

March 25, 2019

Katherine Roe, Ph.D.
Research Associate
Laboratory Investigations Department
People for the Ethical Treatment of Animals
501 Front Street, Norfolk, VA 23510

Dear Dr. Roe:

Thank you for your letter dated March 15, 2019 to Dr. Anthony Fauci and myself. On behalf of Dr. Fauci, and in my role as the Director of the Division of Extramural Activities at NIAID, I am pleased to respond.

West Nile Virus (WNV) was introduced into the Western Hemisphere during the late summer of 1999, when infected individuals were diagnosed in New York State, and spread rapidly throughout North America. From 1999 to 2018, more than 2.5 million people have been infected, with over 22,000 reported cases of encephalitis or meningitis and over 2,200 deaths. WNV is maintained in nature in a cycle between mosquitoes and animal hosts with the predominant and preferred reservoir being birds. Birds of some species become ill, show symptoms of disease, and may die, while others become infected and serve as carriers without showing signs of disease. Understanding the details of transmission between mosquitoes and different species of birds is therefore critical in predicting and controlling outbreaks in humans.

NIAID supports Dr. Ebel to carry out important research on WNV transmission. Dr. Ebel's research is focused on how different birds, mosquitoes and transmission intensities influence WNV genetic diversity within hosts, not, as you suggest, to develop treatments for WNV that can be used in a clinical setting. His studies have clearly demonstrated that different transmission ecologies can have distinct consequences for the viruses that perpetuate within them. He has demonstrated that in wild birds, the degree of WNV diversification is positively associated with bird susceptibility to WNV-induced mortality: disease-resistant American robins imposed stronger selection for WNV fitness compared to disease-susceptible American crows. The significance of this work is that it provides novel data on how different transmission cycles can impact virus genetics, and how this can lead to the emergence of new virus strains. Dr. Ebel's studies have also enabled a better understanding of the transmission dynamics of WNV within and between wild bird populations and the impact of the virus on bird health, as well as factors that may impact transmission of virus from infected birds to humans. This research cannot be conducted by studying WNV replication in cell culture or by *in silico* studies. While it may be true that *in silico* models of WNV biology exist, the algorithms that form the basis for these models can only be developed and validated by data from experiments with live animals such as that collected by Dr. Ebel.

In short, Dr. Ebel's research has increased our understanding, at a mechanistic level, of the fundamental population and evolutionary processes that act on WNV as it cycles between different host environments. Critically, this work has contributed to, and in some cases guided, the development of key technical and computational approaches to quantifying viral population diversity and fitness.

As to whether Dr. Ebel may have engaged in the trapping of crows and other birds without securing the necessary licenses from the Colorado Department of Parks & Wildlife, the Office of Laboratory Animal Welfare (OLAW) investigated the issue and the status of Institutional Animal Care and Use Committee

approval of the activities. OLAW determined that CSU is operating in compliance with the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (Policy) and the Animal Welfare Assurance and that the research conducted under this grant complies with the IACUC approved protocol. The use of sparrows in this study was approved by the IACUC and valid permits are in place for 2019 for the field activities. There have been no concerns associated with this project according to the CSU IACUC. That being said, there was an inadvertent lapse in approval of the Colorado Scientific Collection License for 2018 and 37 crows were collected during that time. All other compliance requirements (federal permit, IACUC approval) were in place. Colorado Parks and Wildlife was notified by the Principal Investigator once the error was recognized and the state agency took no further action. CSU is reconciling grant expenditures associated with the activities conducted during the permit lapse as required by NIH grants policy.

NIH-supported research must be compliant with the provisions of the PHS Policy. As part of receiving this grant, Colorado State University (CSU) accepts the responsibility of complying with all applicable assurances and certifications required for the conduct of the proposed research.

Sincerely,

(b) (6)



Matthew J. Fenton, Ph.D.
Director, Division of Extramural Activities
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane, MSC 9824
Bethesda, MD 20892-9824

cc: Dr. Axel Wolff, OLAW

Wolff, Axel (NIH/OD) [E]

From: Wolff, Axel (NIH/OD) [E]
Sent: Thursday, March 21, 2019 11:50 AM
To: 'gregory.ebel@colostate.edu'; 'Mark.zabel@colostate.edu'
Cc: (b) (6)
Subject: FW: Attached Image
Attachments: 1138_001.pdf

Hello Drs. Ebel and Zabel,

As I shared with Drs. Zabel and (b) (6) NIH/NIAID received a complaint from PETA and shared it with OLAW (see attached). Dr. Fauci has placed the matter on a fast track and requested OLAW to look into the matter. I am currently just gathering facts and do not need a response through the IO or formal reply. From what Dr. Zabel shared this morning, wildlife capture permits were in place for robins and crows for 2017 and 2019. Sparrows were added to the protocol and approved by the IACUC and were covered by a 2019 permit.

What I need to specifically know is whether the field activities were conducted in 2018 when the permit was not in place. Please provide as much information regarding this issue as possible. Dr. Fenton, Director of the Division of Extramural Affairs, is working on a response and needs as much accurate information as possible to address the PETA complaint. The scientific aspects NIH is responding to are coming together well, but the issue of permits still needs to be resolved.

I am open to a conference call with you all and possibly including the OLAW Director, Dr. Patricia Brown. Let me know if this is desirable and convenient times.

Please respond as soon as possible so this can be resolved quickly and without any negative impacts on the grant.

Thanks.

Axel Wolff, M.S., D.V.M.
Deputy Director, OLAW
Office of the Director, NIH
301-594-2061

gregory, zabel @ colostate.edu
(b) (6)

Wolff, Axel (NIH/OD) [E]

From: Wolff, Axel (NIH/OD) [E]
Sent: Thursday, March 21, 2019 10:17 AM
To: 'Zabel, Mark'
Subject: RE: OLAW question

Mark. Zabel @ colostate.edu

Thanks for your response, Dr. Zabel. I am gathering facts to be sure that NIH has the correct information in addressing the complaint. So, if the PI failed to renew in 2018 was field work conducted without a permit in place? If so, this would also violate the terms and conditions of the grant application and could lead to some repercussions. (b) (6) thought that work may have been conducted under another investigator's permit, which I think is acceptable.

The other answers are positive and require no further input.

Just let me know what happened in 2018 and if there were any consequences from the permitting agency if wild bird work was conducted in the absence of a valid permit being in place.

Axel Wolff

From: Zabel, Mark <Mark.Zabel@colostate.edu>
Sent: Thursday, March 21, 2019 10:06 AM
To: Wolff, Axel (NIH/OD) [E] <wolffa@od.nih.gov>
Cc: (b) (6)
Subject: Re: OLAW question

Hello Dr. Wolff,

Thanks for your email and my apologies for our late reply. We are currently drafting a formal response to your questions, but here they are, briefly:

1. Is a state or federal permit required for work with robins and crows? If so, was one in place to cover the field work? Federal permit has been in place. A state permit was issued in 2017 and 2019. The PI failed to renew in 2018.
- 2.
2. The complaint states that sparrows are also going to be used. If so, has this been approved by the IACUC prior to beginning the work? Sparrows are approved on the protocol.
3. If sparrows are to be used, is a permit necessary? If so, is this in place or will it be obtained before work begins? Sparrows were caught in 2019 under the state permit.
4. Have there been any concerns brought to the IACUC regarding this project? None

We hope this helps and will be sending a formal letter shortly.

Sincerely,

Mark Zabel

Chair, Institutional Animal care and Use Committee

Professor, Department of Microbiology, Immunology and Pathology
College of Veterinary Medicine and Biomedical Sciences
Colorado State University, 1619 Campus Drive
Fort Collins, CO, 80521-1619, USA
office: (b) (6) cell: (b) (6)
fax: (b) (6)

On Mar 20, 2019, at 12:20 PM, Wolff, Axel (NIH/OD) [E] <wolffa@od.nih.gov> wrote:

Hello Dr. Zabel,

NIH/NIAID has received a complaint from PETA and has shared it with OLAW. It concerns PI Greg Ebel who is working with wild caught birds and West Nile Virus. PETA alleges that the PI failed to obtain the necessary wildlife permits for this work. Please let me know the following:

1. Is a state or federal permit required for work with robins and crows? If so, was one in place to cover the field work?
2. The complaint states that sparrows are also going to be used. If so, has this been approved by the IACUC prior to beginning the work?
3. If sparrows are to be used, is a permit necessary? If so, is this in place or will it be obtained before work begins?
4. Have there been any concerns brought to the IACUC regarding this project?

Let me know the answers as soon as possible to allow NIH to respond. If you'd like to discuss by telephone, I am available.

Thank you in advance.

Axel Wolff, M.S., D.V.M.
Deputy Director, OLAW

Wolff, Axel (NIH/OD) [E]

From: Wolff, Axel (NIH/OD) [E]
Sent: Thursday, March 21, 2019 1:00 PM
To: 'Zabel,Mark'
Cc: Ebel,Greg; (b) (6)
Subject: RE: Attached Image

Thank you for your work on this. I will indicate to NIAID that the requested information will be available by tomorrow, 3/22.

Axel Wolff

From: Zabel,Mark <Mark.Zabel@colostate.edu>
Sent: Thursday, March 21, 2019 12:38 PM
To: Wolff, Axel (NIH/OD) [E] <wolffa@od.nih.gov>
Cc: Ebel,Greg <Gregory.Ebel@colostate.edu>; Zabel,Mark <Mark.Zabel@colostate.edu>; (b) (6)

(b) (6)

(b) (6)

Subject: Re: Attached Image

Hi Axel,

We are currently reaching out to Dr. Ebel to clarify the information you requested for 2018. We expect to respond to you very soon today.

Thanks for your patience,

M

On Mar 21, 2019, at 9:50 AM, Wolff, Axel (NIH/OD) [E] <wolffa@od.nih.gov> wrote:

Hello Drs. Ebel and Zabel,

As I shared with Drs. Zabel and (b) (6) NIH/NIAID received a complaint from PETA and shared it with OLAW (see attached). Dr. Fauci has placed the matter on a fast track and requested OLAW to look into the matter. I am currently just gathering facts and do not need a response through the IO or formal reply. From what Dr. Zabel shared this morning, wildlife capture permits were in place for robins and crows for 2017 and 2019. Sparrows were added to the protocol and approved by the IACUC and were covered by a 2019 permit.

What I need to specifically know is whether the field activities were conducted in 2018 when the permit was not in place. Please provide as much information regarding this issue as possible. Dr. Fenton, Director of the Division of Extramural Affairs, is working on a response and needs as much accurate information as possible to address the PETA complaint. The scientific aspects NIH is responding to are coming together well, but the issue of permits still needs to be resolved.

I am open to a conference call with you all and possibly including the OLAW Director, Dr. Patricia Brown. Let me know if this is desirable and convenient times.

Please respond as soon as possible so this can be resolved quickly and without any negative impacts on the grant.

Thanks.

Axel Wolff, M.S., D.V.M.
Deputy Director, OLAW
Office of the Director, NIH

(b) (6)

<1138_001.pdf>

Wolff, Axel (NIH/OD) [E]

From: Zabel,Mark <Mark.Zabel@colostate.edu>
Sent: Thursday, March 21, 2019 2:03 PM
To: Wolff, Axel (NIH/OD) [E]
Cc: Ebel,Greg; (b) (6) Rudolph,Alan;
(b) (6)
Subject: Re: Attached Image

Hi Alex,

Thanks for your patience. Dr. Ebel spoke with us. Here's his response with regards to "What I need to specifically know is whether the field activities were conducted in 2018 when the permit was not in place. Please provide as much information regarding this issue as possible"

—response: "Field activities were conducted in 2018; specific to your request, the collection of 37 crows occurred under an expired Colorado Scientific Collection License. All other compliance requirements (federal permit, IACUC approval) were in place. Colorado Parks and Wildlife was notified by Dr. Ebel once the error was recognized. CPW has not followed up with Dr. Ebel. We are working on obtaining associated research and costs specific to the 37 birds collected during this permit lapse. Please let us know if you need any additional information."

We hope this helps. Please reply to this email if we can help further.

Sincerely,

M

Mark Zabel

Chair, Institutional Animal care and Use Committee
Professor, Department of Microbiology, Immunology and Pathology
College of Veterinary Medicine and Biomedical Sciences
Colorado State University, 1619 Campus Deliver
Fort Collins, CO, 80521-1619, USA
office: (b) (6) cell: (b) (6)
fax: (b) (6)

On Mar 21, 2019, at 10:59 AM, Wolff, Axel (NIH/OD) [E] <wolffa@od.nih.gov> wrote:

Thank you for your work on this. I will indicate to NIAID that the requested information will be available by tomorrow, 3/22.

Axel Wolff

From: Zabel,Mark <Mark.Zabel@colostate.edu>
Sent: Thursday, March 21, 2019 12:38 PM
To: Wolff, Axel (NIH/OD) [E] <wolffa@od.nih.gov>
Cc: Ebel,Greg <Gregory.Ebel@colostate.edu>; Zabel,Mark <Mark.Zabel@colostate.edu>; (b) (6)

(b) (6)

Subject: Re: Attached Image

Hi Axel,

We are currently reaching out to Dr. Ebel to clarify the information you requested for 2018. We expect to respond to you very soon today.

Thanks for your patience,

M

On Mar 21, 2019, at 9:50 AM, Wolff, Axel (NIH/OD) [E] <wolffa@od.nih.gov> wrote:

Hello Drs. Ebel and Zabel,

As I shared with Drs. Zabel and (b) (6) NIH/NIAID received a complaint from PETA and shared it with OLAW (see attached). Dr. Fauci has placed the matter on a fast track and requested OLAW to look into the matter. I am currently just gathering facts and do not need a response through the IO or formal reply. From what Dr. Zabel shared this morning, wildlife capture permits were in place for robins and crows for 2017 and 2019. Sparrows were added to the protocol and approved by the IACUC and were covered by a 2019 permit.

What I need to specifically know is whether the field activities were conducted in 2018 when the permit was not in place. Please provide as much information regarding this issue as possible. Dr. Fenton, Director of the Division of Extramural Affairs, is working on a response and needs as much accurate information as possible to address the PETA complaint. The scientific aspects NIH is responding to are coming together well, but the issue of permits still needs to be resolved.

I am open to a conference call with you all and possibly including the OLAW Director, Dr. Patricia Brown. Let me know if this is desirable and convenient times.

Please respond as soon as possible so this can be resolved quickly and without any negative impacts on the grant.

Thanks.

Axel Wolff, M.S., D.V.M.
Deputy Director, OLAW
Office of the Director, NIH

(b) (6)

<1138_001.pdf>

Zabel

(b) (6)

Wolff, Axel (NIH/OD) [E]

From: Wolff, Axel (NIH/OD) [E]
Sent: Wednesday, March 20, 2019 2:20 PM
To: 'Mark.Zabel@colostate.edu'
Subject: OLAW question

Hello Dr. Zabel,

NIH/NIAID has received a complaint from PETA and has shared it with OLAW. It concerns PI Greg Ebel who is working with wild caught birds and West Nile Virus. PETA alleges that the PI failed to obtain the necessary wildlife permits for this work. Please let me know the following:

- 1) Is a state or federal permit required for work with robins and crows? If so, was one in place to cover the field work?
- 2) The complaint states that sparrows are also going to be used. If so, has this been approved by the IACUC prior to beginning the work?
- 3) If sparrows are to be used, is a permit necessary? If so, is this in place or will it be obtained before work begins?
- 4) Have there been any concerns brought to the IACUC regarding this project?

Let me know the answers as soon as possible to allow NIH to respond. If you'd like to discuss by telephone, I am available.

Thank you in advance.

Axel Wolff, M.S., D.V.M.
Deputy Director, OLAW

(b) (6)



Yes, permit in place
under other PI

Wolff, Axel (NIH/OD) [E]

From: Ebel,Greg <Gregory.Ebel@colostate.edu>
Sent: Thursday, March 21, 2019 11:51 AM
To: Wolff, Axel (NIH/OD) [E]
Subject: Automatic reply: Attached Image

Hello,

I will be away from the office with limited email access until 3/25/19. I will respond to your message as soon as possible.

Best regards,

Greg

Wolff, Axel (NIH/OD) [E]

From: Wolff, Axel (NIH/OD) [E]
Sent: Friday, March 22, 2019 7:52 AM
To: Brown, Patricia [OLAW] (NIH/OD) [E]
Cc: Morse, Brent (NIH/OD) [E]
Subject: FW: PETA - concerns regarding NIAID-funded experiments with wild-caught birds

DRAFT REWRITE of last paragraph of Fenton letter:

[REDACTED] (b) (5)

(b) (5) That being said, there was in inadvertent lapse in approval of the Colorado Scientific Collection License for 2018 and 37 crows were collected during that time. All other compliance requirements (federal permit, IACUC approval) were in place. Colorado Parks and Wildlife was notified by the Principal Investigator once the error was recognized but the agency provided no further

[REDACTED] (b) (5)

From: Brown, Patricia [OLAW] (NIH/OD) [E] <brownp@od.nih.gov>
Sent: Wednesday, March 20, 2019 12:58 PM
To: Wolff, Axel (NIH/OD) [E] <wolffa@od.nih.gov>
Cc: Morse, Brent (NIH/OD) [E] <morseb@mail.nih.gov>
Subject: RE: PETA - concerns regarding NIAID-funded experiments with wild-caught birds

That would be great. I am talking with Dr. Fenton at 1 and will let him know.

Patricia Brown, VMD, MS
Director, Office of Laboratory Animal Welfare,
Office of Extramural Research,
Office of the Director, NIH
6700B Rockledge Drive
Bethesda, MD 20892-6910
301-496-7163, fax: 301-480-3394
brownp@mail.nih.gov

Disclaimer: Please note that this message and any of its attachments are intended for the named recipient(s) only and may contain confidential, protected, or privileged information that should not be distributed to unauthorized individuals. If you have received this message in error, please contact the sender.

From: Wolff, Axel (NIH/OD) [E] <wolffa@od.nih.gov>
Sent: Wednesday, March 20, 2019 12:51 PM
To: Brown, Patricia [OLAW] (NIH/OD) [E] <brownp@od.nih.gov>
Cc: Morse, Brent (NIH/OD) [E] <morseb@mail.nih.gov>
Subject: RE: PETA - concerns regarding NIAID-funded experiments with wild-caught birds

Brent just received a copy of the PETA complaint yesterday and showed it to me. I can follow up with the chair to determine whether permits were in place and whether sparrows have been added to the protocol, and if so whether there is a permit for this species.

From: Brown, Patricia [OLAW] (NIH/OD) [E] <brownp@od.nih.gov>
Sent: Wednesday, March 20, 2019 12:00 PM
To: Morse, Brent (NIH/OD) [E] <morseb@mail.nih.gov>; Wolff, Axel (NIH/OD) [E] <wolffa@od.nih.gov>
Subject: FW: PETA - concerns regarding NIAID-funded experiments with wild-caught birds
Importance: High

Last paragraph is a concern. I think that this will need some confirmation directly from the IACUC about the permitting. OLAW needs to weigh in on PHS Policy and the Guide requirements (p. 32) in lieu of referencing the AWA.

Patricia Brown, VMD, MS
Director, Office of Laboratory Animal Welfare,
Office of Extramural Research,
Office of the Director, NIH
6700B Rockledge Drive
Bethesda, MD 20892-6910
301-496-7163, fax: 301-480-3394
brownp@mail.nih.gov

Disclaimer: Please note that this message and any of its attachments are intended for the named recipient(s) only and may contain confidential, protected, or privileged information that should not be distributed to unauthorized individuals. If you have received this message in error, please contact the sender.

From: Fenton, Matthew (NIH/NIAID) [E] <fentonm@niaid.nih.gov>
Sent: Wednesday, March 20, 2019 11:33 AM
To: Lauer, Michael (NIH/OD) [E] <michael.lauer@nih.gov>; Black, Jodi (NIH/OD) [E] <jodi.black@nih.gov>; Brown, Patricia [OLAW] (NIH/OD) [E] <brownp@od.nih.gov>
Subject: RE: PETA - concerns regarding NIAID-funded experiments with wild-caught birds

Here is NIAID's draft response letter to PETA for your review and comment. Timely feedback would be greatly appreciated. Thanks.

Matthew

From: Fenton, Matthew (NIH/NIAID) [E]

Sent: Monday, March 18, 2019 1:54 PM

To: Lauer, Michael (NIH/OD) [E] <Michael.Lauer@nih.gov>; Black, Jodi (NIH/OD) [E] <Jodi.Black@nih.gov>; Brown, Patricia [OLAW] (NIH/OD) [E] <BrownP@OD.NIH.GOV>

Subject: PETA - concerns regarding NIAID-funded experiments with wild-caught birds

Mike et al. – this is a FYI... Tony and I received the attached letter from PETA on Friday. Dr. Gregory Ebel (Colorado State University) holds several NIAID awards as PI or MPI. Only one grant has experiments using wild-caught birds (abstract included below), and he does have up-to-date IACUC approvals for those experiments. While the original grant proposed using wild crows and robins, the latest RPPR mentions that Dr. Ebel plans to also use wild sparrows. NIAID is drafting a response that we will send along to you for comment and review before we send it to PETA. Feel free to contact me if you have any questions at this point. Thanks.

Matthew

Matthew J. Fenton, Ph.D.

Director

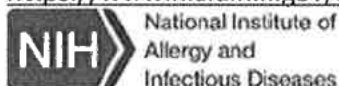
Division of Extramural Activities

National Institute of Allergy and Infectious Diseases, NIH

Tel: 301-496-7291, Fax: 301-402-0369

Email: fentonm@niaid.nih.gov

<https://www.niaid.nih.gov/about/dea>



2R01AI067380-11 (now in 13th year); Quasispecies Dynamics in Arbovirus Persistence, Emergence, and Fitness
PI Name : Ebel, Gregory David, Colorado State University

Local, site-specific characteristics largely control the transmission dynamics of arthropod-borne viruses (arboviruses). Arboviruses, in turn, adapt to local conditions, maximizing their potential to perpetuate and emerge as health threats. The adaptive potential of arboviruses is driven by error-prone replication, which creates a genetically diverse pool of competing virus genotypes within each host. This proposal examines how mosquitoes and birds act in concert to shape WNV evolution and fitness. Our previous research has allowed us to make very clear predictions about the outcome of each proposed aim and has facilitated our ability to translate our previous work to new emerging pathogens such as Zika virus.

In birds, WNV fitness gains are limited by high MOI environments in susceptible vertebrates (e.g. crows) and promoted in birds that limit replication (e.g. robins). Mosquitoes also have species-dependent impacts on WNV diversification and fitness. Ironically, systemic infection of mosquitoes leads to reduced fitness in transmitted WNV populations. Therefore, Aim 1 will attempt to either reduce or increase WNV fitness by forcing it into

transmission cycles with different host assemblages. We predict that crows and *Cx. pipiens* mosquitoes will result in WNV populations that are dramatically reduced in fitness compared to WNV that is maintained by robins and *Cx. quinquefasciatus*.

Our results strongly suggest that the limitations on fitness gains of WNV when it replicates in crows are related to the high viremias that occur in this host relative to robins. At high MOI, coinfection of individual cells is efficient and defective (or low fitness) genomes are complemented by those of high fitness. This suppresses the overall fitness of the population. Aim 2 of the current proposal tests this hypothesis through in vivo and ex vivo studies of WNV loads and diversity in avian PBMCs, a critical site of WNV replication. We predict that at high MOI, clearly deleterious mutations (intra-host length-variants, for example) will persist and fitness will be reduced.

The fitness declines that we observed in WNV during mosquito infection occur because of high virus mutation rates coupled with stochastic reductions in the population (i.e. bottlenecks) as the virus moves from one mosquito tissue to another. It is therefore critical to understand the mechanistic basis for the formation of these "barriers" to arbovirus transmission. Our preliminary data suggests that one critical aspect that contributes to them is RNAi-based targeting of the flavivirus sfRNA. In addition, we have preliminary data suggesting that sfRNA1/2 facilitates virus escape from anatomical barriers. Therefore, in Aim 3 we will examine how mosquito RNAi targets the WNV genome, and in particular the sfRNA1 start site, and how the virus population changes as a result of being "trapped" within a transmission barrier. This aim also will leverage our extensive experience working on WNV-host interactions to more deeply understand the emergent Zika virus. To accomplish this we will use newly developed reverse genetics systems for WNV and ZIKV that lack the ability to produce sfRNA1. Preliminary data on this is provided in the application.

The significance of this work is that it will provide novel data on how different transmission cycles can impact virus genetics, and how this can lead to the emergence of new virus strains. Our proposed work will also provide important mechanistic data on why different birds and mosquitoes have different impacts on virus populations. Translating our WNV-based findings to ZIKV is also critical to this work, as we think it is our job to use what we have learned to address new arboviral threats. Finally, the significance of our work is that we have provided technical and analytical tools that are broadly useful and have permitted us to collaborate effectively with a wide array of investigators. The proposed studies are technically and conceptually innovative due to, for example, our modeling of the WNV transmission cycle, our use of single-cell approaches, and our ability to profile small RNAs within mosquito salivary glands.

From: Katherine Roe <KatherineR@peta.org>

Sent: Friday, March 15, 2019 4:16 PM

To: Fauci, Anthony (NIH/NIAID) [E] <afauci@niaid.nih.gov>; Fenton, Matthew (NIH/NIAID) [E] <fentonm@niaid.nih.gov>

Subject: Concerns regarding NIAID-funded experiments with wild-caught birds

Dear Dr. Fauci and Dr. Fenton,

Good afternoon. I am writing on behalf of People for the Ethical Treatment of Animals (PETA) to express some concerns regarding a series of experiments funded by the National Institute of Allergy and Infectious Diseases.

Please see attached letter for more details.

Thank you very much for your time.

Best,

Katherine V. Roe Ph.D.
Research Associate
Laboratory Investigations Department
People for the Ethical Treatment of Animals
501 Front Street Norfolk, VA 23510

KatherineR@peta.org

(b) (6)

Wolff, Axel (NIH/OD) [E]

A3572

From: Brown, Patricia [OLAW] (NIH/OD) [E]
Sent: Wednesday, March 20, 2019 12:00 PM
To: Morse, Brent (NIH/OD) [E]; Wolff, Axel (NIH/OD) [E]
Subject: FW: PETA - concerns regarding NIAID-funded experiments with wild-caught birds
Attachments: PETA draft response letter v.2.docx

Importance: High

(b) (5)

Patricia Brown, VMD, MS
Director, Office of Laboratory Animal Welfare,
Office of Extramural Research,
Office of the Director, NIH
6700B Rockledge Drive
Bethesda, MD 20892-6910
301-496-7163, fax: 301-480-3394
brownp@mail.nih.gov

Disclaimer: Please note that this message and any of its attachments are intended for the named recipient(s) only and may contain confidential, protected, or privileged information that should not be distributed to unauthorized individuals. If you have received this message in error, please contact the sender.

From: Fenton, Matthew (NIH/NIAID) [E] <fentonm@niaid.nih.gov>
Sent: Wednesday, March 20, 2019 11:33 AM
To: Lauer, Michael (NIH/OD) [E] <michael.lauer@nih.gov>; Black, Jodi (NIH/OD) [E] <jodi.black@nih.gov>; Brown, Patricia [OLAW] (NIH/OD) [E] <brownp@od.nih.gov>
Subject: RE: PETA - concerns regarding NIAID-funded experiments with wild-caught birds

Here is NIAID's draft response letter to PETA for your review and comment. Timely feedback would be greatly appreciated. Thanks.

Matthew

From: Fenton, Matthew (NIH/NIAID) [E]
Sent: Monday, March 18, 2019 1:54 PM
To: Lauer, Michael (NIH/OD) [E] <Michael.Lauer@nih.gov>; Black, Jodi (NIH/OD) [E] <Jodi.Black@nih.gov>; Brown, Patricia [OLAW] (NIH/OD) [E] <BrownP@OD.NIH.GOV>
Subject: PETA - concerns regarding NIAID-funded experiments with wild-caught birds

Mike et al. – this is a FYI... Tony and I received the attached letter from PETA on Friday. Dr. Gregory Ebel (Colorado State University) holds several NIAID awards as PI or MPI. Only one grant has experiments using wild-caught birds (abstract included below), and he does have up-to-date IACUC approvals for those experiments. While the original grant proposed using wild crows and robins, the latest RPPR mentions that Dr. Ebel plans to also use wild sparrows. NIAID is drafting a response that we will send along to you for comment and review before we send it to PETA. Feel free to contact me if you have any questions at this point. Thanks.

Matthew

Matthew J. Fenton, Ph.D.
Director
Division of Extramural Activities
National Institute of Allergy and Infectious Diseases, NIH
Tel: (b) (6) Fax: (b) (6)

Email: fentonm@niaid.nih.gov

<https://www.niaid.nih.gov/about/dea>



2R01AI067380-11 (now in 13th year); Quasispecies Dynamics in Arbovirus Persistence, Emergence, and Fitness
PI Name : Ebel, Gregory David, Colorado State University

Local, site-specific characteristics largely control the transmission dynamics of arthropod-borne viruses (arboviruses). Arboviruses, in turn, adapt to local conditions, maximizing their potential to perpetuate and emerge as health threats. The adaptive potential of arboviruses is driven by error-prone replication, which creates a genetically diverse pool of competing virus genotypes within each host. This proposal examines how mosquitoes and birds act in concert to shape WNV evolution and fitness. Our previous research has allowed us to make very clear predictions about the outcome of each proposed aim and has facilitated our ability to translate our previous work to new emerging pathogens such as Zika virus.

In birds, WNV fitness gains are limited by high MOI environments in susceptible vertebrates (e.g. crows) and promoted in birds that limit replication (e.g. robins). Mosquitoes also have species-dependent impacts on WNV diversification and fitness. Ironically, systemic infection of mosquitoes leads to reduced fitness in transmitted WNV populations. Therefore, Aim 1 will attempt to either reduce or increase WNV fitness by forcing it into transmission cycles with different host assemblages. We predict that crows and *Cx. pipiens* mosquitoes will result in WNV populations that are dramatically reduced in fitness compared to WNV that is maintained by robins and *Cx. quinquefasciatus*.

Our results strongly suggest that the limitations on fitness gains of WNV when it replicates in crows are related to the high viremias that occur in this host relative to robins. At high MOI, coinfection of individual cells is efficient and defective (or low fitness) genomes are complemented by those of high fitness. This suppresses the overall fitness of the population. Aim 2 of the current proposal tests this hypothesis through in vivo and ex vivo studies of WNV loads and diversity in avian PBMCs, a critical site of WNV replication. We predict that at high MOI, clearly deleterious mutations (intra-host length-variants, for example) will persist and fitness will be

reduced.

The fitness declines that we observed in WNV during mosquito infection occur because of high virus mutation rates coupled with stochastic reductions in the population (i.e. bottlenecks) as the virus moves from one mosquito tissue to another. It is therefore critical to understand the mechanistic basis for the formation of these "barriers" to arbovirus transmission. Our preliminary data suggests that one critical aspect that contributes to them is RNAi-based targeting of the flavivirus sfRNA. In addition, we have preliminary data suggesting that sfRNA1/2 facilitates virus escape from anatomical barriers. Therefore, in Aim 3 we will examine how mosquito RNAi targets the WNV genome, and in particular the sfRNA1 start site, and how the virus population changes as a result of being "trapped" within a transmission barrier. This aim also will leverage our extensive experience working on WNV-host interactions to more deeply understand the emergent Zika virus. To accomplish this we will use newly developed reverse genetics systems for WNV and ZIKV that lack the ability to produce sfRNA1. Preliminary data on this is provided in the application.

The significance of this work is that it will provide novel data on how different transmission cycles can impact virus genetics, and how this can lead to the emergence of new virus strains. Our proposed work will also provide important mechanistic data on why different birds and mosquitoes have different impacts on virus populations. Translating our WNV-based findings to ZIKV is also critical to this work, as we think it is our job to use what we have learned to address new arboviral threats. Finally, the significance of our work is that we have provided technical and analytical tools that are broadly useful and have permitted us to collaborate effectively with a wide array of investigators. The proposed studies are technically and conceptually innovative due to, for example, our modeling of the WNV transmission cycle, our use of single-cell approaches, and our ability to profile small RNAs within mosquito salivary glands.

From: Katherine Roe <KatherineR@peta.org>

Sent: Friday, March 15, 2019 4:16 PM

To: Fauci, Anthony (NIH/NIAID) [E] <afauci@niaid.nih.gov>; Fenton, Matthew (NIH/NIAID) [E] <fentonm@niaid.nih.gov>

Subject: Concerns regarding NIAID-funded experiments with wild-caught birds

Dear Dr. Fauci and Dr. Fenton,

Good afternoon. I am writing on behalf of People for the Ethical Treatment of Animals (PETA) to express some concerns regarding a series of experiments funded by the National Institute of Allergy and Infectious Diseases.

Please see attached letter for more details.

Thank you very much for your time.

Best,

Katherine V. Roe Ph.D.
Research Associate
Laboratory Investigations Department
People for the Ethical Treatment of Animals
501 Front Street Norfolk, VA 23510

KatherineR@peta.org

(b) (6)

DATE

Katherine Roe, Ph.D.
Research Associate
Laboratory Investigations Department
People for the Ethical Treatment of Animals
501 Front Street, Norfolk, VA 23510

Dear Dr. Roe:

Thank you for your letter dated March 15, 2019 to Dr. Anthony Fauci and myself. On behalf of Dr. Fauci, and in my role as the Director of the Division of Extramural Activities at NIAID, I am pleased to respond.

West Nile Virus (WNV) was introduced into the Western Hemisphere during the late summer of 1999, when infected individuals were diagnosed in New York State, and spread rapidly throughout North America. From 1999 to 2018, more than 2.5 million people have been infected, with over 22,000 reported cases of encephalitis or meningitis and over 2,200 deaths. WNV is maintained in nature in a cycle between mosquitoes and animal hosts with the predominant and preferred reservoir being birds. Birds of some species become ill, show symptoms of disease, and may die, while others become infected and serve as carriers without showing signs of disease. Understanding the details of transmission between mosquitoes and different species of birds is therefore critical in predicting and controlling outbreaks in humans.

NIAID supports Dr. Ebel to carry out important research on WNV transmission. Dr. Ebel's research is focused on how different birds, mosquitoes and transmission intensities influence WNV genetic diversity within hosts, not, as Dr. Roe suggests, to develop treatments for WNV that can be used in a clinical setting. His studies have clearly demonstrated that different transmission ecologies can have distinct consequences for the viruses that perpetuate within them. He has demonstrated that in wild birds, the degree of WNV diversification is positively associated with bird susceptibility to WNV-induced mortality: disease-resistant American robins imposed stronger selection for WNV fitness compared to disease-susceptible American crows. The significance of this work is that it provides novel data on how different transmission cycles can impact virus genetics, and how this can lead to the emergence of new virus strains. Dr. Ebel's studies have also enabled a better understanding of the transmission dynamics of WNV within and between wild bird populations and the impact of the virus on bird health, as well as factors that may impact transmission of virus from infected birds to humans. This research cannot be conducted by studying WNV replication in cell culture or by *in silico* studies. While it may be true that *in silico* models of WNV biology exist, the algorithms that form the basis for these models can only be developed and validated by data from experiments with live animals such as that collected by Dr. Ebel.

In short, Dr. Ebel's research has increased our understanding, at a mechanistic level, of the fundamental population and evolutionary processes that act on WNV as it cycles between different host environments. Critically, this work has contributed to, and in some cases guided, the development of key technical and computational approaches to quantifying viral population diversity and fitness.

As for whether Dr. Ebel may have engaged in the trapping of crows and other birds without securing the necessary licenses from the Colorado Department of Parks & Wildlife, (b) (5)

(b) (5)

Sincerely,

Matthew J. Fenton, Ph.D.
Director, Division of Extramural Activities
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane, MSC 9824
Bethesda, MD 20892-9824



PEOPLE FOR
THE ETHICAL
TREATMENT
OF ANIMALS

March 15, 2019

Anthony S. Fauci, MD
Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health

Matthew J. Fenton, PhD
Director of the Division of Extramural Activities
National Institute of Allergy and Infectious Diseases
National Institutes of Health

Via email: afauci@niaid.nih.gov, matthew.fenton@nih.gov

Dear Dr. Fauci and Dr. Fenton,

On behalf of People for the Ethical Treatment of Animals (PETA) and our more than 6.5 million members and supporters, I am writing to express several concerns regarding a series of experiments conducted on wild-caught birds in Gregory Ebel's laboratory at Colorado State University (CSU) and funded by the National Institute of Allergy and Infectious Diseases.

These NIAID-funded experiments—in which healthy robins, sparrows, and crows, are captured from the wild, infected with a lethal virus, and killed—prompt several ethical and scientific concerns, which I have summarized below.

Most avian species, including the American crows, American robins, and house sparrows used at the Ebel Laboratory, are highly intelligent animals^{i,ii,iii,iv,v,vi,vii,viii,ix} with complex communication systems^x and social structures.^{xi,xii,xiii} The negative consequences of removing smart, social animals from their natural habitat are well documented.^{xiv,xv,xvi,xvii} It is also well known that laboratory environments and routine experimental procedures cause acute and chronic stress in the animals used.^{xviii} This is particularly true for wild-caught animals,^{xix} including birds.^{xx} Studies using wild-caught birds have found marked changes in stress hormone production in response to both initial capture and subsequent captivity.^{xxi,xxii,xxiii,xxiv} Acute and chronic stress in captive wild-caught birds results in weight loss,^{xxv,xxvi,xxvii} cardiac dysfunction,^{xxviii} behavioral abnormalities,^{xxix,xxx} and DNA damage.^{xxxi} At the Ebel Laboratory, this is all in conjunction with the negative effects of the experimental procedures performed on birds there, which include fever, ataxia, anorexia, multiple organ failure, and death.

In addition to the considerable ethical concerns linked to subjecting healthy wild birds to the myriad negative psychological and physiological effects summarized above, there are several scientific concerns associated with inducing stress in animals in the laboratory. Most important are the known stress-related effects on normal immune system function in wild-caught birds in laboratory

Washington, D.C.
1536 16th St. N.W.
Washington, DC 20036
202-483-PETA

Los Angeles
2154 W. Sunset Blvd.
Los Angeles, CA 90026
323-644-PETA

Norfolk
501 Front St.
Norfolk, VA 23510
757-622-PETA

Berkeley
2855 Telegraph Ave.
Ste. 301
Berkeley, CA 94705
510-763-PETA

Info@peta.org
PETA.org

Affiliates:

- PETA Asia
- PETA India
- PETA France
- PETA Australia
- PETA Germany
- PETA Netherlands
- PETA Foundation (U.K.)

settings.^{xxxii,xxxiii,xxxiv,xxxv,xxxvi,xxxvii,xxxviii,xxxix} Birds who are captured in the wild and held captive in a laboratory setting exhibit hyper-inflammation^{xi} and disrupted innate immune responses to infection.^{xi,xlii}

As wild-caught birds' immune competence is inevitably altered, the response to West Nile virus (WNV) of birds used for experimentation at the Ebel Laboratory is *not* representative of the response of a natural host. This limits the ecological validity of these studies and reduces the likelihood that they will translate to meaningful preventives or treatments for WNV in birds or humans. In other words, the birds experimented on at the Ebel Laboratory cease to be ecologically relevant, natural WNV hosts the moment that they are captured. Additionally, most avian species, including those used in these experiments, exhibit different innate immune response to viruses than humans do,^{xliii,xliv} further reducing the likelihood they will be useful in a clinical setting.

Fortunately, several research groups have successfully investigated WNV using *in silico* and *in vitro* methods as well as human volunteers. For example, *in vitro* research laboratories have measured the effect of serial passage on the evolution of RNA viruses, including WNV, in mosquito, avian, and mammalian host cell lines.^{xlv,xlvi,xlvii,xlviii} *In silico* models of WNV have also been used to study the effects of positive selection pressure on its adaptation,^{xliv} mechanisms of infection in humans,^{li} and treatment development.^{li} Studies with human patients have identified genetic variants that increase susceptibility to the virus^{lii} and elucidated the human immune response to it.^{liii} Similarly, *ex vivo* studies of human primary cells have identified immunophenotypes associated with increased susceptibility to WNV infections.^{liv}

In addition to the issues outlined above, it appears to us—based on a series of Colorado Open Records Act requests filed by our office—that Gregory Ebel may be engaged in the trapping of crows and other birds without securing the necessary licenses from Colorado Parks & Wildlife. If this is true, Ebel would have violated Colorado state law while carrying out activities for a project funded by NIAID. As federal regulations and policies stipulate that NIH grant recipients must comply with federal, state, and local laws, Ebel would also be in violation of the terms and conditions of his grant.

After 14 years of capturing wild birds for experimentation, and \$4,641,014 in funding from NIAID, the Ebel Laboratory has not developed a method to prevent or treat WNV infections in birds or humans. This is not particularly surprising, as animal models of disease rarely translate into human-relevant treatments, and developing human-relevant treatments was never the ultimate goal of these experiments.

Given the extensive harm capture, captivity, and WNV infection have on these intelligent, sentient birds, the considerable economic costs of these experiments, the limited clinical relevance, and the availability of alternative research methods, we ask that the NIAID discontinue funding these experiments.

I would love the opportunity to speak with you on this matter. Thank you for your time.

Sincerely,

Katherine Roe, Ph.D.
 Research Associate
 Laboratory Investigations Department
 People for the Ethical Treatment of Animals
 501 Front Street | Norfolk, VA 23510

ⁱEmery NJ, Clayton NS. 2004. The mentality of crows: Convergent evolution of intelligence in corvids and apes. *Science*. 306(5703):1903-1907.

ⁱⁱBreitwisch R, Breitwisch M. 1991. House sparrows open an automatic door. *Wilson Bull*. 103(4):725-726.

ⁱⁱⁱMarzluff JM, Walls J, Cornell HN, Withey JC, Craig DP. 2010. Lasting recognition of threatening people by wild American crows. *Anim Behav*. 79(3):699-707.

^{iv}Clayton N. 2007. Animal cognition: Crows spontaneously solve a metatool task. *Curr Biol*. 17(20):R894-R895.

^vClucas B, Marzluff JM, Mackovjak D, Palmquist I. 2013. Do American crows pay attention to human gaze and facial expressions? *Ethology*. 119(4):296-302.

^{vi}Klein J. 2008. A though experiment on the intelligence of crows. TED.com. Available from:

https://www.ted.com/talks/joshua_klein_on_the_intelligence_of_crows/up-next?language=en.

^{vii}MacDonald SE, Ritvo S. 2016. Comparative cognition outside the laboratory. *Compar Cogn Behav Rev*. 11(1):49-61.

^{viii}Lamey A. 2012. Primitive self-consciousness and avian cognition. *Monist*. 95(3):486-510.

^{ix}Bekoff M, Elzanowski A. 1997. Collecting birds: The importance of moral debate. *Bird Conserv Int*. 7(4):357-361.

^xKuhl PK. 2003. Human speech and birdsong: Communication and the social brain. *Proc Natl Acad Sci U S A*. 100(17):9645-9646.

^{xi}Kondo N, Izawa EI, Watanabe S. 2012. Crows cross-modally recognize group members but not non-group members. *P Roy Soc Lond B Bio*. 279(1735):1937-1942.

^{xii}Clayton NS, Emery NJ. 2007. The social life of corvids. *Curr Biol*. 17(16):R652-R656.

^{xiii}Swift KN, Marzluff JM. 2015. Wild American crows gather around their dead to learn about danger. *Anim Behav*. 109:187-197.

^{xiv}Dickens MJ, Delehanty DJ, Romero LM. 2009. Stress and translocation: Alterations in the stress physiology of translocated birds. *P Roy Soc Lond B Bio*. 276:2051-2056.

^{xv}Dickens MJ, Earle KA, Romero LM. 2009. Initial transference of wild birds to captivity alters stress physiology. *Gen Comp Endocrinol*. 160(1):76-83.

^{xvi}Morgan K, Tromborg CT. 2007. Sources of stress in captivity. *Appl Anim Behav Sci*. 102(3-4):262-302.

^{xvii}Dickens MJ, Delehanty DJ, Romero LM. 2010. Stress: An inevitable component of animal translocation. *Biol Conserv*. 143(6):1329-1341.

^{xviii}Balcombe JP, Barnard ND, Sandusky C. 2004. Laboratory routines cause animal stress. *Contemp Top Lab Anim Sci*. 43(6):42-51.

^{xix}Morgan, Tromborg.

^{xx}Beaulieu M. 2016. A bird in the house: The challenge of being ecologically relevant in captivity. *Front Ecol Evol*. 4:141.

^{xxi}Romero LM, Romero RC. 2002. Corticosterone responses in wild birds: The importance of rapid initial sampling. *Condor*. 104(1):129-135.

^{xxii}Fischer CP, Wright-Lichter J, Romero LM. 2018. Chronic stress and the introduction to captivity: How wild house sparrows (*Passer domesticus*) adjust to laboratory conditions. *Gen Comp Endocrinol*. 259:85-92.

^{xxiii}Dickens, Earle, Romero.

^{xxiv}Adams NJ, Farnworth MJ, Rickett J, Parker KA, Cockrem JF. 2011. Behavioural and corticosterone responses to capture and confinement of wild blackbirds (*Turdus merula*). *Appl Anim Behav Sci*. 134(3-4):246-255.

^{xxv}Sepp T, Sild E, Hõrak P. 2010. Hematological condition indexes in greenfinches: Effects of captivity and diurnal variation. *Physiol Biochem Zool*. 83(2):276-282.

^{xxvi}Wingfield JC, Smith JP, Farner DS. 1982. Endocrine responses of white-crowned sparrows to environmental stress. *Condor*. 84:399-409.

^{xxvii}Fischer CP et al.

- ^{xxviii}Cyr NE, Dickens MJ, Romero LM. 2009. Heart rate and heart-rate variability responses to acute and chronic stress in a wild-caught passerine bird. *Physiol Biochem Zool.* 82(4):332-344.
- ^{xxix}Fokidis HB, Hurley L, Rogowski C, Sweazea K, Deviche, P. 2011. Effects of captivity and body condition on plasma corticosterone, locomotor behavior, and plasma metabolites in curve-billed thrashers. *Physiol Biochem Zool.* 84(6):595-606.
- ^{xxx}Lattin CR, Pechenko AV, Carson RE. 2017. Experimentally reducing corticosterone mitigates rapid captivity effects on behavior, but not body composition, in a wild bird. *Horm Behav.* 89:121-129.
- ^{xxxi}Gormally BMG, Fuller R, McVey M, Romero LM. 2019. DNA damage as an indicator of chronic stress: Correlations with corticosterone and uric acid. *Comp Biochem Physiol A Mol Integr Physiol.* 227:116-122.
- ^{xxxii}Matson KD, Tieleman BI, Klasing KC. 2006. Capture stress and the bactericidal competence of blood and plasma in five species of tropical birds. *Physiol Biochem Zool.* 79(3):556-564.
- ^{xxxiii}Matson KD, Ricklefs RE, Klasing KC. 2005. A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. *Dev Comp Immunol.* 29(3):275-286.
- ^{xxxiv}Kuhlman JR, Martin LB. 2010. Captivity affects immune redistribution to skin in a wild bird. *Funct Ecol.* 24(4):830-837.
- ^{xxxv}Ewenson EL, Zann RA, Flannery GR. 2001. Body condition and immune response in wild zebra finches: Effects of capture, confinement and captive-rearing. *Naturwissenschaften.* 88(9):391-394.
- ^{xxxvi}Buehler DM, Piersma T, Tieleman I. 2008. Captive and free-living red knots *Calidris canutus* exhibit differences in non-induced immunity that suggest different immune strategies in different environments. *J Avian Biol.* 39(5):560-566.
- ^{xxxvii}Adams *et al.*
- ^{xxxviii}Lattin *et al.*
- ^{xxxix}Millet S, Bennett J, Lee KA, Hau M, Klasing KC. 2007. Quantifying and comparing constitutive immunity across avian species. *Dev Comp Immunol.* 31(2):188-201.
- ^{xl}Martin LB, Kidd L, Liebl AL, Coon CA. 2011. Captivity induces hyper-inflammation in the house sparrow (*Passer domesticus*). *J Exp Biol.* 214(Pt 15):2579-2585.
- ^{xli}Matson, Tieleman, Klasing.
- ^{xlii}Martin LB, Brace AJ, Urban A, Coon CA, Liebl AL. 2012. Does immune suppression during stress occur to promote physical performance? *J Exp Biol.* 215(Pt 23):4097-4103.
- ^{xliii}Ahlers LRH, Goodman AG. 2018. The immune responses of the animal hosts of West Nile Virus: A comparison of insects, birds, and mammals. *Front Cell Infect Microbiol.* 8:96.
- ^{xliv}Cao Y, Huang Y, Xu K, Liu Y, Li X, Xu Y, Zhong W, Hao P. 2017. Differential responses of innate immunity triggered by different subtypes of influenza A viruses in human and avian hosts. *BMC Med Genomics.* 10(Suppl. 4):70.
- ^{xlv}Ciota AT, Ngo KA, Lovelace AO, Payne AF, Zhou Y, Shi PY, Kramer LD. 2007. Role of the mutant spectrum in adaptation and replication of West Nile virus. *J Gen Virol.* 88(Pt 3):865-s874
- ^{xlvi}Combe M, Sanjuán R. 2014. Variation in RNA virus mutation rates across host cells. *PLoS Pathog.* 10(1):e1003855.
- ^{xlvii}Bordería AV, Isakov O, Moratorio G, Henningsson R, Agüera-González S, Organtini L, Gnädig NF, Blanc H, Alcover A, Hafenstein S, *et al.* 2015. Group selection and contribution of minority variants during virus adaptation determines virus fitness and phenotype. *PLoS Pathog.* 11(5):e1004838.
- ^{xlviii}Ngo KA, Rose JT, Kramer LD, Ciota AT. 2019. Adaptation of Rabensburg virus (RBGV) to vertebrate hosts by experimental evolution. *Virology.* 528:30-36.
- ^{xlix}Barzon L, Papa A, Lavezzo E, Franchin E, Pacenti M, Sinigaglia A, Masi G, Trevisan M, Squarzon L, Toppo S, *et al.* 2015. Phylogenetic characterization of Central/Southern European lineage 2 West Nile virus: Analysis of human outbreaks in Italy and Greece, 2013–2014. *Clin Microbiol Infect.* 21(12):1122.e1-10.
- ^lChen J, Sun J, Liu X, Liu F, Liu R, Wang J. 2018. Structure-based prediction of West Nile virus-human protein–protein interactions. *J Biomol Struct Dyn.* 17:1-12.
- ^{li}Hossain MU, Keya CA, Das KC, Hashem A, Omar TM, Khan MA, Rakib-Uz-Zaman SM, Salimullah M. 2018. An immunopharmacoinformatics approach in development of vaccine and drug candidates for West Nile Virus. *Front Chem.* 6:246.
- ^{lii}Long D, Deng X, Singh P, Loeb M, Luring AS, Seielstad M. 2016. Identification of genetic variants associated with susceptibility to West Nile virus neuroinvasive disease. *Genes Immun.* 17(5):298-304.
- ^{liii}Tsioris K, Gupta NT, Ogunniyi AO, Zimnisky RM, Qian F, Yao Y, Wang X, Stern JN, Chari R, Briggs AW, *et al.* 2015. Neutralizing antibodies against West Nile virus identified directly from human B cells by single-cell analysis and next generation sequencing. *Integr Biol (Camb).* 7(12):1587-1597.

^{liv}Qian F, Goel G, Meng H, Wang X, You F, Devine L, Raddassi K, Garcia MN, Murray KO, Bolen CR, *et al.* 2015. Systems immunology reveals markers of susceptibility to West Nile virus infection. *Clin. Vaccine Immunol.* 22(1):6-16.

Morse, Brent (NIH/OD) [E]

From: Brown, Patricia [OLAW] (NIH/OD) [E]
Sent: Monday, March 18, 2019 5:01 PM
To: Morse, Brent (NIH/OD) [E]
Subject: FW: PETA - concerns regarding NIAID-funded experiments with wild-caught birds
Attachments: Letter from PETA to NIH re Gregory Ebel, March 15, 2019[1].pdf

Please review and take any needed action if deemed appropriate.

Patricia Brown, VMD, MS
Director, Office of Laboratory Animal Welfare,
Office of Extramural Research,
Office of the Director, NIH
6700B Rockledge Drive
Bethesda, MD 20892-6910
301-496-7163, fax: 301-480-3394
brownp@mail.nih.gov

Disclaimer: Please note that this message and any of its attachments are intended for the named recipient(s) only and may contain confidential, protected, or privileged information that should not be distributed to unauthorized individuals. If you have received this message in error, please contact the sender.

From: Lauer, Michael (NIH/OD) [E] <michael.lauer@nih.gov>
Sent: Monday, March 18, 2019 2:49 PM
To: Fenton, Matthew (NIH/NIAID) [E] <fentonm@niaid.nih.gov>; Black, Jodi (NIH/OD) [E] <jodi.black@nih.gov>; Brown, Patricia [OLAW] (NIH/OD) [E] <brownp@od.nih.gov>
Cc: Lauer, Michael (NIH/OD) [E] <michael.lauer@nih.gov>
Subject: Re: PETA - concerns regarding NIAID-funded experiments with wild-caught birds

Many thanks Matthew for the heads up and we look forward to seeing your draft.

Best, Mike

From: "Fenton, Matthew (NIH/NIAID) [E]" <fentonm@niaid.nih.gov>
Date: Monday, March 18, 2019 at 1:54 PM
To: "Lauer, Michael (NIH/OD) [E]" <michael.lauer@nih.gov>, "Black, Jodi (NIH/OD) [E]" <jodi.black@nih.gov>, "Brown, Patricia [OLAW] (NIH/OD) [E]" <brownp@od.nih.gov>
Subject: PETA - concerns regarding NIAID-funded experiments with wild-caught birds

Mike et al. – this is a FYI... Tony and I received the attached letter from Gregory Ebel (Colorado State University) who holds several NIAID awards. His grant has experiments using wild-caught birds (abstract included). He has up-to-date IACUC approvals for those experiments. While the original RPPR mentions crows and robins, the latest RPPR mentions that Dr. Ebel plans to use sparrows???)

Need to check
permits against
amendments as
well (added
sparrows???)

NIAID is drafting a response that we will send along to you for comment and review before we send it to PETA. Feel free to contact me if you have any questions at this point. Thanks.

Matthew

Matthew J. Fenton, Ph.D.

Director

Division of Extramural Activities

National Institute of Allergy and Infectious Diseases, NIH

Tel: 301-496-7291, Fax: 301-402-0369

Email: fentonm@niaid.nih.gov

<https://www.niaid.nih.gov/about/dea>



National Institute of
Allergy and
Infectious Diseases

2R01AI067380-11 (now in 13th year); Quasispecies Dynamics in Arbovirus Persistence, Emergence, and Fitness
PI Name : Ebel, Gregory David, Colorado State University

Local, site-specific characteristics largely control the transmission dynamics of arthropod-borne viruses (arboviruses). Arboviruses, in turn, adapt to local conditions, maximizing their potential to perpetuate and emerge as health threats. The adaptive potential of arboviruses is driven by error-prone replication, which creates a genetically diverse pool of competing virus genotypes within each host. This proposal examines how mosquitoes and birds act in concert to shape WNV evolution and fitness. Our previous research has allowed us to make very clear predictions about the outcome of each proposed aim and has facilitated our ability to translate our previous work to new emerging pathogens such as Zika virus.

In birds, WNV fitness gains are limited by high MOI environments in susceptible vertebrates (e.g. crows) and promoted in birds that limit replication (e.g. robins). Mosquitoes also have species-dependent impacts on WNV diversification and fitness. Ironically, systemic infection of mosquitoes leads to reduced fitness in transmitted WNV populations. Therefore, Aim 1 will attempt to either reduce or increase WNV fitness by forcing it into transmission cycles with different host assemblages. We predict that crows and *Cx. pipiens* mosquitoes will result in WNV populations that are dramatically reduced in fitness compared to WNV that is maintained by robins and *Cx. quinquefasciatus*.

Our results strongly suggest that the limitations on fitness gains of WNV when it replicates in crows are related to the high viremias that occur in this host relative to robins. At high MOI, coinfection of individual cells is efficient and defective (or low fitness) genomes are complemented by those of high fitness. This suppresses the overall fitness of the population. Aim 2 of the current proposal tests this hypothesis through in vivo and ex vivo studies of WNV loads and diversity in avian PBMCs, a critical site of WNV replication. We predict that at high MOI, clearly deleterious mutations (intrahost length-variants, for example) will persist and fitness will be reduced.

The fitness declines that we observed in WNV during mosquito infection occur because of high virus mutation rates coupled with stochastic reductions in the population (i.e. bottlenecks) as the virus moves from one mosquito tissue to another. It is therefore critical to understand the mechanistic basis for the formation of these "barriers" to arbovirus transmission. Our preliminary data suggests that one critical aspect that contributes to

them is RNAi-based targeting of the flavivirus sfRNA. In addition, we have preliminary data suggesting that sfRNA1/2 facilitates virus escape from anatomical barriers. Therefore, in Aim 3 we will examine how mosquito RNAi targets the WNV genome, and in particular the sfRNA1 start site, and how the virus population changes as a result of being "trapped" within a transmission barrier. This aim also will leverage our extensive experience working on WNV-host interactions to more deeply understand the emergent Zika virus. To accomplish this we will use newly developed reverse genetics systems for WNV and ZIKV that lack the ability to produce sfRNA1. Preliminary data on this is provided in the application.

The significance of this work is that it will provide novel data on how different transmission cycles can impact virus genetics, and how this can lead to the emergence of new virus strains. Our proposed work will also provide important mechanistic data on why different birds and mosquitoes have different impacts on virus populations. Translating our WNV-based findings to ZIKV is also critical to this work, as we think it is our job to use what we have learned to address new arboviral threats. Finally, the significance of our work is that we have provided technical and analytical tools that are broadly useful and have permitted us to collaborate effectively with a wide array of investigators. The proposed studies are technically and conceptually innovative due to, for example, our modeling of the WNV transmission cycle, our use of single-cell approaches, and our ability to profile small RNAs within mosquito salivary glands.

From: Katherine Roe <KatherineR@peta.org>

Sent: Friday, March 15, 2019 4:16 PM

To: Fauci, Anthony (NIH/NIAID) [E] <afauci@niaid.nih.gov>; Fenton, Matthew (NIH/NIAID) [E] <fentonm@niaid.nih.gov>

Subject: Concerns regarding NIAID-funded experiments with wild-caught birds

Dear Dr. Fauci and Dr. Fenton,

Good afternoon. I am writing on behalf of People for the Ethical Treatment of Animals (PETA) to express some concerns regarding a series of experiments funded by the National Institute of Allergy and Infectious Diseases.

Please see attached letter for more details.

Thank you very much for your time.

Best,

Katherine V. Roe Ph.D.
Research Associate
Laboratory Investigations Department
People for the Ethical Treatment of Animals
501 Front Street Norfolk, VA 23510

KatherineR@peta.org

(b) (6)

Morse, Brent (NIH/OD) [E]

From: Brown, Patricia [OLAW] (NIH/OD) [E]
Sent: Tuesday, March 19, 2019 6:54 AM
To: Fenton, Matthew (NIH/NIAID) [E]
Cc: Lauer, Michael (NIH/OD) [E]; Black, Jodi (NIH/OD) [E]; Morse, Brent (NIH/OD) [E]
Subject: RE: PETA - concerns regarding NIAID-funded experiments with wild-caught birds

Dear Dr. Fenton,

Thank you for sharing the letter from PETA concerning Colorado State University research supported by NIAID. The OLAW Division of Compliance Oversight is reviewing the allegations concerning animal welfare. Dr. Brent Morse will be the point of contact for this case.

Sincerely,

Patricia Brown, VMD, MS
 Director, Office of Laboratory Animal Welfare,
 Office of Extramural Research,
 Office of the Director, NIH
 6700B Rockledge Drive, Suite 2500
 Bethesda, MD 20892
 301-496-7163, fax: 301-480-3394
brownp@mail.nih.gov

Disclaimer: Please note that this message and any of its attachments are intended for the named recipient(s) only and may contain confidential, protected, or privileged information that should not be distributed to unauthorized individuals. If you have received this message in error, please contact the sender.

From: Fenton, Matthew (NIH/NIAID) [E] <fentonm@niaid.nih.gov>
Sent: Monday, March 18, 2019 1:54 PM
To: Lauer, Michael (NIH/OD) [E] <michael.lauer@nih.gov>; Black, Jodi (NIH/OD) [E] <jodi.black@nih.gov>; Brown, Patricia [OLAW] (NIH/OD) [E] <brownp@od.nih.gov>
Subject: PETA - concerns regarding NIAID-funded experiments with wild-caught birds

Mike et al. – this is a FYI... Tony and I received the attached letter from PETA on Friday. Dr. Gregory Ebel (Colorado State University) holds several NIAID awards as PI or MPI. Only one grant has experiments using wild-caught birds (abstract included below), and he does have up-to-date IACUC approvals for those experiments. While the original grant proposed using wild crows and robins, the latest RPPR mentions that Dr. Ebel plans to also use wild sparrows. NIAID is drafting a response that we will send along to you for comment and review before we send it to PETA. Feel free to contact me if you have any questions at this point. Thanks.

Matthew

Matthew J. Fenton, Ph.D.

Director
Division of Extramural Activities
National Institute of Allergy and Infectious Diseases, NIH
Tel: 301-496-7291, Fax: 301-402-0369
Email: fentonm@niaid.nih.gov
<https://www.niaid.nih.gov/about/dea>



2R01AI067380-11 (now in 13th year); Quasispecies Dynamics in Arbovirus Persistence, Emergence, and Fitness
PI Name : Ebel, Gregory David, Colorado State University

Local, site-specific characteristics largely control the transmission dynamics of arthropod-borne viruses (arboviruses). Arboviruses, in turn, adapt to local conditions, maximizing their potential to perpetuate and emerge as health threats. The adaptive potential of arboviruses is driven by error-prone replication, which creates a genetically diverse pool of competing virus genotypes within each host. This proposal examines how mosquitoes and birds act in concert to shape WNV evolution and fitness. Our previous research has allowed us to make very clear predictions about the outcome of each proposed aim and has facilitated our ability to translate our previous work to new emerging pathogens such as Zika virus.

In birds, WNV fitness gains are limited by high MOI environments in susceptible vertebrates (e.g. crows) and promoted in birds that limit replication (e.g. robins). Mosquitoes also have species-dependent impacts on WNV diversification and fitness. Ironically, systemic infection of mosquitoes leads to reduced fitness in transmitted WNV populations. Therefore, Aim 1 will attempt to either reduce or increase WNV fitness by forcing it into transmission cycles with different host assemblages. We predict that crows and *Cx. pipiens* mosquitoes will result in WNV populations that are dramatically reduced in fitness compared to WNV that is maintained by robins and *Cx. quinquefasciatus*.

Our results strongly suggest that the limitations on fitness gains of WNV within mosquitoes are due to the high viremias that occur in this host relative to robins. At high MOI, both efficient and defective (or low fitness) genomes are complemented by those of the other, resulting in the overall fitness of the population. Aim 2 of the current proposal tests the hypothesis that in vivo studies of WNV loads and diversity in avian PBMCs, a critical site of WNV replication, will show that high MOI, clearly deleterious mutations (intra-host length-variants, for example) are reduced.

The fitness declines that we observed in WNV during mosquito infection occur because of high virus mutation rates coupled with stochastic reductions in the population (i.e. bottlenecks) as the virus moves from mosquito tissue to another. It is therefore critical to understand the mechanisms that act as "barriers" to arbovirus transmission. Our preliminary data suggests that one of these barriers is RNAi-based targeting of the flavivirus sfRNA. In addition, we have previously shown that sfRNA1/2 facilitates virus escape from anatomical barriers. Therefore, in Aim 3, we will use RNAi to target the WNV genome, and in particular the sfRNA1 start site, and hope to show that this will act as a result of being "trapped" within a transmission barrier. This aim also will allow us to experience working on WNV-host interactions to more deeply understand the mechanisms that act as barriers. To accomplish this we will use newly developed reverse genetics systems for WNV to produce sfRNA1. Preliminary data on this is provided in the application.

The significance of this work is that it will provide novel data on how different transmission cycles can impact

virus genetics, and how this can lead to the emergence of new virus strains. Our proposed work will also provide important mechanistic data on why different birds and mosquitoes have different impacts on virus populations. Translating our WNV-based findings to ZIKV is also critical to this work, as we think it is our job to use what we have learned to address new arboviral threats. Finally, the significance of our work is that we have provided technical and analytical tools that are broadly useful and have permitted us to collaborate effectively with a wide array of investigators. The proposed studies are technically and conceptually innovative due to, for example, our modeling of the WNV transmission cycle, our use of single-cell approaches, and our ability to profile small RNAs within mosquito salivary glands.

From: Katherine Roe <KatherineR@peta.org>

Sent: Friday, March 15, 2019 4:16 PM

To: Fauci, Anthony (NIH/NIAID) [E] <afauci@niaid.nih.gov>; Fenton, Matthew (NIH/NIAID) [E] <fentonm@niaid.nih.gov>

Subject: Concerns regarding NIAID-funded experiments with wild-caught birds

Dear Dr. Fauci and Dr. Fenton,

Good afternoon. I am writing on behalf of People for the Ethical Treatment of Animals (PETA) to express some concerns regarding a series of experiments funded by the National Institute of Allergy and Infectious Diseases.

Please see attached letter for more details.

Thank you very much for your time.

Best,

Katherine V. Roe Ph.D.
Research Associate
Laboratory Investigations Department
People for the Ethical Treatment of Animals
501 Front Street Norfolk, VA 23510

KatherineR@peta.org

(b) (6)