, as SARS-CoV does (**Fig. 2**), and replicate efficiently in various animal and human cells.”* (D1, p.7-8)

## 5. EHA PLANNED TO INOCULATE WILD BATS WITH AEROSOLIZED VACCINES

The proposal for **wide scale inoculation of bats in the wild** using aerosolized inoculum delivery has never been publicly released or opened to the wider scientific community for discussion as to potential risks associated with this plan.

This is a specialist area of research of Dr. Rocke, Dr. Ainslie and Dr. Unidad (PARC) who have previously researched and developed the technological solutions necessary to make this possible:

**Dr. Jerome Unidad** is a researcher PARC, (2018a) at PARC (owned by Xerox) (PARC, 2018d), who developed the Filament Extension Atomizer (FEA) (PARC, 2019). This technology is used to spray bats with scalable high viscosity mists that stick to their skin or that are edible (PARC, 2018c).

PARC previously partnered with NWHC to develop a vaccine for White Nose Syndrome (WNS) for US bats, using FEA as the technological solution to administer vaccines via aerosol delivery (PARC, 2018b).

**Dr. Tonie Rocke** is a researcher at USGS National Wildlife Health Centre (NWHC in the DEFUSE proposal). She has previously worked on transdermal application of vaccines against rabies in vampire bats “The feasibility of controlling rabies in vampire bats through topical application of vaccines” (USFWS, 2019), also “Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoon pox vectored rabies vaccine in the Brazilian Free-tailed bat” (Stading et al., 2016). There were doubts and concerns about her work:

*“These vaccine candidates use a viral vector (attenuated raccoon poxvirus, RCN) genetically modified to express highly-conserved fungal and specific Pd antigens. While these vaccines and other potential treatments continue to be developed, there is a need for safe and effective methods of treatment delivery”* (USFWS, 2019).

Another similar project:

*“We recently developed a new recombinant rabies vaccine specifically for bats with available sequences from the rabies Phylogroup I glycoprotein. This sequence was cloned into raccoon pox virus (RCN) and the efficacy of this novel RCN-MoG vaccine was tested in big brown bats. Field studies are currently being conducted in Peru and Mexico to test the feasibility of oral and topical delivery of vaccine and transfer rates between vampire bats using biomarker-labelled jelly (without vaccine)”* EEFMVZ (2021).

**Dr. Ainslie** is a Professor at the UNC Department of Biomedical Engineering and the UNC Department of Microbiology and Immunology (Pharmacy UNC, 2021), who works on new polymers for vaccines and electrospray for fabrication of immune targeting microparticles (nanoparticles).

Her publications include “Historical Perspective of Clinical Nano and Microparticle Formulations for Delivery of Therapeutics” (Batty et al., 2021), “Electrospray for generation of drug delivery and vaccine particles applied in vitro and in vivo” (Steipel et al., 2019). “Injectable, Ribbon-Like Microconfetti Biopolymer Platform for Vaccine APPLICATIONS” (Moore et al., 2020), “Considerations for Size, Surface Charge, Polymer Degradation, Co-Delivery, and Manufacturability in the Development of Polymeric Particle Vaccines for Infectious Diseases” (Genito et al., 2020).

One may contrast this fairly aggressive approach with the one described in a recent paper on ‘[Self-disseminating vaccines to suppress zoonoses](#)’. There the recommended approach is to start with captive animals then carefully “perform releases within carefully isolated populations in semi-natural enclosures or on small islands”. More generally [concerns](#) have been raised about such self-disseminating vaccines.

## 6. THE PROPOSAL DOES NOT PROPERLY DISCUSS ETHICAL, LEGAL AND SOCIAL ISSUE

The proposal has about 22 lines on Ethical, Legal and Social Issues (ELSI), most rather vague. Or even rather odd, such as when mentioning ‘*common practice of bat-consumption*’ in Yunnan when bat-consumption is actually not common at all in Yunnan (if it ever occurs), with also a mention of ‘cultural leaders’, which may suggest some hasty editing based on a the text for a similar project in South-East Asia:

*“We will conduct educational outreach to local wildlife authorities and cultural leaders so that there is a public understanding of what we are doing and why we are doing it, particularly because of the common practice of bat-consumption in the region.”* (D1, p.36)

Also worth noting is the mention that ‘*The broader societal impact of this project could be significant, as wildlife immunization against viral zoonoses has been limited to date*’ without further proper consideration (D1, p.36).

The ‘PREEMPT Risk Mitigation Plan’ section seems to suffer from another bad case of hasty editing with a minimalistic 2-line ‘*Risks to the general public section*’ interrupted in mid-air:

**Risks to general public:** The proposed work has minimal risk to the general public, as sampling will be done near the cave sites and not in populous areas. Our team has extensive experience

Full extent of ‘*Risk to general public*’ section (D1, p.34)

## 7. EHA WANTED TO OVERSEE ALL WORK IN CHINA

The PREEMPT proposal to DARPA relied on trusting EHA (a private NGO) for oversight of high risk pathogen research:

*“The lead organisation, EcoHealth, Alliance will oversee all work.”* (D1, p.3)

*“Dr. Shi, Wuhan Institute of Virology will conduct viral testing on all collected samples, binding assays and some humanized mouse work.”* (D1, p.3)

## 8. LIVE BATS WERE MEANT TO BE USED AT THE WIV AND VARIOUS INTERNATIONAL LABS FOR INFECTION EXPERIMENTS, OFTEN USING CAPTIVE BAT COLONIES

**WIV** (Shi) was to work on *Rhinolophus* bats:

*“At WIV, 20 adult wild **Rhinolophus spp.** bats (10 of each sex) will be captured at our test cave site, housed within ABSL3, ACE2 receptor genes sequenced and used to pre-screen spikes as above, then bats will be tested using PCR and serology for current and prior exposure to SARSr-CoVs, and inoculated with WIV1, WIV16 or SHC014.”* (D1, p.20).

*“to Dr. Shi, Wuhan Inst. Virol., to conduct PCR testing, viral discovery and isolation from bat samples collected in China, spike protein binding assays, humanized mouse work, and experimental trials on *Rhinolophus* bats.”* (D1, p.25).

*“Subtask 7.5 Test targeted immune boosting in wild-caught captive Rhinolophus spp: (WIV).” (D1, p.30).*

The WIV was not the only institution meant to work with live bats for infection experiments within its labs. As the proposal explains:

*“Experimental work using bats and or transgenic mice will be conducted at the BSL-3 lab in WIV, Duke-NUS, UNC, or NWHC. Each partner institute will apply for and procure animal research approval from its respective IACUC. All animal work conducted by EcoHealth Alliance in China will be overseen by both the IACUC at WIV and the IACUC at Tufts” (D1, p.35).*

**Duke-NUS** (Linfa Wang) has an Asian cave bat (*Eonycteris spelaea*) breeding colony:

*“Our E. spelaea colony has now reached a sustainable population for infection experiments and the ABSL3 facility has been outfitted with bat-specific cages.” (D1, p.20).*

*“We will use the unique Duke-NUS Asian cave bat (Eonycteris spelaea) breeding colony to conduct initial proof-of-concept tests, extended to small groups of wild-caught Rhinolophus sinicus bats at WIV.” (D1, p.6-7)*

*“Subtask 7.4. Test immune modulation in ‘captive Eonycteris sp. colony, using Malaka virus and SARSr-CoV infections. (Duke-NUS).” (D1, p.30)*

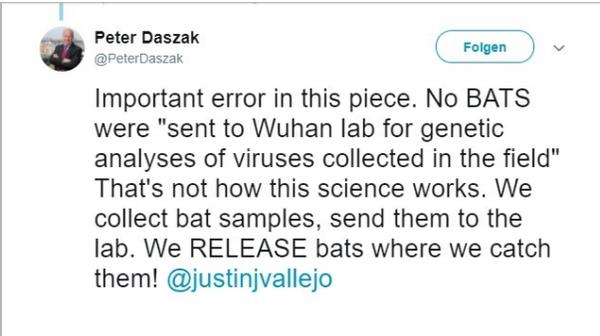
**NWHC** (Rocke) has a captive bat colony colony:

*“We will use the NWHC captive bat colony and wild bats in US caves to trial delivery vehicles using the biomarker rhodamine B (which fluorescently marks hair on consumption) to assess uptake.” (D1, p.7)*

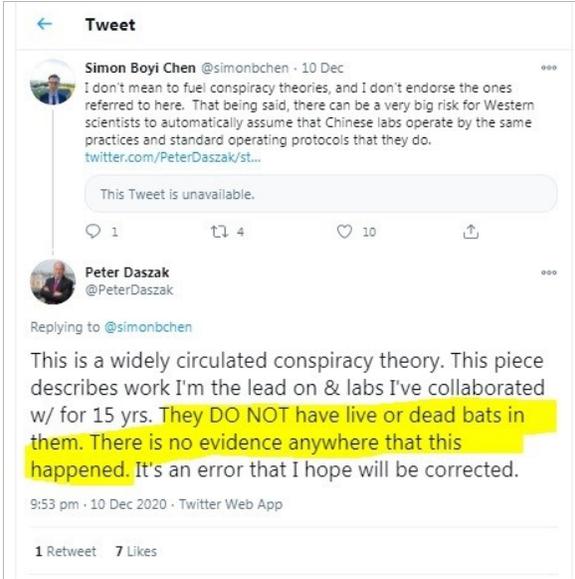
**CSIRO** (Australia, with Linfa Wang at the time) and **University of Queensland** were already using or are planning to be using live bats for experiments

*“Previous infection studies were completed in Pteropus and Rhinolophus bats in Australia by L-F Wang at CSIRO, AAHL and an additional Pteropus infection trial is currently planned through the University of Queensland in Australia.” (D1, p.20)*

As DRASTIC has previously discovered, the WIV was already keeping wild-caught bats more than a decade ago, and established a colony around 2017 - as has been attested by patents, official records and videos (see <https://bit.ly/3pxL8lR>). These revelations have been regularly characterised as ‘conspiracy theories’ by Peter Daszak (see examples in Bostickson & Ghannam, 2021c; Taiwan News, 2021; Sky News, 2021).



Screenshot: Peter Daszak Denies WIV keeps Live Bats. Source: Twitter via Taiwan News (2021).



Screenshot: Peter Daszak Denies WIV keeps Live Bats. Source: Twitter via Taiwan News (2021).

**9. EHA PROPOSED MULTIPLE, REGULAR VISITS TO 3 YUNNAN CAVE SITES**

*“In phase I will sample 60 bats each of R. sinicus, R. ferrumequinum, and R. affinis, (180 bats per cave) every three months non-destructively for 18 months from our three cave sites.” (D1, p.9)*

*“We will conduct pre- and post-intervention sampling (biweekly faecal pellet sampling for 4 months, and 10 male and 10 female bats per species tested every 2 weeks post-intervention for 4 months, prior to- and post-deployment) to monitor SARSr-CoV QS and bat immune status changes in test and control site bats during Phase I (TA2).” (D1, p.9)*

**10. EHA PLANNED TO SEND SAMPLES TO DUKE UNIVERSITY (SINGAPORE) AND UNC CHAPEL HILL**

The proposal states that:

*“Samples will be preserved in viral transport medium, immediately frozen in liquid nitrogen dry shippers, and transported to partner laboratories with a maintained cold chain and under strict biosafety protocols.” (D1, p.9)*

This is further confirmed by items 37 and 38 on page 5 of D2 (Budget).

Incidentally we also know from the recently released documents for the [NIH R01AI110964 grant](#) (“Understanding the Risk of Bat Coronavirus Emergence”) that EHA has plenty of experience shipping samples from and to China.

[grant, p. 15:](#)

*“This gives us unique access to working on-the- ground in countries where surveillance is difficult, such as China, **where our group has proven capacity to export samples from.**”*

p. 141:

*“Drs Shi, Zhang, and Daszak have collaborated together since 2002 and have been involved in running joint conferences, **and shipping samples into and out of China.**”*

This practically means that it is likely that Duke (Singapore) and UNC Chapel Hill have undocumented samples that could help trace the origins of SARS-CoV-2.

## 11. THE PROPOSAL SET A CLEAR PATHWAY FOR CHIMERIC VIRUS CONSTRUCTION

The use of known backbones is specified in the proposal:

*“Synthesis of Chimeric Novel SARSr-CoV QS: We will commercially synthesize<sup>2</sup> SARSr-CoV S glycoprotein genes, designed for insertion into SHC014 or WIV16 molecular clone backbones (88% and 97% S-protein identity to epidemic SARS-Urbani). These are BSL-3, not select agents or subject to P3CO (they use bat SARSr-CoV backbones which are exempt) and are pathogenic to hACE2 transgenic mice.” (D1, p.9)*

However we do not know what additional, unpublished SARS-r CoV and MERS-r CoV research was conducted by the WIV, Wuhan University and other Chinese institutions. Indeed, using analysis of raw metagenomic datasets, unpublished MERS-r CoV infectious clone research in Wuhan has recently been documented (Zhang *et al.* 2021).

## 12. EHA HAS 180 UNPUBLISHED SARSr-CoV STRAINS

*“This will be supplemented by characterization of isolated viruses under DEFUSE (at WIV), approximately 15-20 bat SARSr-CoV spike proteins/year (at UNC, WIV), and >180 bat SARSr-CoV strains sequenced in our prior work and not yet examined for spillover potential.” (D1, p.12)*

Very little of this planned work has been published.

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<sup>2</sup> As per budget (D2, p.5, item 13) the primer synthesis was to be done by [Sangon Biological Engineering Technology & Services](#) (Shanghai).

### 13. ALL CORONAVIRUSES WERE TO BE SCREENED AT THE WIV

*“We will conduct in vitro pseudovirus binding assays, using established techniques<sup>2</sup>, and live virus binding assays (at WIV to prevent delays and unnecessary dissemination of viral cultures) for isolated strains.” (D1, p.12)*

### 14. THREE TO FIVE CHIMERIC CORONAVIRUSES WERE TO BE CREATED PER YEAR

*“We will validate results from chimeric viruses by re-characterizing full-length genome versions, testing whether backbone genome sequence alters full length SARSr-CoV spillover potential. QS for full-genome characterization will be selected to reflect strain differences in antigenicity, receptor usage, growth in human cells and pathogenesis.” (D1, p.13)*

*“We will test growth in primary HAE cultures and in vivo in hACE2 transgenic mice. We anticipate recovering ~3-5 full length genome viruses/year.” (D1, p.13)*

### 15. THE PROPOSAL PLANNED TO IDENTIFY “KEY MINOR DELETIONS” IN THE RECEPTOR BINDING DOMAIN (RBD) TO ALTER HUMAN PATHOGENICITY

*“Testing Synthetic Modifications:*

*“We will synthesize QS with novel combinations of mutations to determine the effects of specific genetic traits and the jump potential of future and unknown recombinants.*

*RBD deletions:*

*Small deletions at specific sites in the SARSr-CoV RBD alter risk of human infection. We will analyze the functional consequences of these RBD deletions on SARSr-CoV hACE2 receptor usage, growth in HAE cultures and in vivo pathogenesis.”*

*(D1, p.13)*

### 16. THE PROPOSAL INCLUDES THE INTRODUCTION OF “HUMAN-SPECIFIC CLEAVAGE SITES”

Human protease-specific site insertion was proposed. The proposal does not specify exactly which protease, but does discuss Furin in the preceding text.

*“We will analyze all SARSr-CoV S gene sequences for appropriately conserved proteolytic cleavage sites in S2 and for the presence of potential Furin cleavage sites<sup>74,75</sup>.”*

*SARSr-CoV S with mismatches in proteolytic cleavage sites can be activated by exogenous Trypsin or Cathepsin L.*

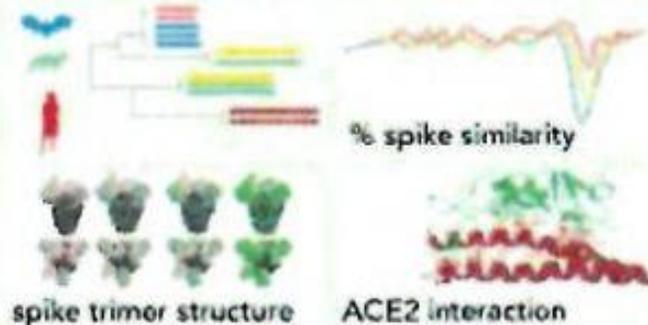
*Where clear mismatches occur, we will introduce appropriate human-specific cleavage sites and evaluate growth potential in Vero cells and HAE cultures.”*

*(D1, p.13)*

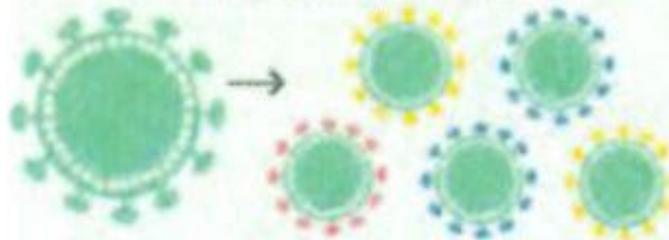
**Predicting SARSr-CoV QS jump potential  
Screen and isolate SARSr-CoV QS<sub>0</sub>**



**Select QS<sub>0</sub> with human infection potential**



**Construct chimeric viruses**



**Evaluate expression in vitro and vivo**



**Input data for predictive modeling**

Furin recognition cleavage motifs are widely used in laboratory research. Furin is an endoprotease which cleaves proteins at a specific motif (RxxR|x) which for virus envelope glycoproteins, can enhance viral fusion with host cell membranes (Coutard *et al.*, 2020).

For SARS-CoV-2 the Furin cleavage site (FCS) has been shown to be key for pathogenicity (Bestle *et al.* 2020; Hoffmann *et al.* 2020; Johnson *et al.*, 2021).

No other sarbecovirus subgenus CoV including SARS-CoV possesses a Furin cleavage site, and as Furin cleavage sites have previously be inserted into coronaviruses in laboratories to increase

tropism and pathogenicity (Cheng et al. 2019), the origin of the FCS has been widely debated (Wade 2021). Wu and Zhao (2021) propose the FCS arose through natural insertion.

Segreto and Deigin (2020) note the “CGGCGG” coding for the two leading arginines is rare for bat origin coronaviruses and note a FCS restriction site just upstream of a leading proline and propose the fcs could have been inserted in a laboratory.

Kaina (2021) proposes that in vitro recombination in human cell culture of a SARS-CoV-2 progenitor with a virus containing the Furin cleavage site as a possible source; Segreto *et al.* (2021) propose laboratory insertion of a more potent motif and insect cell culture passage to generate the “RRAR” FCS sequence.

Given that we find in this EHA proposal, a discussion of the planned introduction of human-specific cleavage sites into novel SARS-r CoVs, a review by the wider scientific community of the plausibility of artificial insertion of an FCS into SARS-CoV-2 or a progenitor is warranted.

## **17. THE PROPOSAL PLANNED TO “INTRODUCE” NATURALLY OCCURRING PROTEOLYTIC CLEAVAGE SITES TO CREATE NOVEL CORONAVIRUSES**

The proposal planned to introduce ‘wild type’ proteolytic cleavage sites from high risk strains into more abundant low risk strains, presumably to increase the pathogenicity of the low risk strains:

*“We will also review deep sequence data for low abundant high risk SARSr-CoV that encode functional proteolytic cleavage sites, and if so, introduce these changes into the appropriate high abundant, low risk parental strain.” (D1, p.13)*

## **18. THE PROPOSAL PLANNED TO RESEARCH ALTERNATE RECEPTORS TO ACE2**

*“To evaluate this, we will sequentially introduce clade 2 disrupting residues of SARS-CoV and SHCO14 and evaluate virus growth in Vero cells, non-permissive cells ectopically expressing DC-SIGN, and in human monocytes and macrophages anticipating reduced virus growth efficiency.” (D1, p.13)*

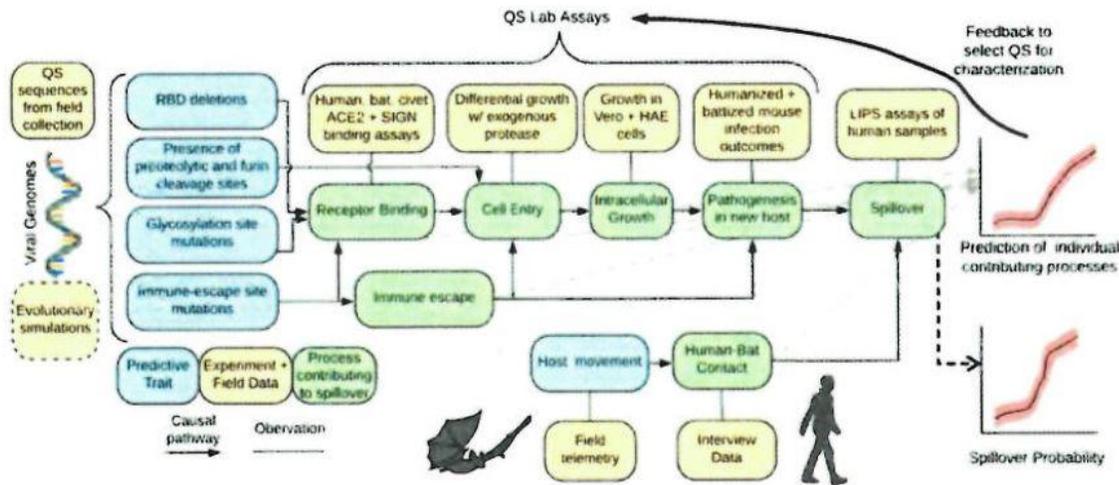
We note that while SARS-CoV was documented to use DC-SIGN as an attachment receptor (Marzi *et al.* 2004), L-SIGN and DC-SIGN act as entry receptors for SARS-CoV-2 (Amraei *et al.* 2020; Thépaut *et al.* 2021).

## **19. THE PROPOSAL PLANNED TO INTRODUCE “KEY RBD RESIDUES” INTO LOW RISK STRAINS TO TEST PATHOGENICITY IN HUMAN AIRWAY-CELLS AND IN hACE2 MICE**

*“Low abundance micro-variations:*

*We will structurally model and identify highly variable residue changes in the SARSr-CoV S RBD, use commercial gene blocks to introduce these changes singly and in combination into the S glycoprotein gene of the low risk, parental strain and test ACE2 receptor usage, growth in HAE and in-vivo pathogenesis”.*

*(D1, p.13)*



**Fig. 7:** A simplified directed graph of a Bayesian network model representing the causal relationships between input data, modeled processes, and outputs.

## 20. THE PROPOSAL “SPOILOVER PROBABILITY” ASSESSMENT DOES NOT INCLUDE LAB-RESEARCH RELATED RISKS

Figure. 7 below (D1, p.14) documents an extensive sampling, and lab experimentation plan. It is quite astounding that the spillover probability calculation incorporates multiple ‘processes contributing to spillover’, all of which require extraction of viruses from bats and experimentation in laboratories, yet does not incorporate a risk factor for field sampling and laboratory experimentation to deliberately increase the pathogenicity of SARS-r CoVs.

## 21. A POTENTIALLY HIGHLY IMPORTANT “SPIKE PROTEIN DATASET” WAS NOT PUBLIC

It is not clear what that ‘dataset of S protein sequences from prior work’ refers to and whether EHA has ever made it public (as is generally required under its grant conditions).

*“We will use a large dataset of S protein sequences and full-length genomes generated from prior work and DEFUSE fieldwork to estimate SARSr-CoV substitution rate and its genome-wide variation.” (D1, p.15)*

## 22. EHA PROPOSED MERS-CORONAVIRUS EXPERIMENTS AND HAD ALREADY INTRODUCED SARS AND MERS INTO BAT CELL LINES

*“First, we will take wing punch biopsies from 3 individuals to sequence their ACE2 receptor gene. This will be inserted into human cell lines to pre-screen viral strains for binding. Those that bind will be used for in vivo experiments. We will use two coronaviruses (SARSr-CoV WIV1 and MERS-CoV) in ABSL3. SARS and MERS infection studies are already underway in Eonycteris and Pteropus cell lines and primary immune cells .“ (D1, p.20)*

Primary cells can also harbour latent viruses that can become reactivated during in vitro cultivation when the cells are outside the host and isolated from other components of the immune system that would otherwise control virus replication” Banerjee *et al.* (2018).

In fact, experiments using non-immune bat cell lines were hallmarked by “subversion of the bat immune system” which contrasts with the effective clearance of bat viruses shown by in vivo captive bat studies into Marburg and Ebola Viruses carried out at Atlanta CDC BSL4, for example (Jones *et al.*, 2015; Schuh *et al.*, 2017a & 2017b).

Research into bat immune systems using bat cell lines at WIV however did not mirror results from in vivo studies, and it was precisely this feature of non-immune bat cell lines that led researchers to create the first bat bone marrow-derived dendritic immune cells (Zhou *et al.*, 2016), bat-mouse bone marrow chimera (Yong *et al.*, 2018) and IFNAR2 knockout bat cell lines using CRISPR/Cas9 technology (Zhang *et al.*, 2017).

These novel cell lines and bat immune system mice were proposed to ensure that in vivo bat cell line and in vivo mouse experiments effectively mirrored in vivo bat immune system response to clearing of viruses (Zhou *et al.*, 2016; Yong *et al.*, 2018).

However, this also suggests that unknown or undetected highly pathogenic bat viruses would have been able to replicate clandestinely in WIV bat cell lines as they would not have been constrained by a bat immune system, and in turn, this may have led to contamination of operators and equipment at the WIV BSL2 Laboratories (Bostickson & Ghannam, 2021c).

## 23. EHA PROPOSED A DATABASE OF ALL FIELD, LAB AND MODELLING WORK

“Data Management and Sharing:  
*EcoHealth Alliance will maintain a central database of data collected and generated via all project field, laboratory, and modelling work.” (D1, p.25)*

This would indicate that EHA has similar databases relating to earlier projects - data that it would have not shared publicly.

## 24. ECOHEALTH ALLIANCE PROPOSED INDUSTRIAL SCALE BAT SAMPLING

### **“Sub-Task 1.2**

*Collect monthly specimens from bats at cave sites in Yunnan, China for SARSr-CoV screening and sequencing. Oral, fecal, and blood sample collected from 360 Rhinolophus spp. bats per month using live- capture and non-invasive sampling. Specimens shipped to laboratory for analysis. Associated morphological, demographic, and physiological data for individual bats collected (EHA, consultant Zhu).” (D1, p.25)*

### **“Deliverables:**

*Specimens from 3,240 bats and fecal pellets collected from high-risk reservoir*

*populations which have been obtained with all proper permits and permissions and shipped to WIV for analysis; real-time telemetry and mark-recapture data uploaded and made available to DARPA collaborators; completed database maintained.” (D1, p.25)*

## 25. ECOHEALTH ALLIANCE MISLED DARPA ABOUT RISKS TO GENERAL PUBLIC

EHA writes about ‘Risks to general public’ section:

### **“Risks to general public:**

*The proposed work has minimal risk to the general public, as sampling will be done near the cave sites and not in populous areas. Our team has extensive experience. ”*

*(D1, p.35)*

That EHA could propose the identification and selection of highest risk SARS-r CoV’s, chimeric CoV construction, serial passage using transgenic hACE2 mice and insertion of human adapted cleavage sites (presumably furin cleavage sites), yet totally ignore the risks of laboratory escape is inconceivable and shows a total lack of understanding of the risks of SARS-r CoV (and MERS-r CoV) laboratory research (Demaneuf 2020).

## 26. EHA PROPOSED TO GENERATE “BATIFIED MOUSE MODELS”

*“We have shown efficient reconstitution of **irradiated mice using bat bone marrow from multiple species, including E. spelaea** (Fig. 10), including reconstitution of bat PBMC’s in the mouse, presence of circulating bat cells and generation of bat-specific antibodies in mice incapable of producing an antibody response.*

***This ‘batified’ mouse model** can be utilized for both circulating infection of SARS-CoV (in the immune compartment only) and as a model for generating bat-specific antibodies against CoV proteins.”*

*(D1, p.18)*

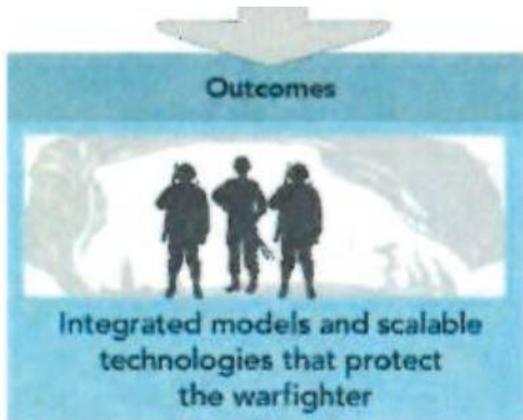
*“PI-TA-02 Task 7: Experimental testing. of ‘Broadscale Immune Boosting’ using **batified mice and captive bat colonies** (Duke-NUS).”*

*(D1, p.30)*

## QUESTIONS TO ECOHEALTH ALLIANCE

Here are some questions that journalists may like to ask EcoHealth Alliance and Peter Daszak. These questions could also be asked under a subpoena process instigated by Congress.

1. Why does EHA refer to one test-site cave and two control caves as “our cave test sites” (D1, p.3 & 5), “*our cave complex*” (D1, p.32; D2, p.3), as “*our three test cave sites*” (D4, slide 2) in Yunnan?
2. Does EHA and the WIV actually own the cave or control access to it?
3. Is EHA trying to suggest that DARPA has to fund EHA if they want to do research that includes these promising sites?
4. Does EHA have an actual evaluation of the ‘*clear and present danger to US defense forces defenses in the region*’ represented by these Yunnan viruses it is focussing on in the proposal (D4, slide 3)?
5. *In particular, can EHA explain under which scenarios this may affect “US warfighters”, given that “Security concerns across Asia make the region a potential deployment site for US warfighters.”? (D4, slide 3).*



6. Was this valuable aspect of the work clearly understood by the Chinese parties to the DEFUSE project?
7. Why did Peter Daszak firmly deny WIV kept live bats when the DEFUSE proposal requires the WIV to do so, and to have the necessary experience to do so - in full agreement with the ample evidence provided [independently by DRASTIC](#)?
8. Why did Peter Daszak deny that keeping live bats was common practice in laboratories, when the DEFUSE proposal shows that many of your partner labs keep bat colonies or wild-caught bats?
9. EHA referred to Eco Health Alliance (EHA) databases in the Proposal with obligation of submission by their Chinese partners. Can EHA share those databases publicly?

10. What does the proposal contain no risk mitigation program at all for DURC (Dual Use Research of Concern) despite aiming to address 'clear and present dangers' to the US 'war-fighters' deployed in Asia, while proposing to hire and pay Shi Zheng Li half-time for two years, a well as Ben Hu and Peng Zhou and other WIV laboratory technicians and employees?
11. Did Scientists at WIV have or see a copy of this DEFUSE proposal and if so, what was the extent of the contribution of the WIV in drafting this proposal?
12. Did Peter Daszak discuss the Proposal with Scientists at WIV, when, who, how, where?
13. Please summarise any discussions with WIV Scientists, ZLS, PZ, BH, regarding proposed experiments and bat sampling.
14. Did you discuss the question of sending live Rhinolophus bats to WIV with Dr. Zhengli Shi or any other WIV Scientists?
15. Page 38 of the DEFUSE Proposal (D1) refers to the introduction of "*appropriate human specific cleavage sites*". Can you explain that in more detail?
16. Can you confirm whether or not any Chinese Scientists have done the sampling / protein S / recombination and animal testing work mentioned in the DEFUSE Project proposal?
17. What are the 180 Coronavirus sequences that resulted from the mentioned prior work by EcoHealth Alliance?  
  
*"180 bat SARSr-CoV strains sequenced in our prior work and not yet examined for spillover potential"*  
(D1, p.7)
18. Why were laboratory accident risk (in a foreign country where EHA could only have limited oversight at best) totally ignored in the proposal?
19. What previous experiments did EHA or its collaborators carry out on irradiated "batified mice models" (D1, p.18)? When, where and for what purpose?
20. The proposal reviewer wrote that "*there are several components of great interest in this proposed effort that are potentially fundable should additional funding become available*".  
Did additional funding become available?
21. Can Dr. Rocke, Dr. Unidad and Dr. Ainslie confirm that they were aware of the DEFUSE project proposal and did they help draft the proposal?
22. Can Dr. Rocke, Dr. Unidad and Dr. Ainslie guarantee that no partial funding was made available after rejection of the DEFUSE proposal, and none of their technology was subsequently used by EHA or WIV or other collaborators in this proposal?
23. Outside of NIH/NIAID the WIV with CAS funding conducted a 2018.01-2021.12 [project](#) studying the evolutionary mechanisms for SARSr-CoV host receptor adaptations and cross-species infection risk (another link [here](#) and [here](#)). Does EHA have any further information as to what work was conducted and any results that could help shed light on the origin of SARS-CoV-2?.

24. Why was discussion of Yunnan miners whose illness was consistent with infection with a SARS-like illness (Rahalkar and Bahulikar, 2020) not discussed in this proposal, especially given the mention of *'Test previously-collected human sera from Yunnan Province to assess SARSr-CoV QS spillover'* (D2, p.3)?
25. Was the Mojiang mine part of 'your' Yunnan cave complex that DEFUSE proposed to use for experiments?
26. Did EHA know of the theses by Xu and Huang (see Rahalkar and Bahulikar, 2020) and the conclusions therein that the miners likely were infected with a SARS-r CoV?
27. If so, did EHA deliberately withhold this information so that the SARS-r CoV's in the Mojiang mine would not be subject to P3CO restrictions?
28. Why does the proposal fail to contain any reference to Regulatory and ELSI (Ethical, Legal, Social) issues, especially given its real-life deployment on Yunnan bat colonies?
29. Would EHA have also ignored Regulatory and ELSI issues if they had planned to deploy immune boosting solutions on bat colonies in Texas?

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