From: Blanca Hurtado
To: Clerk of the Board Public Email; Kennedy. Supervisor; Frost. Supervisor; Supervisor Serna; Rich Desmond; Nottoli. Don
Subject: 8/9/22 meeting item 42
Date: Sunday, August 7, 2022 5:00:16 PM

I'm in supervisor Frost district and do not agree that the county should proclaim any emergency related to monkeypox. There have been zero deaths in our county related to this. Also, aren't there vaccines available for this already? Declaring an emergency seems like it's the popular thing to do. This is setting a standard that will hurt our county. As leaders who have been given power by the people, stop instilling fear into our communities. Instead, empower the community with knowledge about this disease. Educate, raise awareness, but don't continue this cycle of emergency in our county. We are all trying to heal and move on from the past 2-3 years. This supposed emergency seems like another ploy for the county to ask for and use funds as they are doing and have done with covid. As county counsel stated on record, the state of emergency keeps the money coming in (paraphrased). This is fraud.

Blanca

From:	Rachel Kattan
To:	Kennedy. Supervisor; Frost. Supervisor; Supervisor Serna; Rich Desmond; Nottoli. Don; Clerk of the Board Public Email
Subject:	August 9th meeting, Agenda item 42
Date:	Sunday, August 7, 2022 8:27:47 AM

Hello,

I am Supervisor Serna's constiuent and I live in the Natomas area. I am opposed to the ratification of the two proclamations surrounding the Monkeypox emergency. In Sacramento County, there are 65 cases with NO deaths and the vaccine is ready for those who want it. Did we declare an emergency for seasonal flu every year that DOES kill people? No. We need to stop this incessant cycle of fear and bring the population back to reality. Another solution is to educate the public on the risks and treatments of Monkeypox without declaring an emergency much like we have seen in the case of smoking tobacco or drinking and driving. Thank you for all the work you do to try and help keep Sacramento County safe.

Thank you for your consideration,
Rachel Kattan

From: Renee Nielson
To: Clerk of the Board Public Email
Subject: Item 42/ 08-09-2022: Opposed to Advice from Sacramento County Health Officer/Monkey Pox Recommendations - Past Advice Shown to be Ineffective and Even Harmful
Date: Monday, August 8, 2022 7:55:40 AM
Attachments: Large Remdesivir Study Finds No COVID-19 Survival Benefit.pdf
Emergency Use Authorization.pdf

Dear Board Members:

I wanted to take a moment and email you with some important information along with a request to reconsider moving forward on any health mandate our County Health Officer suggests that could impact the citizens of this community through lockdowns, forced medical procedures, removal of medical privacy, and tax dollars being spent on a "hoax" or "inflated" emergency. In the past it has been expressed that we as a county should be following all health advice given by the Sacramento County Health Officer, Ms. Kasirye. Previous votes and discussion to "only" follow the approved treatment for recovery that this individual espoused has been questionable at best. I'd like to request this Board take into consideration the County Health Officer, Ms. Kasirye, is getting direction that may be more political in nature as the conclusions to her recommendations based on evidence are **NOT** necessarily in the best interest of the general population. In hindsight, her recommendations have been **harmful and ineffective to many**. These policies have forced job loss, severe hardships, family splits, and unelected medical procedures and devices on the entire population as a whole. In addition illness has been conflated while our tax dollars are being spent to support what has only been proven in many regards as propaganda. In addition, her impact on this Board has been to discourage the right medicine for the right individual at the right time. She made the argument these COVID and gene altering medical treatments benefit everyone, both kids and adults alike, while unethically not disclosing potential harms or that these shots **aren't FDA approved**. Her "advice" has forced this Board to make decisions outside of it's purview and abilities, allowed our tax dollars to be spent tracking and tracing, dispersing individuals medical records, and for some reason, people now think knowing someone's full health history is their business! Her recommendations have lead to taking away medical freedoms and have led to medical discrimination with many, many abuses (CalGina, HIPAA, ADA, Unruh Civil Rights Act, SEC. 3.5 Section 51 of the Civil Code and per 21 U.S.C. section 360bbb-3(e)(1)(A)(ii)(iii) where individuals must be informed of the **option to accept or refuse administration of an experimental treatment** that is **ONLY** approved through Emergency Use Authorization). This Board has given heavy weight to her words which ended up with the deprivation of our federally secured rights and, for some, have become ill or have died as a reaction to the treatment.

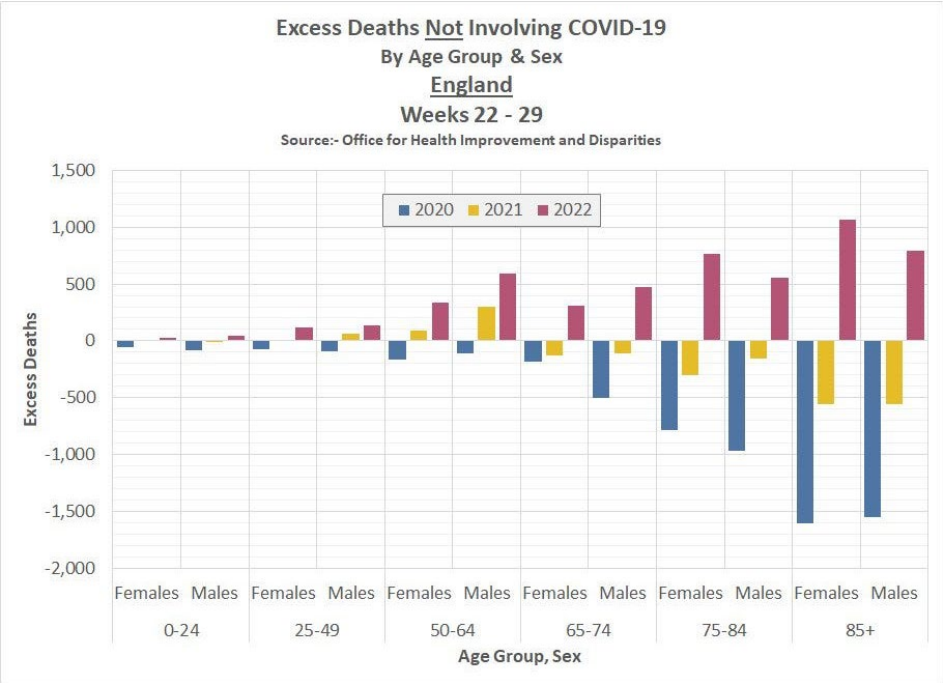
Ms. Kasirye encouraged and recommended only one path of COVID treatment while there were multiple options. Remdesivir was her only recommended treatment of choice even though that was sketchy at best as "the only treatment". A "Large Remdesivir Study Finds No COVID-19 Survival Benefit," (WebMD Health News - see attached). In fact, ivermectin, D3, garlic and sunlight as good health practices were discouraged and stricken from the approved vernacular, along with any other alternatives to the recommended remdesivir treatment. In fact, it's an extremely expensive option in comparison to other treatments. "In the U.S., the price of remdesivir is $3,120 for a course for private insurers and $2,340 for some government plans." (see https://www.statnews.com/2020/11/19/who-recommends-against-remdesivir-

covid-19/).

Her advice led one to believe Pfizer and other shots to resolve COVID are beneficial when Pfizer has already released documentation saying otherwise (http://www.phmpt.org or http://icandecide.org). When this information came to light it would have been important for the narrative to be fixed but it wasn't. These shots are still being pushed as "safe and effective". Ms. Kasirye has detrimentally impacted this community through non-disclosure!

Please also take notice that the COVID mRNA gene therapy she told us would make it so you couldn't get COVID appears to have had the opposite effect. Below studies tracking populations show those who have received the shots and boosters are showing higher death rates as reported by attorney Jeff Childers on his blog. You can't negate the charts showing the heavy death toll and larger impacts of taking experimental treatments:

> *"Britain has a government agency called the Office of National Statistics, or ONS, which also keeps track of all its covid data.*
>
> *The ONS reported 93% of the Covid deaths in April and May, were of vaccinated people (5,276 of 5,678). The 93% figure is almost an exact match for the proportion of vaccinated Brits: of the 12+ population, 93% had a first dose of the vaccine, 87% had a second dose, and 70% had a third dose.*
>
> *In other words, the UK data appears to show ZERO BENEFIT from the jabs in reducing the risk of death from covid. I know, you're shocked.*
>
> *While the UK government continues to claim jabs confer a significant reduction of the risk of dying, using more nuanced analyses, they seem at least to have now abandoned the original lie that jabs would essentially ensure that you "won't die" from covid.*
>
> *Regardless, something else is very wrong in Britain. Excess deaths are at historic highs and nobody knows why. ONS just released data for the week ending July 22nd, and it shows +21% more weekly deaths over the 2015-2019 average, and substantially more over the 2020-2021 average.*

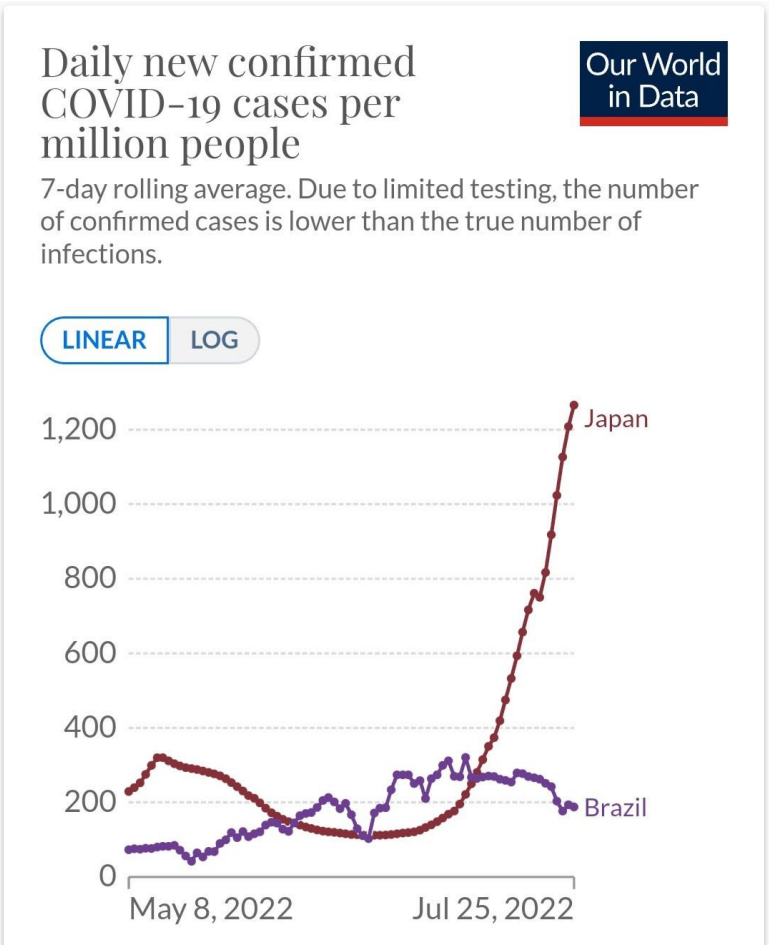


Since the media always LOVES a good scary "mysterious deaths" story, why not this one? They think we don't notice that they can't wait to run a story called "formica — the silent killer," but when 2,000 more people A WEEK are dying than normal: nothing. That's 20,000 mysterious deaths in Britain every ten weeks, assuming the numbers stay flat.

I'm wondering if we need to start a phone and letter campaign demanding investigations into excess deaths. Remember Ontario? "Unknown causes" is now the top killer of Canadians there, far exceeding heart disease, cancer, or even visits from Nancy Pelosi.

If anyone can think of a well-funded government agency that is responsible for looking into threats to public health, let me know.

Folks are starting to compare covid performance in countries that used mRNA vaccines to countries that vaccinated with more traditional vaccines and the differences are striking. For example, take a look at Japan (100% mRNA) versus Brazil (fewer than 50% mRNA):



https://www.coffeeandcovid.com/p/-coffee-and-covid-saturday-august
(credit: Attorney Jeff Childers)

This Board is full of smart and caring individuals so I urge you to see past "medical" advice we are sustaining from the County Health Officer and other politically motivated individuals and recognize their overreaching arguments might be flawed. I humbly request you consider we never again deprive individuals of their bodily autonomy while allowing experimental and forced medical procedures and devices coerced on our populations. While the new monkey pox epidemic is being addressed, I hope we can all see that our CA Legislature may be initiating the monkey pox state of emergency to push an agenda. It's extremely **hypocritical** they are still allowing for San Francisco's Folsom Street Fair (https://www.folsomstreet.org/fs-events), where **behaviors are encouraged that would inflate a spread** if there was one. It's hard to believe it's a serious emergency with that last example. I would hope that we look at these **politically motivated medical recommendations** from a 40,000 foot view when making new mandates that may further detrimentally impact our community. I hope we can help ill individuals find readily available medical assistance but not to the detriment of our bodies, our medical privacy, and our inalienable rights. These impacts over the last few years have been divisive within this community and especially our immediate families! It has also taken time away from solving other truly important problems **as a team**, like homelessness.

Thank you in advance.

Other reference info:
(CalGina: https://www.genome.gov/about-genomics/policy-issues/Genetic-Discrimination)
CIVIL CODE SECTION 51. UNRUH CIVIL RIGHTS ACT: HTTPS://FINDHOALAW.COM/CIVIL-CODE-SECTION-51-UNRUH-CIVIL-RIGHTS-ACT/

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WebMD

Lung Disease & Respiratory Health > Coronavirus > News

WEBMD HEALTH NEWS

Large Remdesivir Study Finds No COVID-19 Survival Benefit

By Damian McNamara

July 16, 2021 -- A lack of consensus regarding the antiviral drug remdesivir to treat people with COVID-19 continues, leaving doctors without clear direction on one of

with a matched group of veterans who did not receive the antiviral, remdesivir did not significantly improve survival rates

The percentages were close: 12.2% of patients in the remdesivir group died within 30 days compared to 10.6% of those in the control group.

At the same time, the study showed remdesivir led to more days in the hospital.

"There is still uncertainty about the role of remdesivir in treatment for people hospitalized with COVID-19," Ohl says.

"It is reasonable to follow the CDC and Infectious Diseases Society of America, "but clinicians should avoid admitting people or keeping people in the hospital solely to receive remdesivir if they do not meet other criteria for hospitalization," says Ohl, lead author and an infectious disease specialist at the Center for Access & Delivery Research and Evaluation, Iowa City Veterans Affairs Health Care System in Iowa City.

The study was published online Thursday in *JAMA Network Open*.

Sticking With the Official Protocol?

The longer hospital stays associated with remdesivir, a median 6 days vs 3 days, could be a result of treating people for 5 or 10 days with the antiviral drug. In other words, it is "possible that clinicians were not discharging patients who otherwise met the criteria for hospital discharge until the remdesivir course was completed," Ohl and colleagues note.

Not doing so, they add, could have resulted in "increased use of scarce hospital beds

IV, they add.

21 USC 360bbb: Expanded access to unapproved therapies and diagnostics
Text contains those laws in effect on August 7, 2022

From Title 21-FOOD AND DRUGS
CHAPTER 9-FEDERAL FOOD, DRUG, AND COSMETIC ACT
SUBCHAPTER V-DRUGS AND DEVICES
Part E-General Provisions Relating to Drugs and Devices
Jump To:

§360bbb. Expanded access to unapproved therapies and diagnostics

(a) Emergency situations

The Secretary may, under appropriate conditions determined by the Secretary, authorize the shipment of investigational drugs or investigational devices for the diagnosis, monitoring, or treatment of a serious disease or condition in emergency situations.

(b) Individual patient access to investigational products intended for serious diseases

Any person, acting through a physician licensed in accordance with State law, may request from a manufacturer or distributor, and any manufacturer or distributor may, after complying with the provisions of this subsection, provide to such physician an investigational drug or investigational device for the diagnosis, monitoring, or treatment of a serious disease or condition if-

(1) the licensed physician determines that the person has no comparable or satisfactory alternative therapy available to diagnose, monitor, or treat the disease or condition involved, and that the probable risk to the person from the investigational drug or investigational device is not greater than the probable risk from the disease or condition;

(2) the Secretary determines that there is sufficient evidence of safety and effectiveness to support the use of the investigational drug or investigational device in the case described in paragraph (1);

(3) the Secretary determines that provision of the investigational drug or investigational device will not interfere with the initiation, conduct, or completion of clinical investigations to support marketing approval; and

(4) the sponsor, or clinical investigator, of the investigational drug or investigational device submits to the Secretary a clinical protocol consistent with the provisions of section 355(i) or 360j(g) of this title, including any regulations promulgated under section 355(i) or 360j(g) of this title, describing the use of the investigational drug or investigational device in a single patient or a small group of patients.

(c) Treatment investigational new drug applications and treatment investigational device exemptions

Upon submission by a sponsor or a physician of a protocol intended to provide widespread access to an investigational drug or investigational device for eligible patients (referred to in this subsection as an "expanded access protocol"), the Secretary shall permit such investigational drug or investigational device to be made available for expanded access under a treatment investigational new drug application or treatment investigational device exemption if the Secretary determines that-

(1) under the treatment investigational new drug application or treatment investigational device exemption, the investigational drug or investigational device is intended for use in the diagnosis, monitoring, or treatment of a serious or immediately life-threatening disease or condition;

(2) there is no comparable or satisfactory alternative therapy available to diagnose, monitor, or treat that stage of disease or condition in the population of patients to which the investigational drug or investigational device is intended to be administered;

(3)(A) the investigational drug or investigational device is under investigation in a controlled clinical trial for the use described in paragraph (1) under an investigational drug application in effect under section 355(i) of this title or investigational device exemption in effect under section 360j(g) of this title; or

(B) all clinical trials necessary for approval of that use of the investigational drug or investigational device have been completed;

(4) the sponsor of the controlled clinical trials is actively pursuing marketing approval of the investigational drug or investigational device for the use described in paragraph (1) with due diligence;

(5) in the case of an investigational drug or investigational device described in paragraph (3)(A), the provision of the investigational drug or investigational device will not interfere with the enrollment of patients in ongoing clinical investigations under section 355(i) or 360j(g) of this title;

(6) in the case of serious diseases, there is sufficient evidence of safety and effectiveness to support the use described in paragraph (1); and

(7) in the case of immediately life-threatening diseases, the available scientific evidence, taken as a whole, provides a reasonable basis to conclude that the investigational drug or investigational device may be effective for its intended use and would not expose patients to an unreasonable and significant risk of illness or injury.

A protocol submitted under this subsection shall be subject to the provisions of section 355(i) or 360j(g) of this title, including regulations promulgated under section 355(i) or 360j(g) of this title. The Secretary may inform national, State, and local medical associations and societies, voluntary health associations, and other appropriate persons about the availability of an investigational drug or investigational device under expanded access protocols submitted under this subsection. The information provided by the Secretary, in accordance with the preceding sentence, shall be the same type of information that is required by section 282(i)(3) of title 42.

(d) Termination

The Secretary may, at any time, with respect to a sponsor, physician, manufacturer, or distributor described in this section, terminate expanded access provided under this section for an investigational drug or investigational device if the requirements under this section are no longer met.

(e) Definitions

In this section, the terms "investigational drug", "investigational device", "treatment investigational new drug application", and "treatment investigational device exemption" shall have the meanings given the terms in regulations prescribed by the Secretary.

(June 25, 1938, ch. 675, §561, as added Pub. L. 105–115, title IV, §402, Nov. 21, 1997, 111 Stat. 2365 ; amended Pub. L. 109–482, title I, §102(f)(2), Jan. 15, 2007, 120 Stat. 3685 .)

Editorial Notes

Amendments

2007-Subsec. (c). Pub. L. 109–482 substituted "section 282(i)(3)" for "section 282(j)(3)" in concluding provisions.

Statutory Notes and Related Subsidiaries

Effective Date of 2007 Amendment

Amendment by Pub. L. 109–482 applicable only with respect to amounts appropriated for fiscal year 2007 or subsequent fiscal years, see section 109 of Pub. L. 109–482, set out as a note under section 281 of Title 42, The Public Health and Welfare.

Effective Date

Section effective 90 days after Nov. 21, 1997, except as otherwise provided, see section 501 of Pub. L. 105–115, set out as an Effective Date of 1997 Amendment note under section 321 of this title.

Investigational Drugs

Pub. L. 115–52, title VI, §610(a), (b), Aug. 18, 2017, 131 Stat. 1051 , 1053, provided that:

"(a) Patient Access to Investigational Drugs.-

"(1) Public meeting.-

"(A) In general.-The Secretary of Health and Human Services (referred to in this section as the 'Secretary'), acting through the Commissioner of Food and Drugs, in coordination with the Director of the National Institutes of Health, and in consultation with patients, health care providers, drug sponsors, bioethicists, and other stakeholders, shall, not later than 270 days after the date of enactment of this Act [Aug. 18, 2007], convene a public meeting to discuss clinical trial inclusion and exclusion criteria to inform the guidance under paragraph (3). The Secretary shall inform the Comptroller General of the United States of the date when the public meeting will take place.

"(B) Topics.-The Secretary shall make available on the internet website of the Food and Drug Administration a report on the topics discussed at the meeting described in subparagraph (A) within 90 days of such meeting. Such topics shall include discussion of-

"(i) the rationale for, and potential barriers for patients created by, research clinical trial inclusion and exclusion criteria;

"(ii) how appropriate patient populations can benefit from the results of trials that employ alternative designs;

"(iii) barriers to participation in clinical trials, including-

"(I) information regarding any potential risks and benefits of participation;

"(II) regulatory, geographical, and socioeconomic barriers; and

"(III) the impact of exclusion criteria on the enrollment in clinical trials of particular populations, including infants and children, pregnant and lactating women, seniors, individuals with advanced disease, and individuals with co-morbid conditions;

"(iv) clinical trial designs and methods, including expanded access trials, that increase enrollment of more diverse patient populations, when appropriate, while facilitating the collection of data to establish safe use and support substantial evidence of effectiveness, including data obtained from expanded access trials; and

"(v) how changes to clinical trial inclusion and exclusion criteria may impact the complexity and length of clinical trials, the data necessary to demonstrate safety and effectiveness, and potential approaches to mitigating those impacts.

"(2) REPORT.-Not later than 1 year after the Secretary issues the report under paragraph (1)(B), the Comptroller General of the United States shall report to the Committee on Health, Education, Labor, and Pensions of the Senate and the Committee on Energy and Commerce of the House of Representatives on individual access to investigational drugs through the expanded access program under section 561(b) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360bbb(b)). The report shall include-

"(A) a description of actions taken by manufacturers and distributors under section 561A of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360bbb–0);

"(B) consideration of whether Form FDA 3926 and the guidance documents titled 'Expanded Access to Investigational Drugs for Treatment Use-Questions and Answers' and 'Individual Patient Expanded Access Applications: Form FDA 3926', issued by the Food and Drug Administration in June 2016, have reduced application burden with respect to individuals and physicians seeking access to investigational new drugs pursuant to section 561(b) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360bbb) and improved clarity for patients, physicians, and drug manufacturers about such process;

"(C) consideration of whether the guidance or regulations issued to implement section 561 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360bbb) have improved access for individual patients to investigational drugs who do not qualify for clinical trials of such investigational drugs, and what barriers to such access remain;

"(D) an assessment of methods patients and health care providers use to engage with the Food and Drug Administration or drug sponsors on expanded access; and

"(E) an analysis of the Secretary's report under paragraph (1)(B).

"(3) GUIDANCE.-

"(A) IN GENERAL.-Not later than 1 year after the publication of the report under paragraph (1)(B), the Secretary, acting through the Commissioner of Food and Drugs, shall issue one or more draft guidances regarding eligibility criteria for clinical trials. Not later than 1 year after the public comment period on each such draft guidance ends, the Secretary shall issue a revised draft guidance or final guidance.

"(B) CONTENTS.-The guidance documents described in subparagraph (A) shall address methodological approaches that a manufacturer or sponsor of an investigation of a new drug may take to-

"(i) broaden eligibility criteria for clinical trials and expanded access trials, especially with respect to drugs for the treatment of serious and life-threatening conditions or diseases for which there is an unmet medical need;

"(ii) develop eligibility criteria for, and increase trial recruitment to, clinical trials so that enrollment in such trials more accurately reflects the patients most likely to receive the drug, as applicable and as appropriate, while establishing safe use and supporting findings of substantial evidence of effectiveness; and

"(iii) use the criteria described in clauses (i) and (ii) in a manner that is appropriate for drugs intended for the treatment of rare diseases or conditions.

"(b) IMPROVING INSTITUTIONAL REVIEW BOARD REVIEW OF SINGLE PATIENT EXPANDED ACCESS PROTOCOL.-Not later than 1 year after the date of enactment of this Act [Aug. 18, 2017], the Secretary, acting through the Commissioner of Food and Drugs, shall issue guidance or regulations, or revise existing guidance or regulations, to streamline the institutional review board review of individual patient expanded access protocols submitted under [section] 561(b) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360bbb(b)). To facilitate the use of expanded access protocols, any guidance or regulations so issued or revised may include a description of the process for any person acting through a physician licensed in accordance with State law to request that an institutional review board chair (or designated member of the institutional review board) review a single patient expanded access protocol submitted under such section 561(b) for a drug.

The Secretary shall update any relevant forms associated with individual patient expanded access requests under such section 561(b) as necessary."

From:	Stand Up Sacramento County
To:	Clerk of the Board Public Email
Cc:	Frost. Supervisor; Kennedy. Supervisor; Supervisor Serna; Nottoli. Don; Rich Desmond
Subject:	Public Comment - August 9th: Agenda Item 42
Date:	Monday, August 8, 2022 9:12:52 AM
Attachments:	Package Insert - JYNNEOS.pdf Monkeypox Global Pandemic Simulation Held Last Year.pdf Did You Know That A 2021 Report Predicted The Monkeypox Outbreak On May 15th 2022 - Copy.pdf A review of experimental and natural infections of animals with monkeypox.pdf Viral Shedding form Vaccines.pdf

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Dear Board,

With Newsom's emergency declaration on Monkeypox and the fast track into the County Agenda for Ratification of the Proclamations of Local State of Emergency and a Local Public Health Emergency for Monkeypox, we wonder if you've learned anything in the last 2 years on the Covid19 response that will have you pause and refuse to ratify these proclamations?

Our hope is that you are wiser, shrewder about the information being presented to you: filtering out emotion from facts, fear mongering from truth. By now at least you should realize that those of us that members of this Board called conspiracy theorists in 2020 when we sounded the alarm on coming mandates around vaccines and more, are in fact well educated researchers. Our critical thinking hats function very well. Here is what we know about monkeypox:

For over a year, Monkeypox has been pushed in the media as the next pandemic. For over a year, people have watched it, dug into research on it, and monitored what the World Economic Forum, WHO and others are doing surrounding it. JUST LIKE COVID19, there was a Pandemic Simulation done (see Monkeypox Global Pandemic Simulation attached). Just like Covid19, the outbreak was predicted, except this time, they called the case spike date of May 2022 exactly, as though they know those in a position to do anything in halting government overreach on this, will brush that off as crazy (see Did you know that a 2021 Report Predicted...).

Ther are no deaths from monkeypox at this time nor is there an emergent level of cases. All these proclamations will do is allow for the flow of money, tax dollars from federal to state, further increasing inflation, and further increasing the size of agencies like Public Health to track and trace a non-emergent disease. County Council through conversations with Public health has already advised twice that the Locate State of Emergency Proclamation for COVID19 be left in place for the acquisition of grant funding. Twice on record it has been acknowledged that there is no actual emergency to the people. The death rate for covid being 0.22% per the Health Services report in this same agenda packet. There is no Covid19 "emergency" and keeping it in place violates Government Code 8558(c). We know that. You know that. County Counsel knows it and it stays in place under the shelter of the state not rescinding theirs. Yet here we are, with it still in place and another two being attempted to go in place for another NON-emergent disease.

We ask that you OPPOSE this ratification. Please read the attached articles, two referenced above, one going over Experimental and Natural Infection of years of study, the package insert for JYNNEOS, the live viral smallpox vaccine that is being pushed to help, and lastly a paper on Viral Shedding from Vaccines. The last paper attached because we fully expect that what is now predominantly being seen in the Male, homosexual community, will be seen in all sectors

of the community after JYNNEOS is more widely available. The solution to a small problem will be the creator of a more wide-spread one.

The precedent that will be set with this ratification, for a non-emergent disease, will allow for so many other Proclamations down the road. With such a low case rate and significantly low risk to the population at large, we could apply the same parameters in declaring the Influenza A a pandemic, the common cold, spikes in mosquito populations leading to increased bites, the options are endless to 1) open the county up to no-bid contracts, 2) open up accessibility to funding applications to cushion the budget, and 3) transfer a significant amount of power to an Appointed agency such as Public Health.

There will always be a Pandemic from here on out with so low a bar to access money, contracts, resources and power. Covid19 was the canary in the coal mine. You ratify these Proclamations and you set the precedent.

Please vote No or Abstain.

Thank you,
Gabrielle Ingram,
Founder of Stand Up Sacramento County, representing thousands in the community.

P.S. There are effective therapies, natural ones, that will quickly deal with monkeypox without the need for anti-virals or JYNNEOS. We aren't dumb enough to put that information here though and have what was done with effective therapeutics surrounding Covid19, done here. There never needed to be any death with Covid but there was, and it was at the hand of tyrannical government and Public Health. Let's not repeat history with monkeypox.
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Stand Up Sacramento County
Facebook: https://www.facebook.com/groups/standupsaccounty/
Telegram: https://t.me/standupsacramentocounty

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use JYNNEOS safely and effectively. See full prescribing information for JYNNEOS.

JYNNEOS (Smallpox and Monkeypox Vaccine, Live, Non-replicating) suspension for subcutaneous injection
Initial U.S. Approval: 2019

----------INDICATIONS AND USAGE----------
JYNNEOS is a vaccine indicated for prevention of smallpox and monkeypox disease in adults 18 years of age and older determined to be at high risk for smallpox or monkeypox infection. (1)

----------DOSAGE AND ADMINISTRATION----------
For subcutaneous injection only.
Administer two doses (0.5 mL each) 4 weeks apart. (2.1, 2.2)

----------DOSAGE FORMS AND STRENGTHS----------
Suspension for injection. Each dose (0.5 mL) is supplied in a single-dose vial. (3)

----------ADVERSE REACTIONS----------
- In smallpox vaccine-naïve healthy adults, the most common (> 10%) solicited injection site reactions were pain (84.9%), redness (60.8%), swelling (51.6%), induration (45.4%), and itching (43.1%); the most common solicited systemic adverse reactions were muscle pain (42.8%), headache (34.8%), fatigue (30.4%), nausea (17.3%) and chills (10.4%). (6.1)
- In healthy adults previously vaccinated with a smallpox vaccine, the most common (> 10%) solicited injection site reactions were redness (80.9%), pain (79.5%), induration (70.4%), swelling (67.2%), and itching (32.0%); the most common solicited systemic adverse reactions were fatigue (33.5%), headache (27.6%), and muscle pain (21.5%). (6.1)
- The frequencies of solicited local and systemic adverse reactions among adults with HIV-infection and adults with atopic dermatitis were generally similar to those observed in healthy adults. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Bavarian Nordic at toll-free phone 1-800-675-9596 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION

Revised: 06/2021

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

JYNNEOS is a vaccine indicated for prevention of smallpox and monkeypox disease in adults 18 years of age and older determined to be at high risk for smallpox or monkeypox infection.

2 DOSAGE AND ADMINISTRATION

For subcutaneous injection only.

2.1 Dose and Schedule

Administer two doses (0.5 mL each) of JYNNEOS 4 weeks apart.

2.2 Preparation and Administration

Allow the vaccine to thaw and reach room temperature before use. Once thawed, the vaccine may be kept at +2°C to +8°C (+36°F to +46°F) for 12 hours. Do not refreeze.

When thawed, JYNNEOS is a milky, light yellow to pale white colored suspension.
Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered.

Swirl the vial gently before use for at least 30 seconds. Withdraw a dose of 0.5 mL into a sterile syringe for injection.

Administer JYNNEOS by subcutaneous injection, preferably into the upper arm (deltoid).

3 DOSAGE FORMS AND STRENGTHS

JYNNEOS is a suspension for injection. Each dose (0.5 mL) is supplied in a single-dose vial.

5 WARNINGS AND PRECAUTIONS

5.1 Severe Allergic Reactions

Appropriate medical treatment must be available to manage possible anaphylactic reactions following administration of JYNNEOS.

Persons who experienced a severe allergic reaction following a previous dose of JYNNEOS or following exposure to any component of JYNNEOS may be at increased risk for severe allergic reactions after JYNNEOS. The risk for a severe allergic reaction should be weighed against the risk for disease due to smallpox or monkeypox.

5.2 Altered Immunocompetence

Immunocompromised persons, including those receiving immunosuppressive therapy, may have a diminished immune response to JYNNEOS.

5.3 Limitations of Vaccine Effectiveness

Vaccination with JYNNEOS may not protect all recipients.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine, and may not reflect the rates observed in practice. There is the possibility that broad use of JYNNEOS could reveal adverse reactions not observed in clinical trials.

The overall clinical trial program included 22 studies and a total of 7,859 individuals 18 through 80 years of age who received at least 1 dose of JYNNEOS (7,093 smallpox vaccine-naïve and 766 smallpox vaccine-experienced individuals).

Solicited Adverse Reactions

Solicited Adverse Reactions in Smallpox Vaccine-Naïve Individuals:

The safety of JYNNEOS in smallpox vaccine-naïve individuals was evaluated in Study 1 [1], a randomized, double-blind, placebo-controlled study conducted in the US in which vaccinia-naïve adults ages 18 to 40 years received either two doses of JYNNEOS (N=3003), or two injections of Tris-Buffered Saline (placebo, N=1002) four weeks apart.

In the total study population, the mean age was 28 years; 47.9% of the subjects were men; 77.4% were white/Caucasian, 17.8% black/African American, 1.9% Asian, 0.5% American Indian/Alaska Native, 0.4% Native Hawaiian/Other Pacific, 1.9% other racial groups; and 11.4% of subjects were of Hispanic/Latino ethnicity. The demographic compositions of JYNNEOS and placebo groups were similar.

In Study 1, subjects were monitored for local and systemic adverse reactions using diary cards for an 8-day period starting on the day of each vaccination. The frequencies of solicited local and systemic adverse reactions following any dose of JYNNEOS are presented in Table 1.

Table 1: Percentages of Subjects with Solicited Local Injection Site Reactions and Systemic Adverse Reactions within 8 Days of Administration of Any Dose of JYNNEOS in Adults 18 to 40 Years of Age, Study 1[x]

Reaction	JYNNEOS N=2943 %	Placebo N=980 %
Local (Injection site)	--	--
Pain	84.9	19.1
Pain, Grade 3[a]	7.4	1.0
Redness	60.8	17.7
Redness ≥ 100 mm	1.5	0.0
Swelling	51.6	5.6
Swelling ≥ 100 mm	0.8	0.0
Induration	45.4	4.6
Induration ≥ 100 mm	0.3	0.0
Itching	43.1	11.7
Itching, Grade 3[b]	1.6	0.2
Systemic	--	--
Muscle Pain	42.8	17.6
Muscle Pain, Grade 3[b]	2.6	0.7
Headache	34.8	25.6
Headache, Grade 3[b]	2.4	2.1
Fatigue	30.4	20.5
Fatigue, Grade 3[b]	3.0	1.3
Nausea	17.3	13.1
Nausea, Grade 3[b]	1.5	1.2
Chills	10.4	5.8
Chills, Grade 3[b]	1.0	0.3
Fever[c]	1.7	0.9
Fever, Grade ≥ 3[c]	0.2	0.0

[X] NCT01144637

[a] Grade 3 pain defined as spontaneously painful

[b] Grade 3 itching, muscle pain, headache, fatigue, nausea and chills defined as preventing routine daily activities

[c] Fever defined as oral temperature ≥ 100.4°F (≥ 38°C), Grade ≥ 3 fever defined as ≥ 102.2°F (≥ 39.0°C)

N=number of subjects

In Study 1, the majority of solicited local and systemic adverse reactions reported with JYNNEOS had a median duration of 1 to 6 days. In general, there were similar proportions of subjects reporting solicited local or systemic reactions of any severity after Dose 2 of JYNNEOS compared with Dose 1, with the exception of injection site pain, which was more commonly reported following Dose 1 (79.3%) than Dose 2 (69.9%).

Solicited Adverse Reactions in Persons Previously Vaccinated with a Smallpox Vaccine:

Three studies (Study 2, Study 3, and Study 4, [2-4]) conducted in the US and Germany evaluated the safety of JYNNEOS in 409 persons previously vaccinated with a smallpox vaccine who received one or two doses of JYNNEOS (mean age 39 years, range 20-80 years; 59% women; 98.8% white/Caucasian; 0.7% Asian; 0.5% black/African American). Subjects were monitored for local and systemic adverse reactions using diary cards for an 8-day period starting on the day of each

vaccination. Across all three studies, solicited local adverse reactions reported following any dose of JYNNEOS were redness (80.9%), pain (79.5%), induration (70.4%), swelling (67.2%), and itching (32.0%) at the injection site; solicited systemic adverse reactions reported following any dose of JYNNEOS were fatigue (33.5%), headache (27.6%), muscle pain (21.5%), nausea (9.8%), chills (0.7%), and fever (0.5%).

Solicited Adverse Reactions in HIV-infected Individuals:

The safety of JYNNEOS in HIV-infected individuals was evaluated in Study 5 [5], an open label trial conducted in the US that included 351 HIV-infected smallpox vaccine-naïve subjects, 131 HIV--infected subjects who previously received smallpox vaccine, 88 non-HIV-infected smallpox vaccine-naïve subjects and 9 non-HIV-infected subjects who had previously received a smallpox vaccine. The racial/ethnic and gender compositions of HIV-infected smallpox vaccine-naïve subjects and those who had previously received smallpox vaccine were similar and overall were 17.0% women; 45.8% white/Caucasian; 0.4% Asian; 33.2% black/African American; 19.0% Hispanic/Latino ethnicity; the HIV-infected smallpox vaccine-naïve group tended to be younger (mean age 37 years) compared to those who had previously received a smallpox vaccine (mean age 45 years). Subjects had CD4 counts ≥ 200 and ≤ 750 cells/μL at study entry.

Solicited local and systemic adverse reactions were reported at similar or lower frequencies in HIV-infected smallpox vaccine-naïve subjects as compared to those seen in non-HIV-infected smallpox vaccine-naive individuals in this study.

In HIV-infected subjects with previous smallpox vaccine exposure, fever and chills were reported in 1.5% and 8.4% of subjects respectively. Frequencies of other solicited local and general adverse reactions in this population were similar to those reported in Studies 2-4 in non-HIV-infected subjects who had previously received smallpox vaccination.

Solicited Adverse Reactions in Individuals with Atopic Dermatitis:

The safety of JYNNEOS in smallpox vaccine-naïve subjects with currently active or a history of atopic dermatitis (AD) was evaluated in a multicenter, open-label clinical study (Study 6 [6]) conducted in the US and Mexico that included 350 subjects with AD and 282 subjects without AD. In the overall study the mean age of subjects was 27 years (range 18-42 years), and subjects were 59.0% women, 39.4% white/Caucasian, 10.9% Asian, 9.0% black/African American, 2.2% Other, and 38.4% Hispanic/Latino ethnicity. Demographic compositions were similar between subjects with and without AD. In subjects with AD, solicited local and systemic adverse reactions were reported at similar frequencies as those in subjects without AD in this study, with the exception of redness (61.2% with AD vs. 49.3% without AD), swelling (52.2% with AD vs. 40.8% without AD), chills (15.9% with AD vs. 7.8% without AD) and headache (47.2% with AD vs. 34.8% without AD).

Serious Adverse Events

The integrated analyses of serious adverse events (SAEs) pooled safety data across 22 studies, which included a total of 7,093 smallpox vaccine-naïve subjects and 766 smallpox vaccine-experienced subjects who received at least 1 dose of JYNNEOS and 1,206 smallpox vaccine-naïve subjects who received placebo only. SAEs were monitored from the day of the first study vaccination through at least 6 months after the last study vaccination.

Among the smallpox vaccine-naïve subjects, SAEs were reported for 1.5% of JYNNEOS recipients and 1.1% of placebo recipients. Among the smallpox vaccine-experienced subjects enrolled in studies without a placebo comparator, SAEs were reported for 2.3% of JYNNEOS recipients. Across all studies, a causal relationship to JYNNEOS could not be excluded for 4 SAEs, all non-fatal, which included Crohn's disease, sarcoidosis, extraocular muscle paresis and throat tightness.

Cardiac Adverse Events of Special Interest

Evaluation of cardiac adverse events of special interest (AESIs) included any cardiac signs or symptoms, ECG changes determined to be clinically significant, or troponin-I elevated above 2 times the upper limit of normal. In the 22 studies, subjects were monitored for cardiac-related signs or symptoms through at least 6 months after the last vaccination.

The numbers of JYNNEOS and placebo recipients, respectively, with troponin-I data were: baseline level (6,376 and 1,203); level two weeks after first dose (6,279 and 1,166); level two weeks after second dose (1,683 and 193); unscheduled visit, including for clinical evaluation of suspected cardiac adverse events (500 and 60).

Cardiac AESIs were reported to occur in 1.3% (95/7,093) of JYNNEOS recipients and 0.2% (3/1,206) of placebo recipients who were smallpox vaccine-naïve. Cardiac AESIs were reported to occur in 2.1% (16/766) of JYNNEOS recipients who were smallpox vaccine-experienced. The higher proportion of JYNNEOS recipients who experienced cardiac AESIs was driven by 28 cases of asymptomatic post-vaccination elevation of troponin-I in two studies: Study 5, which enrolled 482 HIV-infected subjects and 97 healthy subjects, and Study 6, which enrolled 350 subjects with atopic dermatitis and 282 healthy subjects. An additional 127 cases of asymptomatic post-vaccination elevation of troponin-I above the upper limit of normal but not above 2 times the upper limit of normal were documented in JYNNEOS recipients throughout the clinical development program, 124 of which occurred in Study 5 and Study 6. Proportions of subjects with troponin-I elevations were similar between healthy and HIV-infected subjects in Study 5 and between healthy and atopic dermatitis subjects in Study 6. A different troponin assay was used in these two studies compared to the other studies, and these two studies had no placebo controls. The clinical significance of these asymptomatic post-vaccination elevations of troponin-I is unknown.

Among the cardiac AESIs reported, 6 cases (0.08%) were considered to be causally related to JYNNEOS vaccination and included tachycardia, electrocardiogram T wave inversion, electrocardiogram abnormal, electrocardiogram ST segment elevation, electrocardiogram T wave abnormal, and palpitations.

None of the cardiac AESIs considered causally related to study vaccination were considered serious.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically

recognized pregnancies is 2% to 4% and 15% to 20%, respectively. Available human data on JYNNEOS administered to pregnant women are insufficient to inform vaccine-associated risks in pregnancy.

The effect of JYNNEOS on embryo-fetal and post-natal development was evaluated in four developmental toxicity studies conducted in female rats and rabbits. In two studies, rats were administered a single human dose of JYNNEOS (0.5 mL) once prior to mating and on one or two occasions during gestation. In the third study, rats were administered a single human dose of JYNNEOS (0.5 mL) on two occasions during gestation. In the fourth study, rabbits were administered a single human dose of JYNNEOS (0.5 mL) once prior to mating and on two occasions during gestation. These animal studies revealed no evidence of harm to the fetus *[see Data]*.

Data

Animal Data
Developmental toxicity studies were conducted in female rats and rabbits. In one study, female rabbits were administered a single human dose of JYNNEOS (0.5 mL) by the subcutaneous route on three occasions: prior to mating, and on gestation days 0 and 14. Three studies were conducted in female rats administered a single human dose of JYNNEOS (0.5 mL) by the subcutaneous route on two or three occasions: prior to mating, and on gestation days 0 and 14; or prior to mating, and on gestation day 0; or on gestation days 0 and 6. No vaccine-related fetal malformations or variations and adverse effects on female fertility or pre-weaning development were reported in these studies.

8.2 Lactation

Risk Summary

It is not known whether JYNNEOS is excreted in human milk. Data are not available to assess the effects of JYNNEOS in the breastfed infant or on milk production/excretion.

The development and health benefits of breastfeeding should be considered along with the mother's clinical need for JYNNEOS and any potential adverse effects on the breastfed child from JYNNEOS or from the underlying maternal condition. For preventive vaccines, the underlying condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of JYNNEOS have not been established in individuals less than 18 years of age.

8.5 Geriatric Use

Forty-two smallpox vaccine-experienced adults 65 to 80 years of age received at least one dose of JYNNEOS (Study 4).

Clinical studies of JYNNEOS did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects.

11 DESCRIPTION

When thawed, JYNNEOS (Smallpox and Monkeypox Vaccine, Live, Non-replicating) is a milky, light yellow to pale white colored suspension for subcutaneous injection.

JYNNEOS is a live vaccine produced from the strain Modified Vaccinia Ankara-Bavarian Nordic (MVA-BN), an attenuated, non-replicating orthopoxvirus. MVA-BN is grown in primary Chicken Embryo Fibroblast (CEF) cells suspended in a serum-free medium containing no material of direct animal origin, harvested from the CEF cells, purified and concentrated by several Tangential Flow Filtration (TFF) steps including benzonase digestion. Each 0.5 mL dose is formulated to contain 0.5×10^8 to 3.95×10^8 infectious units of MVA-BN live virus in 10 mM Tris (tromethamine), 140 mM sodium chloride at pH 7.7. Each 0.5 mL dose may contain residual amounts of host-cell DNA (≤ 20 mcg), protein (≤ 500 mcg), benzonase (≤ 0.0025 mcg), gentamicin (≤ 0.163 mcg) and ciprofloxacin (≤ 0.005 mcg).

JYNNEOS is a sterile vaccine formulated without preservatives. The vial stoppers are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

JYNNEOS is an attenuated, live, non-replicating smallpox and monkeypox vaccine that elicits humoral and cellular immune responses to orthopoxviruses. Vaccinia neutralizing antibody responses in humans were evaluated to establish the effectiveness of JYNNEOS for prevention of smallpox and monkeypox.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

JYNNEOS has not been evaluated for carcinogenic or mutagenic potential, or for impairment of male fertility in animals. Developmental toxicity studies conducted in rats and rabbits vaccinated with JYNNEOS revealed no evidence of impaired female fertility *[see Use in Specific Populations (8.1)]*.

13.2 Animal Toxicology and/or Pharmacology

The efficacy of JYNNEOS to protect cynomolgus macaques (*Macaca fascicularis*) against a monkeypox virus (MPXV) challenge was evaluated in several studies. Animals were administered Tris-Buffered Saline (placebo) or JYNNEOS (1×10^8 $TCID_{50}$) sub-cutaneously on day 0 and day 28. On day 63, animals were challenged with MPXV delivered by aerosol (3×10^5 pfu), intravenous (5×10^7 pfu) or intratracheal (5×10^6 pfu) route. Across all studies, 80-100% of JYNNEOS-vaccinated animals survived compared to 0-40% of control animals.

14 CLINICAL STUDIES

14.1 Vaccine Effectiveness

Vaccine effectiveness against smallpox was inferred by comparing the immunogenicity of JYNNEOS to a licensed smallpox vaccine (ACAM2000) based on a Plaque Reduction Neutralization Test (PRNT) using the Western Reserve strain of vaccinia virus and was supported by efficacy data from animal challenge studies. *[see Nonclinical Toxicology (13.2)]*

Vaccine effectiveness against monkeypox was inferred from the immunogenicity of JYNNEOS in a clinical study and from efficacy data from animal challenge studies. *[see Nonclinical Toxicology (13.2)]*

14.2 Immunogenicity

Study 7 [7] (N=433) was a randomized, open-label study conducted at US military facilities in South Korea to compare the immunogenicity of JYNNEOS to ACAM2000 in healthy smallpox vaccine-naïve adults 18 through 42 years of age. Subjects were randomized to receive either two doses of JYNNEOS (N=220) administered 28 days apart or one dose of ACAM2000 (N=213). In the total study population, the mean age was 24 years and 23 years in subjects receiving JYNNEOS and ACAM2000, respectively; 82.3% and 86.4% of the subjects were men; 57.3% and 63.8% were white/Caucasian, 21.8% and 18.8% black/African American, 6.4% and 5.6% Asian, 3.6% and 2.8% American Indian/Alaska Native, 2.3% and 1.4% Native Hawaiian/Other Pacific, 8.6% and 7.5% other racial groups, and 24.5% and 18.8% of Hispanic/Latino ethnicity (JYNNEOS and ACAM2000, respectively).

The primary immunogenicity endpoint was geometric mean titer (GMT) of vaccinia neutralizing antibodies assessed by PRNT at “peak visits” defined as two weeks after the second dose of JYNNEOS and four weeks after the single dose of ACAM2000. Analyses of antibody responses were performed in the per-protocol immunogenicity (PPI) population, consisting of individuals who received all vaccinations and completed all visits up until the peak visit without major protocol violations pertaining to immunogenicity assessments. Table 2 presents the pre-vaccination and “peak visit” PRNT GMTs from Study 7.

Table 2: Comparison of Vaccinia-Neutralizing Antibody Responses Following Vaccination with JYNNEOS or ACAM2000 in Healthy Smallpox Vaccine-Naïve Adults 18 through 42 Years of Age, Study 7[x], Per Protocol Set for Immunogenicity[y]

Time Point	JYNNEOS[a] (N=185) GMT[b] [95% CI]	ACAM2000[a] (N=186) GMT[b] [95% CI]
Pre-Vaccination	10.1 [9.9, 10.2]	10.0 [10.0, 10.0]
Post-Vaccination "Peak Visit"[y]	152.8[c] [133.3, 175.0]	84.4[c] [73.4, 97.0]

[x] NCT01913353

[y] Per Protocol Set for Immunogenicity included subjects who received all vaccinations, completed all visits up until the specified "peak visits" (two weeks after the second dose of JYNNEOS or 4 weeks after the single dose of ACAM2000) without major protocol violations pertaining to immunogenicity assessments.

[a] JYNNEOS was administered as a series of two doses given 28 days apart, and ACAM2000 was administered as a single dose.

[b] GMT of vaccinia-neutralizing antibody titers assessed by plaque reduction neutralization test (PRNT) using the Western Reserve vaccinia strain. Values below the assay lower limit of quantitation (LLOQ) of 20 were imputed to a titer of 10; the proportions of subjects with pre-vaccination titers less than the assay lower limit of detection were 98.9% among subjects randomized to JYNNEOS and 97.8% among subjects randomized to ACAM2000, respectively.

[c] Non-inferiority of the "peak visit" PRNT GMT for JYNNEOS compared to ACAM2000 was demonstrated as the lower bound of the 1-sided 97.5% CI for the GMT ratio (JYNNEOS/ACAM2000) was > 0.5.

N: Number of subjects in the specified treatment group; GMT: Geometric Mean Titer; 95% CI: 95% confidence interval, lower limit and upper limit.

PRNT GMTs were also evaluated at pre-specified time points post-vaccination and prior to the "peak visits". The PRNT GMTs at two and four weeks after the first dose of JYNNEOS (prior to the second dose), were 23.4 (95% CI: 20.5, 26.7) and 23.5 (95% CI: 20.6, 26.9), respectively. The PRNT GMT at two weeks after the single dose of ACAM2000 was 23.7 (95% CI: 20.9, 26.8).

15 REFERENCES

1. Study 1: NCT01144637
2. Study 2: NCT00316524
3. Study 3: NCT00686582
4. Study 4: NCT00857493
5. Study 5: NCT00316589
6. Study 6: NCT00316602
7. Study 7: NCT01913353

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

Package of 20 single-dose vials (Package NDC number: 50632-001-02; Vial NDC number: 50632-001-01)

16.2 Storage Conditions

Keep frozen at -25°C to -15°C (-13°F to +5°F).
Store in the original package to protect from light.
Do not re-freeze a vial once it has been thawed.
Once thawed, the vaccine may be kept at +2°C to +8°C (+36°F to +46°F) for 12 hours.
Do not use the vaccine after the expiration date shown on the vial label.

17 PATIENT COUNSELING INFORMATION

- Inform vaccine recipient of the potential benefits and risks of vaccination with JYNNEOS.
- Inform vaccine recipient of the importance of completing the two dose vaccination series.
- Advise vaccine recipient to report any adverse events to their healthcare provider or to the Vaccine Adverse Event Reporting System at 1-800-822-7967 and www.vaers.hhs.gov.

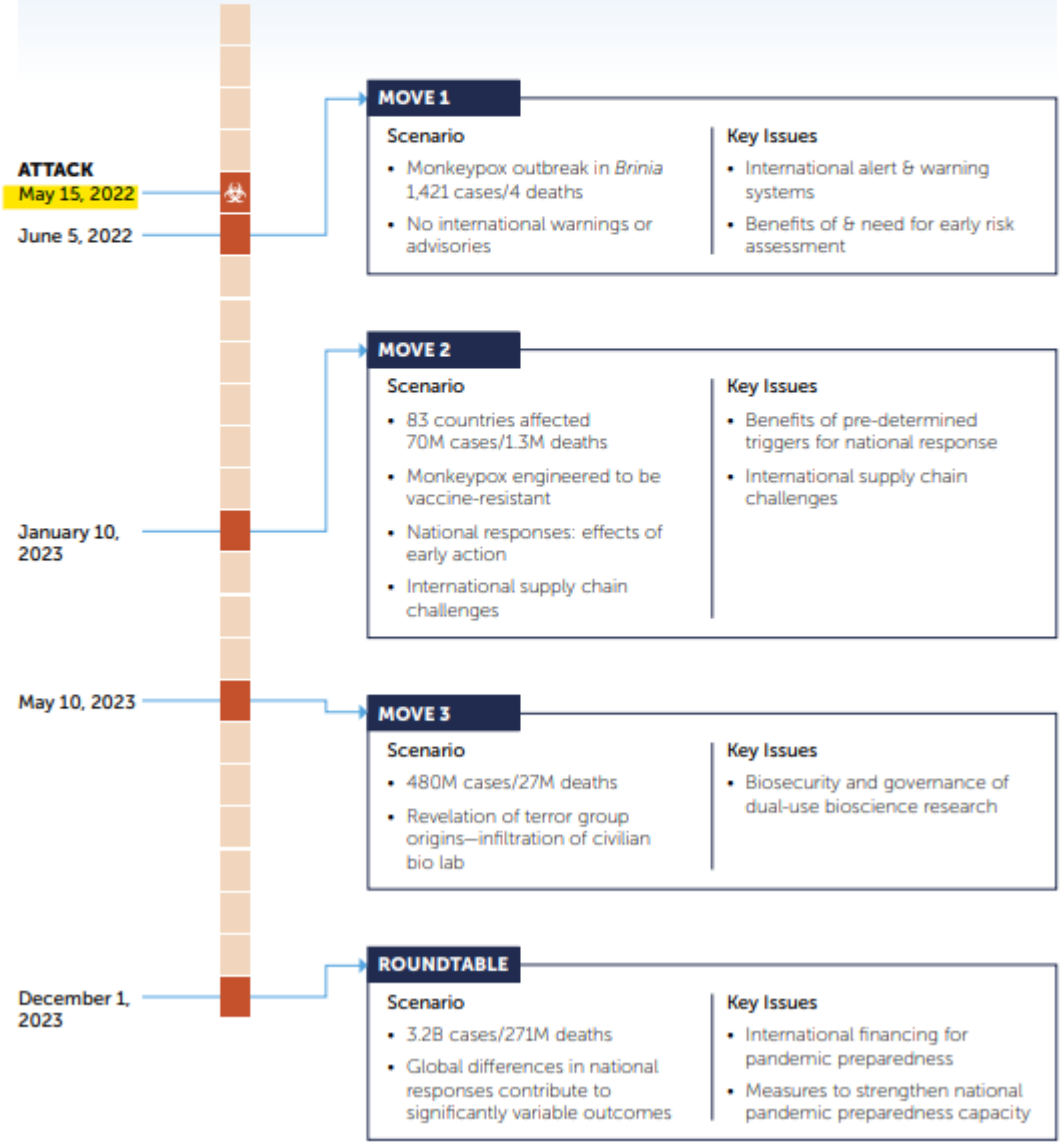
Manufactured by:
Bavarian Nordic A/S
Hejreskovvej 10a
DK-3490 Kvistgaard
Denmark

Here We Go Again: Monkeypox ‘Global Pandemic’ Simulation Held Just Last Year

Elite media outlets around the world are on red alert over the world’s first-ever global outbreak of Monkeypox in mid-May 2022 – just one year after an international biosecurity conference in Munich held a simulation of a “global pandemic involving an unusual strain of Monkeypox” beginning in mid-May 2022.

The discussion was organized into three sequential "moves" corresponding with scenario developments, followed by a roundtable discussion of broader biosecurity and pandemic preparedness issues. The step-by-step approach to revealing scenario developments reflected the limitations of information available to real-world decision makers, as well as the resulting uncertainty associated with a pandemic of unknown origin (see Figure 1).

Figure 1. Scenario Design Summary



Monkeypox was first identified in 1958, but there's never been a global Monkeypox outbreak outside of Africa until now—in the exact week of the exact month predicted by the biosecurity folks in their pandemic simulation. Take these guys to Vegas!

Ed Yong, who's penned dozens of hysterical articles on Covid for The Atlantic including such gems as COVID-19 Long-Haulers Are Fighting for Their Future, Even Health-Care Workers With

Long COVID Are Being Dismissed, How Did This Many Deaths Become Normal? and The Final Pandemic Betrayal, is hot on the scene of the new Monkeypox outbreak.

Eric Feigl-Ding is also all over this.

> *BREAKING—The first confirmed case of #monkeypox in the United States this year just confirmed in a Boston individual who recently travelled to Canada, officials said, as concern rises over the spread of the infectious virus in multiple countries, now US.https://t.co/W0OXFYemYT pic.twitter.com/jwJgbJ8G8q*
>
> *— Eric Feigl-Ding (@DrEricDing) May 18, 2022*

Epidemiologists Jennifer Nuzzo and Bill Hanage are on the scene — but still no word from them as to whether they see anything strange about the first-ever global Monkeypox outbreak occurring in mid-May 2022, a year after they acted as advisers on an international biosecurity simulation of a global Monkeypox outbreak occurring in mid-May 2022.

Appendix A. Expert Contributors to Scenario Development

NTI convened a diverse group of experts in December 2020 to advise on the tabletop exercise scenario. These experts participated as individuals—not as representatives of their respective organizations—and they do not necessarily endorse the recommendations in this report.

Dr. Hillary Carter
Senior Advisor in the Countering Weapons of Mass Destruction Office
Department of Homeland Security

Dr. Sarah Carter
Principal
Science Policy Consulting, LLC

Dr. Bradley Dickerson
Senior Manager, Global Chemical and Biological Security
Sandia National Laboratories

Dr. Diane DiEuliis
Senior Fellow
National Defense University

Dr. James Diggans
Director, Data Science and Biosecurity
Twist Biosciences

Dr. Jessica Dymond
Assistant Program Area Manager, Health Protection and Assurance, National Health
John Hopkins Applied Physics Laboratory

Dr. Dylan George
Vice President
Ginkgo Bioworks
Former Vice President, Technical Staff
In-Q-Tel

Dr. John Glass
Professor and Leader, JCVI Synthetic Biology Group
J. Craig Venter Institute

Amanda Glassman
Executive Vice President and Senior Fellow
Center for Global Development

Dr. William Hanage
Associate Professor of Epidemiology
Harvard T.H. Chan School of Public Health

Jeremy Konyndyk
Executive Director of the COVID-19 Task Force and Senior Advisor
United States Agency for International Development (USAID)

Amb. (ret.) Bob Mikulak
Expert Advisor on Chemical and Biological Weapons Issues
U.S. Department of State

Ryan Morhard
Director, Policy and Partnerships, Concentric
Ginkgo Bioworks

Dr. Jennifer Nuzzo
Senior Scholar and Visiting Faculty, Center for Health Security
John Hopkins Bloomberg School of Public Health

Dr. Megan Palmer
Executive Director of Bio Policy & Leadership Initiatives, Department of Bioengineering
Stanford University

Chris Park
Senior Advisor, International Security and Nonproliferation
Office of the Under Secretary for Arms Control and International Security
U.S. Department of State

Carolyn Reynolds
Co-Founder
Pandemic Action Network

Deborah Rosenblum
Executive Vice President
Nuclear Threat Initiative

Jonas Sandbrink
Biosecurity Researcher
Future of Humanity Institute

The US Government is hot on the scene with an order of 13 million Monkeypox vaccine doses from Bavarian Nordic.

The WHO is on the scene.

Monkeypox: WHO convenes emergency meeting as UK cases 'double'

It comes as the WHO convenes a meeting of monkeypox experts to discuss the worldwide outbreak

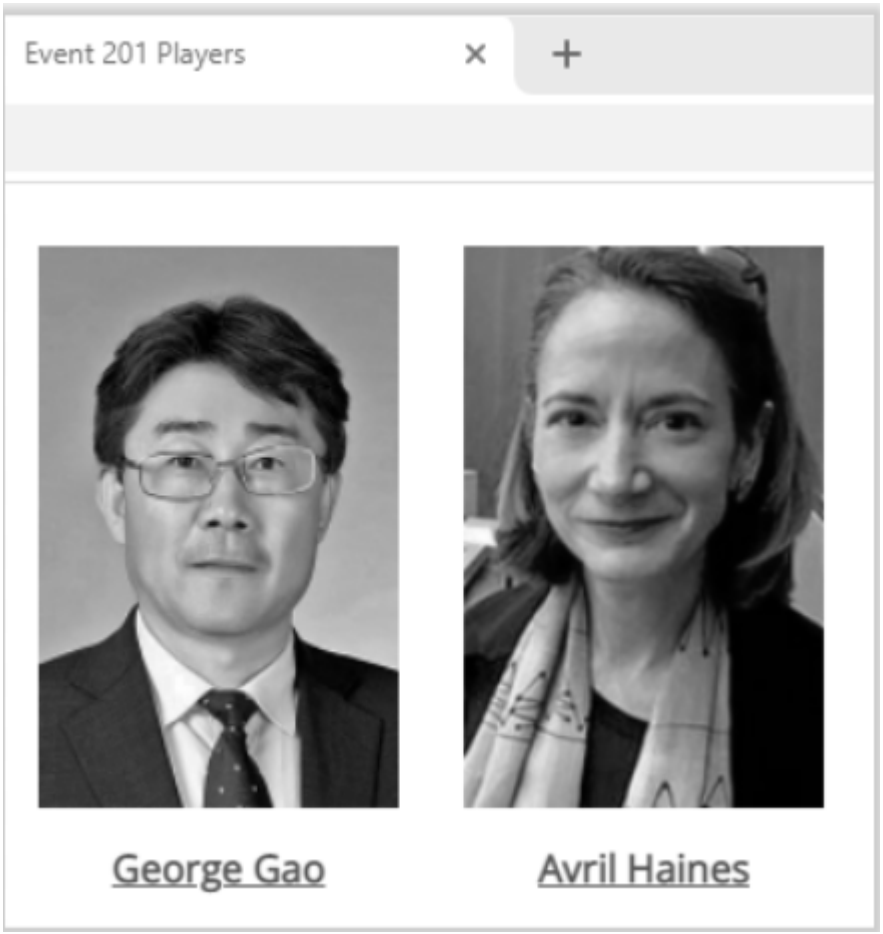
The global Monkeypox outbreak – occurring on the exact timeline predicted by a biosecurity simulation of a global Monkeypox outbreak a year prior – bears a striking resemblance to the outbreak of COVID-19 just months after Event 201, a simulation of a coronavirus pandemic almost exactly like COVID-19.

Event 201 was hosted in October 2019 – just two months before the coronavirus was first revealed in Wuhan – by the Gates Foundation, the World Economic Forum, Bloomberg, and Johns Hopkins. As with the Event 201, the participants at the Monkeypox simulation have thus far been stone silent as to their having participated in a pandemic simulation the facts of which happened to come true in real life just months later.

One person who was present at both Event 201 and the Monkeypox simulation is George Fu Gao, director of the Chinese Center for Disease Control. At event 201, Gao specifically raised the point of countering "misinformation" during a "hypothetical" coronavirus pandemic.

> *Never forget. 37/ pic.twitter.com/4b7QkVO26o*
>
> *– LLadany (@lladany) March 29, 2022*

Here's Gao at Event 201 right next to our very own Avril Haines, Director of National Intelligence—technically the highest-level intelligence official in the United States. Look at these cuties. Doesn't that make you feel all warm and fuzzy? Phew. Making Kim Philby jealous.



That said, I won't sit here and debate wild conspiracy theories that there might be anything unusual about a global pandemic occurring just months after a simulation of a global pandemic of exactly that kind, followed shortly after by the first-ever global outbreak of an even-more-obscure virus just months after a simulation of an outbreak of exactly that kind.

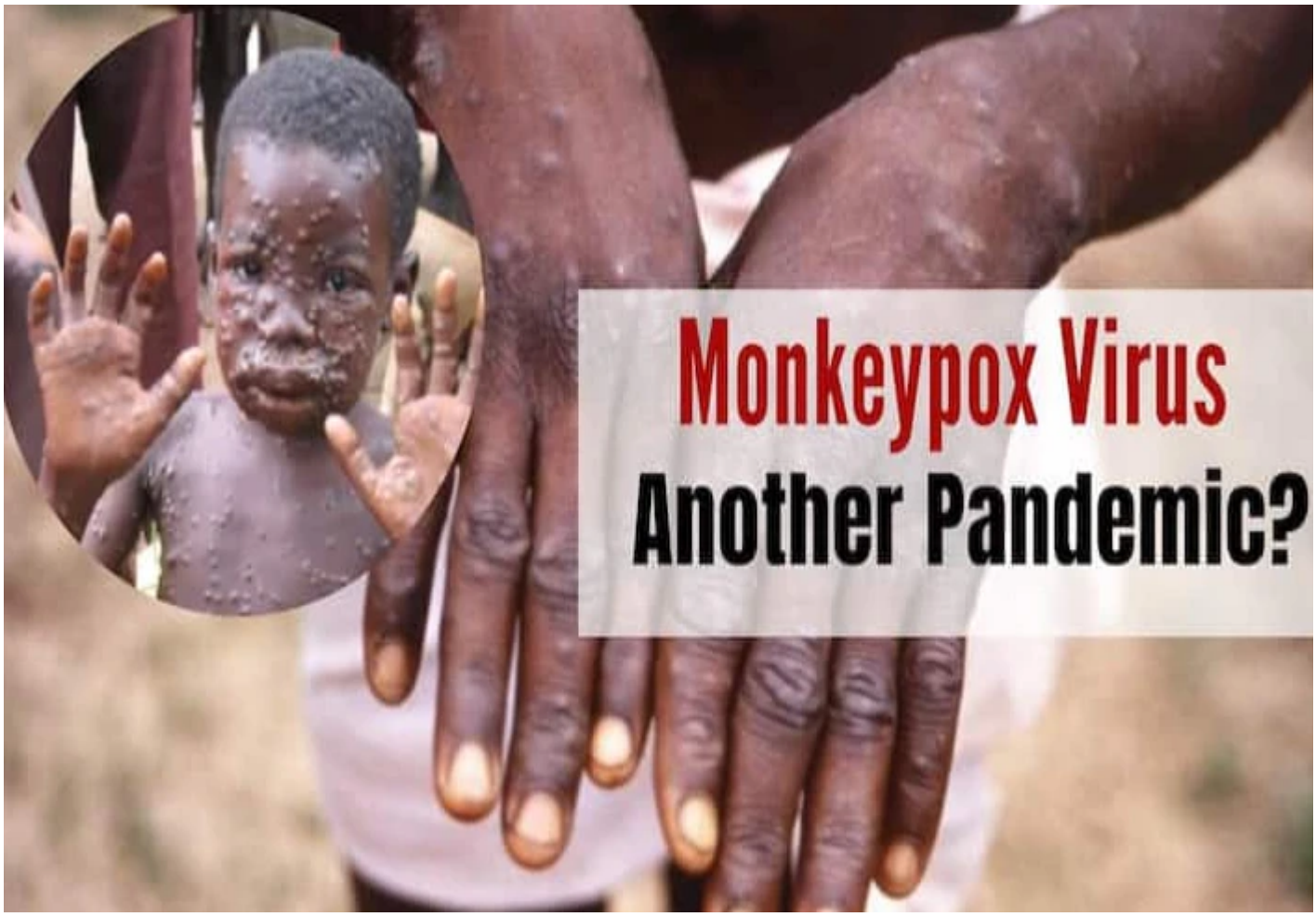
If you want to be a good American and make a six-figure salary — or be friends with people who make six-figure salaries—then do as your government tells you: Sit down, shut up, stay home, save lives, take your shots, show your papers and muzzle your kids.

Republished from the Brownstone Institute.

Did You Know That A 2021 Report Predicted The Monkeypox Outbreak On May 15th 2022 ? Both Companies Involved In The Report Have Received Millions From The Bill & Melinda Gates Foundation.

threadsirish
May 20

In March 2021, the National Threat Initiative (NTI) partnered with the Munich Security Conference to conduct a tabletop exercise on reducing high-consequence biological threats. The report focused specifically on a Monkeypox outbreak.



This tabletop exercise can be added to a long list of other pandemic table top exercises. In a previous thread that I wrote back in November 2021 (before my twitter account was banned) I

wrote a thread entitled “Who Are The Johns Hopkins Center for Health Security And Why Did They Publish A Document Called The SPARS Pandemic 2025-2028”. In that thread I spoke of 4 pandemic tabletop exercises that have taken place over the last twenty years such as Operation Dark Winter, Atlantic Storm, Clade X & most famously Event 201.

threadsirish

Who Are The Johns Hopkins Center for Health Security And Why Did They Publish A Document Called The SPARS Pandemic 2025-2028 In 2017

Read more

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We now have another one that we can add to the list called “Strengthening Global Systems to Prevent and Respond to High-Consequence Biological Threats". Open Philanthropy funded the report. One of its main funders is Dustin Moscovitz who founded Facebook along with Mark Zuckerberg.

We are grateful to Open Philanthropy. The exercise and report would not have been possible without their generous support.

Open Philanthropy

Before I jump into a brief summary of the document it is important to go down the rabbit hole to see how the dots are connected. The report was a partnership between The Nuclear Threat

Initiative (NTI) and the Munich Security Conference. The Munich Security Conference has received funding of $1.2 mil from The Bill & Melinda Gates Foundation.

Foundation Munich Security Conference

Division	Date	Region served	Committed amount
Global Development +1	**NOVEMBER 2019**	**GLOBAL** +1	**$800,000**

Grant topic	Duration (months)	Grantee location
Delivery of Solutions to Improve Global Health +2	**30**	**Munich, Germany**

Foundation Munich Security Conference

Division	Date	Region served	Committed amount
Global Policy and Advocacy	**NOVEMBER 2017**	**GLOBAL** +1	**$400,000**

Grant topic	Duration (months)	Grantee location
Global Health and Development Public Awareness and Analysis	**25**	**Munich, Germany**

The Nuclear Threat Initiative (NTI) has also received $3.5 mil from The Bill & Melinda Gates Foundation in the name of Vaccine Development.

Nuclear Threat Initiative

Division	Date	Region served	Committed amount
Global Health	**JULY 2020**	**GLOBAL**	**$1,000,045**
Grant topic	Duration (months)	Grantee location	
Vaccine Development	**25**	**Washington, District of Columbia, United States**	

Nuclear Threat Initiative

Division	Date	Region served	Committed amount
Global Health	**OCTOBER 2017**	**GLOBAL** +1	**$250,000**
Grant topic	Duration (months)	Grantee location	
Vaccine Development	**20**	**Washington, District of Columbia, United States**	

Nuclear Threat Initiative

Division	Date	Region served	Committed amount
Global Health	**DECEMBER 2004**	**GLOBAL**	**$2,250,000**
Grant topic	Duration (months)	Grantee location	
Neglected Tropical Diseases	**36**	**Washington, District of Columbia, United States**	

On September 20, 2017, the Nuclear Threat Initiative (NTI) and the World Economic Forum (WEF) hosted a roundtable discussion on the current landscape of biological risks presented by technology advancement in the context of the Fourth Industrial Revolution

https://www.nti.org/news/nti-and-world-economic-forum-partner-discuss-biological-risks-posed-advances-technology/

Fast forward 3 years and in January 2020, NTI and the World Economic Forum released a report called “Biosecurity Innovation and Risk Reduction: A Global Framework for Accessible, Safe and Secure DNA Synthesis”

https://www.nti.org/wp-content/uploads/2020/01/Biosecurity_Innovation_and_Risk_Reduction.pdf

From the World Economic Forum press release they speak of

“Rapid advancements in commercially available DNA synthesis technologies – used for example to artificially create gene sequences for clinical diagnosis and treatment – pose

growing risks, with the potential to cause a catastrophic biological security threat if accidentally or deliberately misused”

https://www.nti.org/news/nti-and-world-economic-forum-release-new-report-dna-synthesis-technologies/

Now that we’ve established who is funding these reports let’s have a closer look at the 2021 report itself which predicted the Monkeypox outbreak (and yes the exact date is predicted in the document)

https://www.nti.org/wp-content/uploads/2021/11/NTI_Paper_BIO-TTX_Final.pdf

On Page 6 of the 36 page report in the Executive Summary it says

“The exercise scenario portrayed a deadly, global pandemic involving an unusual strain of monkeypox virus that emerged in the fictional nation of Brinia and spread globally over 18 months. Ultimately, the exercise scenario revealed that the initial outbreak was caused by a terrorist attack using a pathogen engineered in a laboratory with inadequate biosafety and biosecurity provisions and weak oversight. By the end of the exercise, the fictional pandemic resulted in more than three billion cases and 270 million fatalities worldwide”

Discussion among exercise participants led to the following key findings:

- Weak global detection, assessment, and warning of pandemic risks
- Gaps in national-level preparedness.
- Gaps in biological research governance
- Insufficient financing of international preparedness for pandemics.

To address these findings, the authors developed the following 5 recommendations.

1. Bolster international systems for pandemic risk assessment, warning, and investigating outbreak origins
2. Develop and institute national-level triggers for early, proactive pandemic response
3. Establish an international entity dedicated to reducing emerging biological risks associated with rapid technology advances
4. Develop a catalytic global health security fund to accelerate pandemic preparedness capacity building in countries around the world
5. Establish a robust international process to tackle the challenge of supply chain resilience

What is especially interesting about these recommendations is the role the UN, WHO and Banks will have to play (all are mentioned in the report). This seems like a direct nod to the WHO Pandemic treaty which is being discussed at the World Health Assembly May 22-28.

https://www.forbes.com/sites/brucelee/2022/05/18/the-who-to-discuss-global-pandemic-treaty-at-world-health-assembly-may-22-28/

Isn’t it interesting as well that the World Economic Forum 2022 meeting is also taking place from May 22-26.

I mentioned earlier the exercise participants in the tabletop exercise but who are they. It will come as no surprise that it was the usual runners and riders which coincidentally were involved in Event 201. Here are just a few from Page 9..

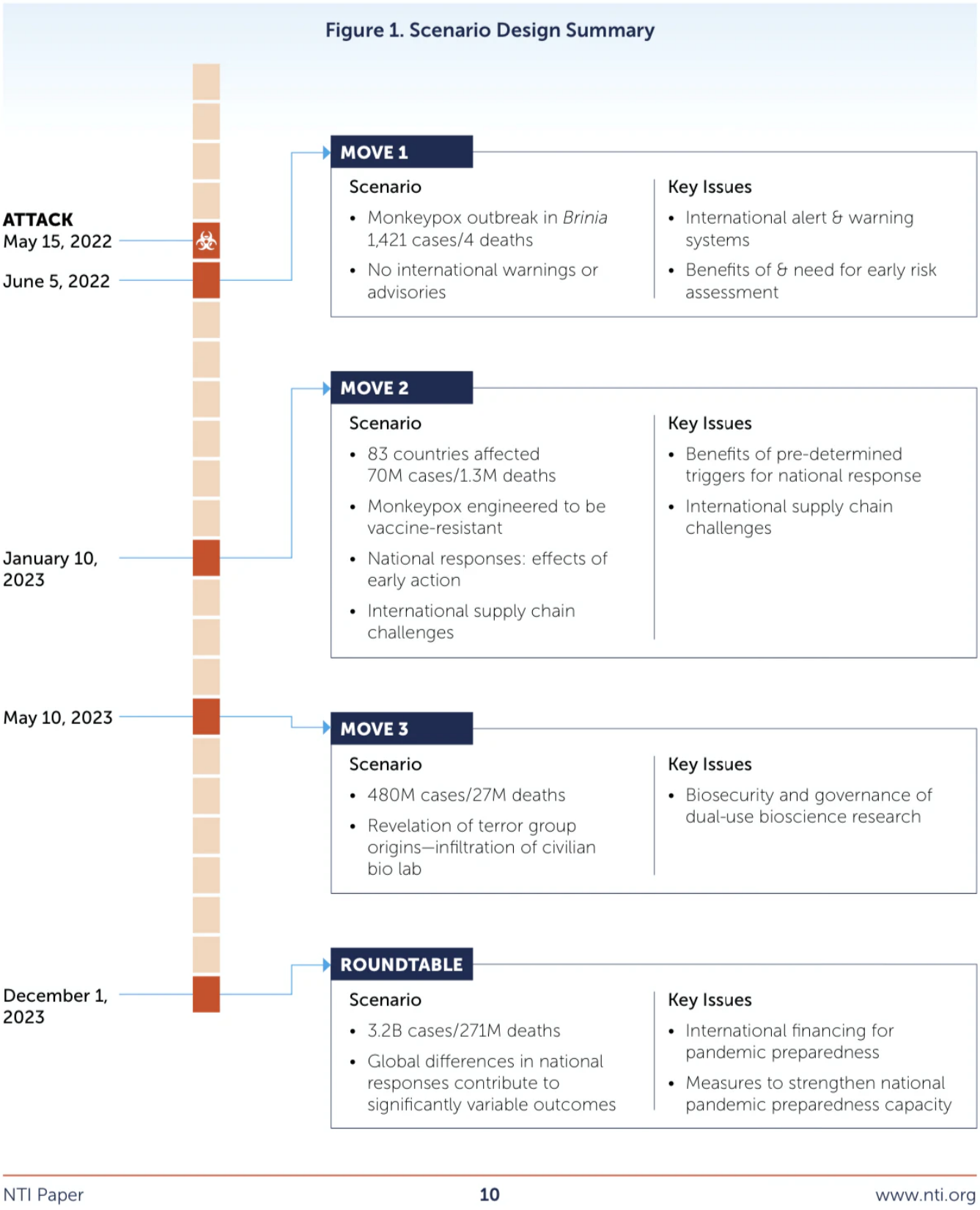
Dr. Michael Ryan, Executive Director, WHO Health Emergencies Programme

Dr. Ruxandra Draghia-Akli, Global Head, Johnson & Johnson Global Public Health R&D Janssen Research & Development

Dr. Chris Elias, President, Global Development Division Bill & Melinda Gates Foundation

Sir Jeremy Farrar, Director Wellcome Trust

Most fascinating of all though was the predicted date from the document of the Monkeypox outbreak (Page 9). Funny how unerringly accurate they are with their predictions. Just a coincidence of course.



The report also speaks of future triggers. Page 17

“In national pandemic response plans, specific readiness measures would be “triggered” based on factors related to the potential severity of the outbreak, expected delays in situational

awareness, and the time it would take to implement response measures and see results”

As was evident in Covid yet again they speak of flattening the curve, using mask mandates and ceasing mass gatherings as well as health screening measures for travel (vaccine passports).

“Although triggered actions would vary depending upon the particular needs of the country, in most cases the goals are the same: slow the spread of disease to buy time and flatten the epidemiological curve, while using that time to scale up public health and medical systems to keep up with growing caseloads and save lives. NPIs such as mask mandates and ceasing mass gatherings were deemed to be critical for blocking chains of disease transmission. Participants generally did not endorse travel restrictions such as border closures, but travel health screening measures were viewed as valuable”

One other coincidence I noticed in the document is the address of the Nuclear Threat Initiative in Washington. 1776 Eye Street. Wasn’t 1776 the date of the American Declaration Of Independence. The document is also 33 pages long but maybe that’s another coincidence and a discussion for another day. Make of it what you will.

If anybody thinks these pandemic tabletop exercises are a coincidence they need their head examined. This is far from over.

Joseph similia May 20

FAMINES

Like a voice in the midst of the four living creatures say: “A QUART OF WHEAT FOR A de·nar′i·us and three quarts of barley for a de·nar′i·us; and do not harm the olive oil and the wine"- Revelation 6:6.

Note, food would become very expensive, 2 pounds of wheat for a denarius which was a days wage in bible times. Don't even think about buying olive oil it says.

We are in the end of times for this old wicked system. We need to stay awake because he will come as a thief in the night, Jesus said. Read the bible , pray for God's kingdom to come, seek out those that have faith.

Don't be caught sleeping.Those calling on the name of Jehovah and have faith in Jesus will be saved, says Romans is 10:13-17 " For “everyone who calls on the name of Jehovah will be saved.” 14 However, how will they call on him if they have not put faith in him? How, in turn, will they put faith in him about whom they have not heard? How, in turn, will they hear without someone to preach? 15 How, in turn, will they preach unless they have been sent out? Just as it is written: “How beautiful are the feet of those who declare good news of good things!” 16 Nevertheless, they did not all obey the good news. For Isaiah says: “Jehovah, who has put faith in the thing heard from us?” 17 So faith follows the thing heard. In turn, what is heard is through the word about Christ

Only God's kingdom can bring an equitable human society with plenty of food and plenty of good things for all.

Pray for God's kingdom to come and God's will to be done on earth as it is in heaven.

♡ 9 Reply

Jay 22 hr ago

The fastest way I know of to end a pandemic is to put the likes of Bill Gates and George Soros in jail.

♡ 7 Reply

2 replies

28 more comments...

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A review of experimental and natural infections of animals with monkeypox virus between 1958 and 2012

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Abstract

Monkeypox virus (MPXV) was discovered in 1958 during an outbreak in an animal facility in Copenhagen, Denmark. Since its discovery, MPXV has revealed a propensity to infect and induce disease in a large number of animals within the mammalia class from pan-geographical locations. This finding has impeded the elucidation of the natural host, although the strongest candidates are African squirrels and/or other rodents. Experimentally, MPXV can infect animals via a variety of multiple different inoculation routes; however, the natural route of transmission is unknown and is likely to be somewhat species specific. In this review we have attempted to compile and discuss all published articles that describe experimental or natural infections with MPXV, dating from the initial discovery of the virus through to the year 2012. We further discuss the comparative disease courses and pathologies of the host species.

Keywords

aerosol; animals; infection; intrabronchial; intradermal; intramuscular; intranasal; intratracheal; intravenous; outbreak; primates; subcutaneous

Orthopoxviruses (OPVs) have host specificities ranging from narrow (e.g., ectromelia and variola [VARV]) to broad (e.g., cowpox and vaccinia [VACV]). Monkeypox virus (MPXV) has a broad host-range and is capable of infecting many species from across the globe. In nature, the major environs of MPXV are restricted to the Congo Basin (CB) and West Africa (WA). The MPXV virion is a brick-shaped enveloped virus of 200–250 nm, characterized by surface tubules and a dumbbell-shaped core. Humans and highly susceptible nonhuman primates (NHPs) infected with MPXV have near identical clinical manifestations compared to humans infected with VARV. For humans, the only obvious difference in clinical signs is the absence of lymphadenopathy in smallpox patients [1,2]. Despite the similarity, MPXV remained primarily of academic interest throughout the 1960s; however, this attitude changed when the scientific community realized that MPXV could lethally infect humans in known smallpox-free locales.

Although there are clinical similarities in humans, MPXV is not considered to be the direct ancestor to VARV; instead, both viruses are believed to have evolved from progenitor

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No writing assistance was utilized in the production of this manuscript.

poxviruses most similar to the cowpox virus (CPXV) lineage [3,4]. Genomic differences amongst MPXV isolates have been mapped using restriction fragment length polymorphisms [5] and DNA sequencing techniques [6,7]. Like other OPVs, MPXV strains demonstrate gene variation towards the terminal regions, with conservation towards the center of the genome.

In this review, records pertaining to natural or experimental infections of animals with MPXV have been extensively researched. The authors have given the origin of the isolate (either CB or WA) and the specific strain name where known; if the strain and origin are unknown, it has simply been referred to as 'MPXV'. Doses and routes of inoculation are given where known. The authors have taken effort to focus on 'confirmed' cases of MPXV, therefore, cases of likely MPXV infections in animals may only be discussed transiently where appropriate; for example, several OPV-specific serological surveys of animals in various regions have been conducted in an attempt to elucidate MPXV host species [8-11], and some literature report on natural 'smallpox' in various NHPs (reviewed in [12]).

Natural history

Field studies have revealed that MPXV infects many species that inhabit all strata of the lowland tropical forest within central and west Africa [13-20]. The infected species have some similar and dissimilar traits based on diet and habitat preferences; approximately 40% are arboreal, 40% are semiterrestrial and 20% are terrestrial [13].

Several studies suggest that there may be no one reservoir of MPXV; rather, several animal species may support MPXV. The only reported case of MPXV being isolated from a wild animal consisted of MPXV being isolated from a diseased squirrel, *Funisciurus anerythrus* [17]. In the 2003 shipment of African rodents that introduced MPXV into the USA, cell culture demonstrated MPXV in three out of six *Funisciurus* sp. (rope squirrel), one out of 15 *Cricetomys* sp. (Gambian-pouched rat) and eight out of ten *Graphiurus* sp. (African dormouse) [21] (as well as several other animals that were found to be positive by PCR; see below). The lack of MPXV antibodies in sera from certain animal species is also informative. Sera from terrestrial rodents of the genera *Lophuromys, Lemniscomys, Oenomys, Thamnomys* and *Praomys* were negative (579 sera samples) [16]. One study tested the transmission of MPXV from infected squirrel tissue to other naive squirrels via ants – no transmission was reported [16]. However, *Petrodromus tetradactylus* (four-toed elephant-shrew), an insectivore that consumes minimal plant material and yet is seropositive for OPV antibodies, suggests that the role of insects in the natural lifecycle of MPXV may be worth evaluating. The presence of MPXV antibodies in so many distinct species and virus detection in specimens from *Funisciurus* sp., *Cricetomys* sp. and *Graphiurus* sp. suggest that the natural lifecycle is a complex interaction of reservoir hosts and incidental species.

Virulence differences between WA & CB strains

Sequence analysis of the genomes of several CB and WA MPXV isolates revealed approximately 95% identity amongst all MPXV isolates. This value approaches 99% when comparing between CB or WA isolates, allowing separation into two clades based on geographical origin, sequence homology and disease severity [6,7]. Consistent with the aforementioned restriction fragment length polymorphism analysis, the greatest DNA sequence diversity between the two clades is localized to the terminal regions that encode for predicted host-response modifier proteins. Several animal studies have shown that CB isolates of MPXV have increased virulence compared with WA isolates. In the intranasal (in.) CAST/EiJ mouse strain, disease severity (which is a commonly accepted biomarker of morbidity in OPV-induced disease [22-26]) is similar between viral strains at doses of 10^5–

10^6 PFU, and both strains cause 100% mortality; however, at 10^4 PFU the mortality rate of the WA-infected mice decreases to 50%, while it remains at 100% in CB-infected animals. Further more, at a 10^3 PFU dose, 12% of WA-infected animals die compared with 60% of CB-infected animals, and the maximum weight loss is 10% in WA animals compared with 22% in CB animals. These studies revealed that the median lethal dose (LD_{50}) for the WA strain in CAST/EiJ mice is 7600 PFU, approximately 1 log higher than that of the CB strain [27]. Following a footpad (FP) infection of BALB/c and C57BL/6 mice, it was observed that edema occurred following infection with both the CB and WA strains; however, the level of edema was less and resolved quicker in WA infected animals. Furthermore, following an in. infection of these mice, it was reported that CB animals experienced weight loss whereas WA animals did not; and in the case of the in. infected BALB/c strain, ruffled fur was seen on CB-infected but not on WA-infected animals [28]. No animals died in these mouse experiments. In squirrels, the intradermal (id.) LD_{50} values for both strains are approximately the same (~0.5 PFU); however, the disease courses are different, with more severe clinical signs, earlier symptom onset and earlier mortality characterizing the CB-infected animals [29]. For prairie dogs, a mortality rate of 25 and 50% was recorded in animals infected with a CB strain via the in. or subcutaneous (sc.) route, compared with 0% mortality in WA-infected animals. A higher temperature and increased weight loss was also recorded in CB-infected animals, again indicating increased virulence of CB strains [30]. These results were confirmed by a study that calculated that the WA in. LD_{50} value is approximately 100-times higher than that of the CB strain [31]. In NHPs, it was found that disease severity and mortality was higher in cynomolgus macaques (*Macaca fascicularis*) infected with 10^6 PFU of a CB strain (ZR-599) via the sc. route compared with animals challenged with a WA strain (Liberia) [32].

The CB strain has also been suggested to be more virulent in humans. Virulence differences between WA and CB MPXV are supported by epidemiological analyses that observed a similar prevalence of antibodies in non-vaccinated humans in both regions [16]; however, 90% of reported cases and 100% of fatalities occurred in the Congo Basin, compared with 0% of fatalities in the WA cases [33].

Discovery of MPXV & natural outbreaks

Initial outbreak of MPXV in Denmark (1958)

MPXV was first discovered during a nonfatal outbreak at an animal facility in Copenhagen, Denmark, in 1958. The facility received a continual supply of Asian monkeys (mostly *M. fascicularis*) and rhesus macaques (*Macaca mulatta*), which were used for polio vaccine research. The first outbreak occurred 2 months after the monkeys had been received and the second outbreak occurred 4 months after the initial outbreak. The outbreaks occurred in *M. fascicularis* that had arrived from Singapore. Upon arrival, monkeys were treated with antibiotics and appeared in satisfactory health. The outbreak manifested itself with vesiculopustular skin eruptions that were observed over the entire trunk, tail, face, limbs, palms of the hands and soles of the feet. Despite the disease, the general health of the animals appeared relatively normal and the lesions formed crusts, healed and fell off, leaving a scar. No lesions were observed in tissues upon autopsy. The outbreak lasted approximately 2 weeks and involved six out of 32 animals. The second outbreak occurred in another shipment of animals from Singapore. The initial health of the animals appeared normal; however, the pox disease was observed in 11 out of 120 animals. Animals were examined and scattered healed lesions were observed in a further 12 monkeys 1 month later. Several weeks later, a further two animals developed the disease [34]. MPXV was isolated from the pustules of diseased animals by incubation in eggs. After incubation, the pocks were reported to resemble those of VARV, but were distinctly different to those of VACV and CPXV. Neutralization experiments with VACV antisera from humans and rabbits were

used to confirm that the virus belonged to the OPV genus. Further experiments (complement-fixation, hemagglutination inhibition (HAI), electron microscopy and diffuse-precipitation) also suggested that the virus belonged to the OPV genus. Interestingly, MPXV was reported to be isolated from the kidneys of healthy animals that had been sacrificed for other reasons. The origin of the isolate is thought to be WA, based on genome sequence comparisons [6]. Further studies in other animals were performed and are described below [34].

Outbreak of MPXV in the USA (1959)

A second MPXV outbreak was also reported the same year as the Von Magnus *et al.* [34] report in a colony of captive monkeys in Philadelphia, USA. Small numbers of animals of all ages and both sexes were infected; however, because of the large numbers of animals in a given cage and the inability to examine all animals, it was impossible to determine the exact number of diseased animals [35]. This outbreak predominantly affected the *M. fascicularis* species; however, unlike the case reported by Von Magnus *et al.* [34], *M. mulatta* was also affected, although less severely. Subsequent serological findings revealed that a large number of *M. mulatta* had been infected without showing clinical evidence. In the outbreak, two distinct types of disease occurred; the first consisted of an acute disease in *M. fascicularis* animals only. The disease was characterized by facial edema that extended to the cervical region. Severe difficulty in breathing was also observed, which apparently led to death by asphyxiation. At the same time, papular eruptions were present over various parts of the body, with ulcerative lesions in the oral mucosa and generalized lymphadenopathy. The second form of the disease, which presented in *M. fascicularis* and *M. mulatta*, was more common and resulted in cutaneous eruptions with no other obvious clinical signs. Initially, the lesions formed a single crop of papules which became pustular and then crusted over after 7–10 days, leaving small scars. Hemorrhagic lesions were particularly associated with fatal cases. All parts of the body surface were involved, with the most commonly affected areas being the buttocks, hands, feet, face and hind limbs. Histopathology of the skin revealed focal proliferation of the epidermis, followed by necrosis and a recruitment of inflammatory cells, with the thickness of the epidermis increasing from three to four layers to 25–30. MPXV was isolated from naturally infected animals using embryonated chicken eggs and tissue culture. Similar to the findings by Von Magnus *et al.* [34], in chorioallantoic membranes the lesions typically spread along the blood vessels, were smaller than those produced by VACV, and were not typical of those associated with CPXV infection.

Outbreak of MPXV in the USA (1962)

A second outbreak of MPXV was reported in the USA in two *M. fascicularis* monkeys approximately 45 days after exposure to whole-body irradiation [36]. Lesions were clinically similar to those described by Von Magnus *et al.* and Prier *et al.* [34,37], that is: pox-like eruptions, severe facial and cervical edema, hemorrhagic ulcerations, dyspnea and bloody diarrhea. Both infected monkeys died after 12 days. One nonirradiated monkey also became sick with cervical edema and ulcerated areas on the arms and forehead; however, this animal survived. HAI titers from animals housed in the same room as those exhibiting disease revealed that 89% of animals were positive; in contrast, only 11% of animals held in separate rooms were positive [36].

Outbreak of MPXV at Rotterdam Zoo (1964)

One of the first indications of the broad host-range of MPXV, and its propensity to also infect larger species in pan-geographical locations, came from an outbreak at Rotterdam Zoo. MPXV was apparently introduced by Central/South American giant anteaters (*Myrmecophaga tridactyla*), which became ill approximately 12 days after arrival; however, it is suspected that the anteaters contracted the virus from previous contact with monkeys

elsewhere. Following a pox-like illness, characterized by multiple skin lesions, the animals were sacrificed. The anteaters had been housed close to the Asian orangutan (*Pongo pygmaeus*) enclosure and these orangutans (n=10) also became severely ill with erythema and had purulent nasal discharge on the mucous membranes. Lesions were also observed on the face, legs and body. Six out of ten animals died after a few days of clinical signs and the remainder survived following a long period of convalescence. It is not clear if the virus was transmitted by aerosol or fomites. Several other species also became infected, with various degrees of mortality and morbidity, including both African gorillas (*Gorilla gorilla*) and most (n unknown) chimpanzees (*Pan troglodytes*), which became ill and presented with pox lesions; the only Asian gibbon (*Hylobates lar*) in the park, which died after 18 days of severe illness and presented with vesicles on the face, trunk and limbs; three (total n unknown) South American squirrel monkeys (*Saimiri sciureus*) died, with one out of three presenting with pox lesions; four (total n unknown) African owl-faced monkeys (*Cercopithecus hamlyni*), which became sick and presented with lesions on the lips that resolved within a few days; and a South American common marmoset (*Hapale jacchus*), which became sick and died following reddening and swelling of the area around the nose and eyes with lesions on the face and belly. Some other *Hapale* also presented with similar swelling, but had no further clinical signs [38,39].

Outbreak of MPXV in the USA (2003)

A third outbreak of MPXV in the USA occurred via the importation of rodents from Ghana, WA (see below). Infected animals went on to infect native prairie dogs, which were housed in close proximity to the African rodents. The prairie dogs proved to be highly susceptible to the virus and transmitted MPXV to approximately 40 humans – the first known case of human monkeypox (MPX) outside of Africa [21,40]. Interestingly, humans were only infected by the prairie dogs – which acted as amplifying hosts – and not by any of the rodents imported from WA. More detailed descriptions of animal infections are discussed below.

Nonhuman primates as models of MPXV

Evidence of MPXV infection of NHPs has been reported in several African serological surveys. In a 1986 survey in north Zaire, 39 primates were tested for MPXV specific antibodies; of which, two out of 22 crowned monkeys (*Cercopithecus ascanius*) and one out of three red-tailed monkeys (*Cercopithecus pogonias*) were positive. The remaining animals, all of different species, were negative [19]. During serological surveys in WA, two lesser white-nosed monkeys (*Cercopithecus petaurista*) were found to be positive for MPXV-specific antibodies, as well as one western colobus monkey (*Colobus badius*) [14]. A second serological survey tested 13 wild-caught monkeys for MPXV-specific antibodies, two *Chlorocebus aethiops* and one *Cercopithecus petaurista* were positive [15]. This could indicate that monkeys of the *Cercopithecus* genus have a propensity for MPXV infection.

MPXV has only been detected in NHPs in nature in Africa, and many African primates appear to have been exposed at some point. It is therefore unfortunate that, due to lack of availability, experimental studies have not been performed in African primates; rather, the bulk of studies have been performed in cynomolgus and rhesus macaques, which are native to various parts of Asia where MPXV has never been reported. This choice of primate was likely made for several reasons: because these animals had been observed to be susceptible to the disease in previous outbreaks; because these animals are readily available; because these primates are well studied; and because they thrive well in a research vivarium.

Early experimental studies on MPXV pathogenesis in *M. fascicularis* & *M. mulatta*

The original report by Von Magnus *et al.* included an id. inoculation of MPXV (dose unreported) in the palm of the hand of two (presumably seronegative) *M. mulatta* monkeys which resulted in no signs of illness [34]; however, similar inoculation in an *M. fascicularis* monkey resulted in a local pustule surrounded by edema 7 days post-infection (p.i.) and a slightly elevated temperature between days 5 and 9 p.i. [34]. These id. findings were confirmed by Prier, who also found that an intravenous (iv.) challenge resulted in generalized eruptions and virus recovery from skin lesions [35,37,41].

One of the earliest studies on experimental infection of *M. mulatta* investigated the protective capability of vaccination (by traditional scarification [SCR]) with VACV against an iv. challenge with $10^{5.5}$ median tissue culture infective dose ($TCID_{50}$) of MPXV isolated from the second US outbreak [36]. Challenge at 35 days postvaccination resulted in no disease manifestations in five out of six monkeys; however, one animal developed bloody diarrhea and died 9 days p.i.; but necropsy did not reveal any patho logical manifestations of MPX. In unvaccinated animals, severe MPX disease was reported, with characteristic vesicles, pustules, and necrotic lesions on the dermis of the face, arms, legs and tail – one out of five monkeys died on day 9 p.i. [42].

Gispen *et al.* challenged *M. fascicularis* via SCR or via the in. route with MPXV (Utrecht 64-7255) [38] isolated from animals from the Rotterdam Zoo outbreak [39]. The scarified monkey showed a temperature rise on day 6 and a local eruption of pox lesions 1 day later that had crusted over and fallen off by day 20. A second temperature rise was also reported on day 11 followed by secondary eruption of a few discrete vesicles on the inner side of the thigh, arms, chest and belly. The in. inoculated animal had a febrile reaction on day 7 and 8, followed by the appearance of discrete vesicles on the neck, chest, belly, inner-thigh and the hollow of the knees. Both animals survived [38].

By far the most comprehensive early studies on MPXV pathogenesis were conducted in a series of experiments by Wenner *et al.* in the late 1960s. Challenge (with a strain of MPXV originally recovered from a pustule of *M. fascicularis*) of *M. fascicularis* with 10^5 PFU via the iv., intramuscular (im.) and sc. routes revealed strikingly similar disease patterns marked by an abrupt temperature increase from day 2–4 that lasted only 1–2 days followed by a second temperature increase on day 6 p.i. that lasted for approximately 5 days [34,43]. Eleven out of 12 animals developed a rash at this time with a generalized exanthema on day 7–11 characterized by the typical papule, vesicle, pustule and scab appearance over a period of 3–7 days. Lesions were also observed on the soles of the feet, palms, buccal mucosa and soft palate. No animals died. A second experiment using the same dose of MPXV repeated the im. study but also included im. inoculations of *M. mulatta.* Generally, the disease progression in *M. mulatta* followed that of *M. fascicularis*, but with reduced severity and a delay in disease biomarker appearance by approximately 2 days: that is, seven out of nine and nine out of nine animals developed a rash for *M. mulatta* and *M. fascicularis* from day 9–16 and 8–20, respectively, and lesions were far less pronounced in the former. Virus could not be detected in tissues before day 6 and could rarely be found in visceral organs after rash onset. HAI and neutralizing antibodies (NA) developed and reached maximum levels at 3 weeks p.i., and all animals subsequently challenged with VACV via the im. route were resistant, whereas control animals developed typical VACV lesions and generalized vaccinia [1,2].

Further studies by Wenner *et al.* investigated the effect of increasing the virus dose on MPX disease in *M. fascicularis* inoculated via the im. route with a strain derived previously from

blood obtained on the fifth day from a monkey that developed MPX (strain not identified). Generally, disease progression mirrored those of the previous studies [1,2]. Animals regularly developed MPX from 10^{-1}–10^{-5} virus dilutions (from tissue culture-prepared virus). Emergent clinical features at each dilution were almost indistinguishable; however, the variability in viremia was high and was not dose-dependent. By day 6 there was a high concentration of MPXV in the tonsil, spleen, lymph nodes, bone marrow and skin lesions. Ten out of 34 (doses not given) animals died at day 10–15, characterized by a rash more intense than in survivors and marked by a more severe illness from days 4–11, with progressive dehydration and weight loss. Sentinel monkeys remained healthy until days 18–20, when three out of four developed fever followed by MPX disease (no indication of the route of infection was provided). Three out of four sentinel animals developed rash; however, all sentinels were HAI positive by day 23 following clinical signs of illness [44,45].

The im. model was also used to evaluate the drug methisazone for the treatment of smallpox in the *M. fascicularis* model. Infected controls presented with a disease course similar to that reported above [44,45]. The infected and drug-treated group had a mortality rate of 20% (one out of five) with death on day 18 p.i. The infected and untreated group had a mortality rate of 66% (two out of three) with death on days 15 and 23 p.i. The drug failed to prevent or modify the disease course [46].

These early studies revealed that *M. fascicularis* is highly susceptible to MPXV, and that inoculation via several routes of infection (iv., SCR, in., im. and sc.) elicit a similar disease course that typically results in little or no mortality. Challenge of *M. mulatta* via the iv. route resulted in low mortality (one out of five) and again, a disease course similar to that produced by im. challenge. *M. mulatta* appears to be more resistant to an im. challenge compared with *M. fascicularis*, as indicated by disease severity; however, both species are infected, as evidenced by antibodies. The low mortality rates in these studies compared with more recent studies (see below) indicates that a lower inoculum was used and/or a less-virulent strain of MPXV was administered.

Experimental infections of *M. fascicularis* & *M. mulatta* via the iv. route

With the realization that the Soviet Union had weaponized VARV [47], the USA initiated a research program to license modern smallpox vaccines, produce additional stocks of vaccinia immunoglobulin and license at least two antiviral drugs targeted at different stages of the replication cycle. The most thoroughly utilized model for these developments is the iv. challenge of *M. fascicularis* and *M. mulatta*.

The iv. route

MPXV iv. inoculations in NHPs have been extensively evaluated as a model for smallpox and human monkeypox. The major disadvantage to this route is that the initial infection of respiratory tissue, incubation and prodromal phases are completely bypassed, and thus it does not accurately model the natural route of transmission. The iv. route is primarily used to test medical countermeasures against the most rigorous challenge route. Indeed, the majority of vaccination and antiviral development studies have utilized the iv. route of exposure in *M. fascicularis* and *M. mulatta*. Figure 1 shows the development of four key disease biomarkers in *M. fascicularis* and *M. mulatta* challenged with 10^7 PFU of CB (Z79). At high challenge doses the disease patterns were similar between both NHP species.

iv. inoculation of *M. fascicularis*

Details of iv. challenges are found in Table 1 and Figure 1. Similar responses following an iv. challenge with 10^7 PFU of CB (Z79) have been reported by several groups: viral load

increases rapidly from approximately day 4, fever occurs at approximately day 3 p.i., with lymphadenopathy at day 3–4. A vesiculopustular rash develops from approximately day 4, initially on the oral mucosa followed by axillary and inguinal regions. Weight loss and a typical skin lesion count of >1000 lesions is frequently reported, followed by euthanasia or death between days 9 and 15 [23,48,49]. Goff *et al.* used a slightly attenuated CB (Z79-GFP) recombinant to further analyze the disease progression and found that they could detect lesions 1–2 days earlier under fluorescence [50]. Fluorescence was brightest in lesions of the nonkeratinized lining of the oral cavity and least prominent in the thickly keratinized skin of the soles and palms. Necropsy revealed lesions in the skin, mouth and upper airway, the lining of the GI tract, enlargement of lymph nodes, the spleen and pulmonary congestion. Immunohistochemistry (IHC) on euthanized animals using anti-VACV or anti-GFP antibodies detected antigens in numerous sites, including the pox lesions of the skin, mouth, nose, mandibular and axillary lymph nodes, esophagus, lung, liver, spleen and testis [50]. Multiple studies have examined the efficacy of vaccines and antivirals in the iv. model and are summarized in Table 2.

iv. inoculation of *M. mulatta*

Several studies have utilized the iv. model in *M. mulatta* (Figure 1 & Table 3). Hooper *et al.* showed that animals infected with 10^8 PFU of CB (Z79) caused organ-hemorrhagic MPX with death on day 6 [51]. The likely cause of death was cardiovascular collapse secondary to multiple organ failure. It was subsequently determined that this dose did not accurately model smallpox or natural infections with MPXV. A lower dose of 10^6 PFU caused an eruptive rash on day 6, which progressed to disseminated exanthema with >100 lesions per animal; however, all animals survived challenge. A 10^7-PFU dose resulted in the development of grave MPX, and animals succumbed on days 7, 10 and 14 p.i., with associated fever commencing on days 2–4 and viral DNA (vDNA) being detected from days 2–6. The cause of death in the day-7 monkey was likely hemorrhage of the lymph nodes, heart, lungs, urinary bladder, uterus and digestive tract. In addition, there was hepatopathy, splenomegaly, lymphadenopathy, diffuse pulmonary edema, and degeneration and necrosis of the bone marrow. Animals that died on days 10 and 14 presented with a disseminated exanthematous rash, marked lymphadenopathy, mild splenomegaly, mild pulmonary edema and a notable absence of remarkable pathology in other organs. The most noticeable manifestation was the generalized vesiculopustular rash that became evident on day 6 that followed typical macule, papule, vesicle, pustule progression until crusts at day 10. Lesions were primarily distributed on the hands and face, but rarely on the abdomen [51]. Multiple studies have examined the efficacy of vaccines and antivirals in the iv. model and are summarized in Table 2.

In summary, both *M. mulatta* and *M. fascicularis* are highly susc eptible to MPXV administered via the iv. route at $\geq 10^6$ PFU and respond well to vaccines and antivirals. Fever develops at approximately the same time (~day 2–4), but skin lesions and death are slightly delayed in the *M. mulatta* model (Figure 1). Blood vDNA is detected earlier in the *M. mulatta* model compared with *M. fascicularis*, which has a delay of approximately 2 days with significant variability in detection. One key observation is the interspecies variability between animals – particularly in mortality and lesion onset – which makes direct comparisons between *M. fascicularis* and *M. mulatta* difficult; moreover, only four studies have evaluated the iv. *M. mulatta* model compared with nine for *M. fascicularis*.

Experimental infections of *M. fascicularis* & *M. mulatta* via respiratory routes

Experimental challenges via respiratory routes are outlined below. Figure 2 presents a schematic of the presentation of fever, vDNA detection in blood, euthanasia/death and the appearance of skin lesions in *M. fascicularis*. Table 4 summarizes the key findings from respiratory challenges.

Aerosol

Aerosol exposure of *M. fascicularis* was first evaluated in 1961. Administration of virus (dose unspecified) resulted in 50% mortality (day of death not specified) and the development of a typical rash by day 9–10 p.i. An elevation in temperature was noted between day 5–8 p.i. as well as bronchiolitis, bronchitis and peri bronchitis. Fibrinous necrosis was found in the bronchial walls, peri bronchial lymph oid tissues and bronchopulmonary lymph nodes [52].

Exposing *M. fascicularis* to a ~6.5×10^4 dose of CB (Z79) resulted in death or euthanasia as a result of fibrinonecrotic bronchopneumonia [53]. Exanthema, enanthema, anorexia, fever, cough and nasal discharge began to present on days 6–7 p.i. By days 9–10 p.i. all animals had exanthema, enanthema, depression and weakness which worsened until death. The lower airway epithelium served as the principal target for primary infection and the tonsil, mediastinal and mandibular lymph nodes were also infected early in the course of infection. Lesions affecting lymph nodes, thymus, spleen, skin, oral mucosa, the GI tract and the reproductive system were caused by a monocytic cell-associated viremia; however, there was no cell-free viremia detected at any time. Detectable poxvirus antigen was limited to sites exhibiting obvious morphological involvement and was most prominent in epithelial, macrophage, dendritic and fibroblast cells of affected tissues. The presence of antigen as determined by IHC correlated with virus-infected tissues as observed by ultrastructural examination. Necropsy findings revealed that the lungs were congested, failed to collapse and presented with edema atelectasis and necrosis throughout the lobes. The oral cavity of most animals presented with glossitis and stomatitis, with gingivitis in 50% of animals; generally, the dorsal surface of the tongue and hard palate were involved and presented with depressed foci of necrosis, erosion or ulceration. Infectious virus was isolated from the buffy coats of samples taken from most animals at day 9 p.i. [53].

A separate study also evaluated aerosol exposure of *M. fascicularis* with CB (Z79) at doses ranging from 10^4–10^6 PFU [54]. Unlike the Zaucha *et al.* study [53], which focused primarily on histopathological and IHC findings, Nalca *et al.* focused on disease biomarkers; however, overlapping pathological and disease progression/presentation studies revealed strikingly similar results, with fibrinonecrotic bronchopneumonia being the most distinctive lesion observed and being the attributed cause of death [54]. Interestingly, lesion numbers were not dose-dependent but were generally higher in survivors compared with nonsurvivors, and it was noted that heavier animals had increased protection. Similar to the study by Hahon and McGavran, fever occurred from day 5; however, fever was significantly longer, with conclusion not being reached until 13–15 days later [52]. Interestingly, groups receiving lower-dose inoculums had lower temperature elevations and fevers of shorter duration. Serum chemistry was evaluated and it was found that total protein, albumin levels, LDH levels and CRP were altered with disease progression. vDNA levels, which were slightly higher in nonsurvivors compared with survivors, could be detected in the blood and throat-swabs from day 4 and peaked at day 10 p.i., with levels typically returning to normal over the following 4–12 days. In tissue, DNA from the lung and pox lesions had the highest

viral loads; high loads were also reported in the spleen, gonads, axillary lymph nodes and inguinal lymph nodes [54].

Bronchoscope-microsprayer aerosol delivery

The main disadvantage of typical aerosol delivery is that the inhaled dose of virus for each monkey varies considerably. The bronchoscope-microsprayer aerosol delivery (BMAD) method results in a dose-dependent incubation period before disease onset, the development of a disease that resembles smallpox with systemic dissemination, and results in pathology that is consistent with inhalation MPX. Use of the microsprayer reduces the incidence of lobar pneumonia and allows for the production of a clinical disease with similar pathology to that produced using aerosol challenges, albeit with a fraction of the required virus volume. BMAD allows for a more precise administration of virus inoculum. Goff *et al.* delivered MPXV CB (Z79) via the BMAD method to *M. fascicularis* (Figure 2 & Table 4); 10–100-fold (10^6–10^7) increases in dose only doubled mortality, indicating a poor dose response [24]. Those animals in the high-dose group followed a similar but accelerated disease course compared with the lower-dose animals, and two out of three animals succumbed to infection by day 8. Fever developed in two animals on day 4 and on day 6 in the third animal. Temperature remained elevated for 40 h; in the surviving animal, temperature returned to baseline, whereas the other two animals had temperature drops below baseline at the terminal stages. All animals lost approximately 10% body weight. Lymphadenopathy was first observed on day 6 and lesions developed from day 4, with total lesion counts being 350, 85 and 22. A typical pox lesion pattern was observed and higher lesion counts were observed in animals infected at the lower inoculums. In the lowest inoculum groups, vDNA was detected in the blood from day 4 and increased steadily until day 12. Animals that succumbed or were euthanized had similar gross pathological findings. Fibrinonecrotic bronchopneumonia was a consistent finding; the lungs were edematous and red and failed to collapse. Often, multiple necrotic foci were present. The trachea contained bloody froth and had multifocal or coalescing necrotic, dark-red mucosal lesions. Other gross lesions observed included the typical vesiculopustular, umbilicated and scabbed skin lesions, oral ulcers, enlarged peripheral lymph nodes, and proliferative and necrotizing or ulcerative lesions in the esophagus, stomach, and urinary bladder. Histological analysis revealed lesions consistent with fibrinonecrotic bronchopneumonia, necrosis of the trachea and surrounding mediastinal tissues, pulmonary edema, and pleuritis. Other lesions were noted in a large variety of tissues including stomach, intestines, liver, testes, bone marrow, skeletal muscle, thymus and spleen. IHC largely confirmed pathological findings.

Intrabronchial

The intrabronchial (ib.) route of infection delivers virus in a similar fashion to aerosol delivery; however, it has the advantage that it is easier to perform the inoculation and does not require the specialized equipment and facilities inherent to aerosol experiments. Furthermore, a more accurate inoculum dose can be delivered. Johnson *et al.* evaluated the disease course in *M. fascicularis* infected by the ib. route with 10^4–10^6 PFU of CB (Z79). End point criteria/death was observed in 66 and 15% of the NHPs challenged at the 10^6–10^5-PFU doses, respectively. In the 10^6 PFU-infected animals, the mean time to death was 20 days. Oxygen saturation (SpO_2; in circulating blood and the peripheral tissue) and respiratory rate experiments revealed elevated respiratory rates from day 5 p.i. – that did not return to normal levels in animals that succumbed to disease – and SpO_2 levels that dropped from day 14. Fever developed at day 6.3 and lasted until day 14, and lesions developed at approximately day 7.6. vDNA in the blood was present from days 3–20 with peak levels at day 13.7 and virus detection in oral and nasal swabs occurred at days 5–9 and 7–9, respectively, with peak levels at days 11–14 and 9–12, respectively, and NA could be detected from days 7–8. The overall cytokine/chemokine response to infection was

proinflammatory; however, the levels within groups were highly variable [55]. Generally, animals receiving the lower-dose inoculums (10^5 and 10^4 PFU) presented with biomarker manifestations at similar times to those in the higher dose groups; however, clinical signs were less severe, with few lesions reported (Table 4).

In an ib. study utilizing a sublethal infection (10^5 PFU of CB [Z79]) of *M. mulatta*, it was found that pustular lesions developed by day 7–12 on the skin and oral mucosa. These numerous and widespread lesions progressed from the pustular to the crusting stage at day 12–14 before finally scabbing and healing at day 18–28. Animals also developed coughs and labored breathing between day 7 and 14. All infected animals developed fever, with a peak temperature recorded between days 7 and 14; interestingly, temperature remained elevated until week 3 or 4 p.i. vDNA levels were measured in whole blood, peripheral blood mononuclear cells and bronchoalveolar lavage (BAL) fluids, and generally it was found that elevated viral loads paralleled the appearance of skin lesions and the development of fever. A direct correlation was observed between viral load and clinical signs. Detection of vDNA in blood was highly variable at approximately days 4–14 and lasted approximately 10 days before becoming undetectable. In BAL fluid, vDNA was detected between days 7 and 10 and remained elevated until day 42, when levels were undetectable [56]. Interestingly, the proteome from the BAL fluid was also characterized, and it was found that elevated levels of proteins indicative of inflammation were produced, along with a dramatic decrease of structural and metabolic proteins [57].

Intratracheal

Infection of *M. fascicularis* with 10^6 PFU of MPXV (CB) via the intratracheal (it.) route is summarized in Figure 2 [52]. Infection of *M. fascicularis* via the it. route with 10^7 PFU of CB (MSF#6) resulted in death/euthanasia by day 15-19 p.i. (Table 4). Lesions developed by day 8–10 p.i. and were accompanied by anorexia and dyspnea. By day 14 the lesions were characterized as pustules and clinical signs of illness became more severe. Similar to other studies, histopathological examination of the lungs of the dead animals revealed macroscopic lesions characterized as fibrinonecrotic bronchopneumonia and cutaneous lesions characterized by acanthosis, necrosis and intracytoplasmic amphophilic inclusion bodies. Other changes to tissues were reported: tracheitis, necrotizing glossitis, lymphadenitis and splenitis with lymphoid depletion. vDNA could be detected in the blood and saliva from day 4 and increased rapidly until death, with peak levels reported at days 11–15. Fever was recorded from day 5 until death.

The it. challenge route has been used to demonstrate the efficacy of vaccination with MVA (IMVAMUNE®) and the classical VACV-based vaccines (Elstree-RIVM and Elstree-BN) in various combinations. All vaccinated animals presented with a brief elevation in body temperature between days 5 and 8 and slight elevations in blood and throat viral loads. Only one animal developed pocks; all other animals had no clinical signs of disease other than the temperature elevation [58]. In another study, *M. fascicularis* was infected via the it. route (10^7 PFU of CB [MSF#6]) and several different treatment regimens were evaluated: vaccination with RIVM; treatment with 5 mg/kg of CDV; and treatment with 5 mg/kg HPMPO-DAPy. Animals in the control group died by day 15 and presented with similar clinical signs to those in Stittelaar *et al.*'s studies [58]. These experiments revealed that antiviral treatment is more efficacious than post-exposure vaccination following MPXV infection of *M. fascicularis* (Table 4) [59].

Intranasal

An in. infection (Utrecht strain) of *M. fascicularis* was first evaluated in 1967 by Gispen *et al.* who observed a febrile reaction on days 7–8, followed by the appearance of vesicles on

the neck, chest, belly, inner-thigh and the hollow of the knees [38]. Both animals survived. A second study by Noble (also using *M. fascicularis*) reported different results; two monkeys were inoculated with 10^8 PFU of MPXV (Utrecht 65–32), but failed to demonstrate skin lesions, facial edema or death; however, animals did seroconvert by 3 weeks p.i. [60]. More recently, Saijo *et al.* evaluated disease in *M. fascicularis* challenged with either 10^8 PFU of WA (Liberia) or CB (Zr-599) [32]. In Liberia strain-infected animals, body-weight decreased by approximately 10% following challenge, and clinical signs included loss of appetite, rhinorrhea and conjunctival discharge, diarrhea, irritability and a typical skin rash. The structures of the mucous membranes in the nasal cavity were damaged due to necrosis and inflammatory cell accumulation. vDNA could be detected in the blood from day 4 and peaked by day 9. In monkeys infected with 10^6 PFU of CB (Zr-599), one out of two presented with severe clinical signs and the other had very mild clinical signs; however, both survived. In the more severe cases, 178 skin lesions were recorded; these ulcerative lesions were still exudative on day 18 and were more severe compared with Liberia-infected animals, whose lesions had dried and were covered in scar tissue by day 18. vDNA levels peaked between days 5–10 and were 2 logs higher than those recorded for Liberia animals. Inappetence was also increased in these animals. Gross MPX-associated lesions were observed in the lymphoreticular system (tonsil, spleen, thymus and radial, inguinal, axillary, and submandibular lymph nodes), in the liver, pancreas and ileum [32].

The efficacy of vaccination with VACV strain Lister and LC16m8 was evaluated in *M. fascicularis* challenged in. with 10^8 PFU of WA (Liberia). Nonvaccinated animals presented with typical clinical signs (as above), whereas vaccinated animals presented with no clinical signs, no histopathological changes and no changes to viral load [61].

Experimental infections of *M. fascicularis* & *M. mulatta* via other routes

Subcutaneous

Prier and Sauer were the first to demonstrate that a sc. infection of *M. fascicularis* and *M. mulatta*, with a virus isolated from the 1959 US outbreak, caused local lesions without spread to other body parts [37]. Olsen *et al.* found that, following a $10^{4.6}$ PFU WA (Cop) sc. challenge, a *M. mulatta* (n = 1) became ill with generalized MPX disease from day 7; this animal also survived [62]. Unfortunately, these findings are not consistent with more contemporary studies. Recently, *M. fascicularis* was challenged with 10^6 PFU of CB (Zr-599) via the sc. route [61]. Infection with MPXV was lethal and monkeys lost approximately 15% of their body weight. Papulovesicular skin lesions appeared on day 7 and animals presented with 390–1150 lesions. vDNA was detected by day 4 and reached a peak by day 12–15. Clinical signs were so severe that monkeys were euthanized on day 18. Lesions were detected in the lymphoid system, lung, trachea, stomach, small intestine, colon, rectum, liver, urinary bladder and uterus. CRP was measured as an indicator of inflammation and was significantly increased. Lymphopenia and thrombocytopenia were also detected. IFN-γ was elevated from day 4 and reached a peak at day 7 [61].

In a second study, Saijo *et al.* compared the disease of *M. fascicularis* inoculated sc. with 10^6 PFU of WA (Liberia) or CB (ZR-599) [32]. Generally, the sc. challenge caused milder disease, with findings similar to those of earlier studies [62]. Infection of three out of four ZR-599 animals was fatal and body mass decreased by 10–20% without any sign of recovery (except in the animal which survived). One out of three Liberia-infected animals died. The typical papulovesicular rash appeared on days 7–9 with higher lesion numbers in ZR-599-infected animals (390–1150 in fatal cases and 95 in surviving animal) compared with Liberia-infected animals (881 in fatal case and 29–196 in surviving animals); however, the morphologies of the lesions were similar. The most noticeable clinical signs in both

groups were anorexia and diarrhea. The lymphoreticular system (radial, inguinal, axillar and submandibular lymph nodes, tonsil, thymus, spleen and pharynx) of both groups presented with the most significant lesions. The most significant differences between the two groups were the appearance of lesions with granulomatous inflammation in the stomach, small intestine and colon in ZR-599-infected animals. Antigen detection revealed that ZR-599 was present in organs that presented with lesions, as well as in the genitourinary tract, respiratory tract and gastrointestinal organs. Liberia infection was generally restricted to the skin, lymphoid and reticuloendothelial systems. The lungs of ZR-599-infected animals were entirely and diffusely affected by the infection but were unaffected in Liberia-infected animals.

Saijo *et al*. also evaluated the protection provided by vaccination with VACV Lister and LC16m8 in a *M. fascicularis* 10^6 sc. challenge with CB (Zr-599) [61]. Following vaccination a 'take' was reported in both groups. Following challenge (5 weeks post vaccination), animals vaccinated with either vaccine presented with no indications of disease except for local cutaneous lesions at the site of infection in LC16m8 animals; however, these lesions were much milder than those observed in the nonvaccinated group. Animals generally presented with no clinical signs of MPX. vDNA could be detected in the blood of vaccinated animals; however, the levels were lower than those of naive animals and persisted for a shorter time. Nonvaccinated animals (two out of two) all died with typical disease.

Comparison of inoculation routes

It is difficult to determine which inoculation route best recapitulates natural transmission of MPXV in *M. fascicularis* and *M. mulatta.* Early reports of natural outbreaks are rather vague and the viral strains are usually unknown; this presents a problem because many studies have indicated markedly different pathologies between CB and WA strains. Furthermore, it is likely that the inoculum in natural outbreaks is far lower than those administered experimentally. It is interesting that the 1958 outbreak in Denmark occurred 2 months after the animals were received. This could indicate that MPXV had been transmitted via fomites that had been in the vicinity for some time, or it is possible that MPXV had been amplifying in the colony for several months before the outbreak – although this does not explain why so few of the animals presented with clinical signs. id. inoculation from the Denmark outbreak and sc./id. from 1959 US outbreak caused few or no clinical signs, which indicates that infection did not occur via the skin. It is interesting that the 1959 US outbreak indicated that many *M. mulatta* did not present with disease but were seropositive. This is consistent with other findings that found that *M. mulatta* is less susceptible to the virus than *M. fascicularis*.

Since OPVs utilize various transmission mechanisms, one cannot assume that MPXV favors one mechanism over another; however, human infections in the US 2003 outbreak suggest that the virus gains access through the skin and likely through the respiratory tract too. The majority of *M. fascicularis* and *M. mulatta* studies have been geared toward modeling smallpox and therefore have utilized respiratory infection routes including aerosol, BMAD, ib., in. and it. It is difficult to evaluate which of these routes is the best method for inoculation because the transmission route for MPXV is unknown and, to some extent, may be species-specific. None of the investigated routes adequately mimic natural infection with MPXV and VARV. The main feature of most of the respiratory tract routes (except in.) was an infection with a high level of mortality with the presentation of a fibrinonecrotic bronchopneumonia; interestingly, a high incidence of fibrinonecrotic bronchopneumonia and interstitial pneumonitis was reported in fatal cases of human MPX [16,63,64]. Consideration should be given to the fact that the mechanical activity of inserting the delivery-apparatus (except for in aerosol challenges) could also scratch the tissue and initiate an inflammatory response at the inoculation site. The advantage of aerosol delivery over it.

delivery is that it. deposits virus directly in the airways without regard to particle size and the physiological deposition that occurs during the process of inhalation. That said, the it. route led to 100% mortality in both studies, whereas mortality was variable in the aerosol studies. The drawbacks of aerosol infection are the very high dose of virus required for the nebulizing application and the variability of the exposure dose [24]. The aerosol route deposits virus to the distal airways and alveoli, which may more closely mimic exposure from biowarfare compared with natural transmission, which likely deposits virus in the upper respiratory tract. The ib. route delivers virus in a similar fashion to aerosol delivery and disease clinical signs are also similar. Inoculation is based on an anatomical landmark and the quantity of inoculums can be readily measured and completely administered. The procedure delivers the inoculum into the left tertiary bronchus using a pediatric bronchoscope, which is also inexpensive compared with aerosol delivery and likely makes the ib. route more appealing; it also gives similar mortality values as aerosol at similar doses. Two studies have made a direct comparison between lethal ib. and iv. challenges, which found that the maximum tolerated dose was double that experienced by iv.-challenged animals, and fever developed significantly later (2.6 days compared with 6.3) in ib. animals [55,65]. Furthermore, the number of lesions in the ib. model was significantly lower than those of the iv. model (range 194–644 and 60–1552 for ib. and iv., respectively). vDNA in the blood was present from days 3–20 in the ib. group and from days 2–11 in the iv. group; peak vDNA levels were at days 13.7 and 8.2, respectively. It should be noted from this data that lesions developed in the iv. and ib. models at approximately 2 days and approximately 4 days following the onset of viremia, respectively. In the iv. model, MPXV from oral and nasal swabs was detected at days 2–7 and 4–6, respectively; which was approximately 3 days earlier than in the ib. model. Cytokine/chemokine responses in the ib. model were also delayed by several days (~6). In general the distribution and level of virus replication in tissue was comparable between both routes, although levels were higher in the lymphoid and reticuloendothelial tissues from the iv. group. Notable differences were observed in the axillary, popliteal and cervical lymph nodes, where titers were 100-fold higher in the iv. groups. Other tissues with detectable virus included the liver, testes, ovaries, uterus, kidney and spinal cord. In summary, several key events were delayed in the ib. model compared with iv.: fever, lesion development, peak viremia, viral shedding in oral and nasal excretions, peak cytokine levels and end-point criteria. These data indicate that disease progression in ib. animals is delayed, which is understandable as the iv. route bypasses many early components of respiratory disease. iv. animals also experienced no changes in respiratory rates compared with ib., which was elevated [55].

When comparing the ib. to the it. route, it was found that it. animals had 100% mortality and a shorter time to death (16.3 vs 20 days in ib.); however, histopathology indicated similar lung pathology with fibrinonecrotic pneumonia reported in both it. and ib., thus, it would seem that the it. and ib. routes are comparable. BMAD allows for delivery directly above the tracheal carina of a large-particle aerosol via a microsprayer attached to a bronchoscope, resulting in a similar disease course to that of an aerosol-inoculated monkey. It also offers advantages over the aerosol route, that is: a dose-dependent incubation period before disease onset, the ability to accurately deliver a fixed dose of virus, and the fact that the equipment required for inoculation is inexpensive and easy to use. The disadvantage to BMAD is that it only has 30–60% mortality at a 10^6–10^7 PFU dose compared with it., which has 100% mortality at a similar dose. The in. route is difficult to evaluate because dose volume can affect the degree of infection of the respiratory tract; with low volumes it is localized to the upper respiratory tract, whereas larger volumes result in greater involvement of the lower respiratory tract. Past and recent studies have not demonstrated mortality even at doses of 10^8 PFU [32,38,60,61]. Therefore, it would appear that for lower respiratory tract inoculation each route has some advantage over the other. Only the it. route so far has

demonstrated 100% lethality, although this level of lethality is not a feature of human MPX or smallpox.

By far the most pathogenic route appears to be sc., which causes 100% mortality (in CB strain) at a relatively low dose of 10^6 PFU. Given that the natural route of transmission of MPXV is possibly via the skin – as is the case with other poxviruses – consideration should be given to this route for antiviral and vaccine studies instead of the less physiologically relevant iv. route. That said, if the overall goal of a MPXV animal model is to develop a better human/variola model, then respiratory challenges would be more appropriate.

Experimental & natural MPX in small animals

Prairie dogs: early finding

Prairie dogs (*Cynomys ludovicianus*), native to the USA, were infected with MPXV when housed in close contact with various rodent species imported from Africa [40,66,67]. Approximately 110 prairie dogs were sold and 15 became ill, of which ten died rapidly. Analysis of animals from the initial outbreak revealed ocular and nasal mucoid discharge, swollen eyes, anorexia, tongue ulcers and red-brown consolidations involving 50% of the pulmonary parenchyma [68,69]. Two animals presented with livers that were red with scattered with mottled areas. OPV antigens were identified in areas with grossly and microscopically identified lesions. The lungs had concentric, coalescing bronchoalveolar pneumonia and inflammation extending to the bronchiolar walls and alveoli. Active viral replication was demonstrated in the lungs and tongue, and viral antigen was abundant in the lung bronchial epithelial cells [68]. In a third animal, a systemic MPXV infection with extensive and severe lesions in numerous organs was reported. Ulcers were noted on the tongue and hard palate, the cranial and middle lobes of the lungs were red, depressed and firm. Remaining parenchyma was pale tan and failed to collapse. Multiple white plaques were observed in the visceral pleura. Cervical and thoracic lymph nodes were swollen and small. White, firm foci with umbilicated necrotic centers were sparsely distributed throughout the glandular portion of the stomach and small intestine [69]. Multifocal, necrotizing lesions were reported in a diverse range of organs, such as thymus, brown fat, colon, liver, uterus and vagina, among others [69].

Prairie dogs: in. & intraperitoneal challenges

The observation of disease in the US MPX outbreak provided the impetus to establish a prairie dog model. Following intraperitoneal (ip.) inoculation with 10^5 PFU of WA (US-2003) most animals became lethargic and anorexic. All infected animals died 8–11 days p.i. and did not develop skin lesions. MPXV could be detected in the blood and throat from day 5–6 p.i. with levels increasing until death. Highest titers were reported in the livers and spleens; low levels were detected in the kidneys and lungs. Hematoxylin and eosin staining at necropsy revealed that spleens showed moderate–severe necrosis, the livers showed centrilobular necrosis and hepatocellular inclusion bodies, and the lungs exhibited mild-to-moderate thickening of the interstitium with infiltration by mononuclear inflammatory cells. The spleen and liver inclusion bodies were positive for OPV antigens by IHC [70].

Three studies have evaluated the in. route of infection. Xiao *et al.* evaluated a $10^{5.1}$ PFU WA (US-2003) challenge and found that three out of five infected animals died by day 11–14 p.i. Surviving animals developed vesicular lesions on the lips and tongue and had nasal congestion and discharge. In the three fatal infections, MPXV appeared in the throat from day 3 p.i. and in the blood from days 8–9 p.i. At death, animals had the highest levels of virus in the lungs and lower levels in the liver, spleen, kidney and heart. No virus was detected in the brain. No hepatic lesions or splenic necrosis was observed. The lungs of dead

animals showed marked edema, hemorrhage and necrosis. Surviving animals were in apparent good health but with some histological foci of inflammation in the skin. By IHC, viral antigen could be detected in the liver, lungs, mediastinum, bronchus and mediastinal lymph nodes [70]. The pattern of experimental infection in the in. group concurs with the clinical and pathological observations made during the 2003 US MPXV outbreak in prairie dogs, with similar mortality rates and similar clinical signs [68]. Hutson *et al.* compared the response to disease following in. or id. challenges with WA (US-2003) and CB (358) viruses at $10^{4.5}$ PFU [30]. in. and id. WA-infected animals all survived and had similar disease presentation and clinical signs, with lesions developing at 9–12 days p.i.; however, fewer lesions were observed in in.-infected animals and id.-infected animals developed a lesion at the inoculation site by 6–9 days p.i. The id. CB-infected animals also developed a lesion at the inoculation site and again the lesion count was slightly fewer for in.-infected animals compared with id. infected animals. Lesions again occurred at days 9–12, with mortality rates of 50% for id.-infected and 25% for in.-infected animals. MPXV DNA could be detected in the blood from days 3–15 and 6–15 for id.- and in.-infected animals, respectively. Detection of DNA by qPCR, showed similar onset, kinetics and cessation in CB- and WA-infected animals.

Animals challenged in in. dose-escalation (10^2–10^5 PFU) experiments with WA and CB strains again experienced the development of lesions that were higher in number at higher doses. An earlier disease-onset and an increase in mortality, morbidity and viral shedding was observed as doses were increased, with 25–75% and 50–100% mortality at 10^3–10^5 dose for WA and CB, respectively. Dose-escalation studies revealed that the CB and WA strains had LD_{50} values of approximately 10^3 and 10^5, respectively [31]. These data reveal that the CB strain is more virulent than the WA strain in the prairie dog model, as evidenced by a higher lesion count, a greater trend towards weight loss, a significantly higher percent increase in temperature and higher mortality. Furthermore, the id. route appeared to be more pathogenic than the respiratory (in.) challenges, as evidenced by a slightly more severe disease with increased mortality (in CB-infected animals) [28,31].

Prairie dogs: transmission studies

Hutson *et al.* evaluated the transmission of the WA strain (US-2003-044) between prairie dogs via three different experimental routes: contaminated bedding (fomites); cohousing of animals; and respiratory/nasal discharge [25]. Primary animals were inoculated with a sublethal dose of 10^3 PFU via the in. route and developed clinical signs similar to those reported previously. To evaluate transmission via bedding (fomites), the primary animal was infected and placed in a trough until day 16 p.i. Upon removal of the challenged animal, 3 naive animals were cohoused in the vacated trough. These contact animals all developed ten to 14 lesions from days 11–18, lost 8–14% body weight and had a mortality rate of one out of three. Contact animals in the cohoused studies and in the respiratory/nasal-discharge studies also had mortality rates of one out of three and one out of two, respectively. In general, contact animals developed similar, but often more severe, clinical signs as in. challenged animals, indicating that virus was transmitted to the naive animals and produced a more virulent disease than in the in. challenged animals. These observations are supported by the finding that contact-infected animals often shed larger quantities of virus in oral secretions and in blood – which is in agreement with the findings of the US 2003 MPXV outbreak.

Prairie dogs: vaccination studies

A study by Keckler *et al.* evaluated the protective capability of Dryvax, ACAM2000 and IMVAMUNE against an in. CB (Congo-23) challenge [71]. A ‘take’ was observed after vaccination with Dryvax and ACAM2000 on days 2–7 and scab detachment on day 19–25.

All vaccinated animals developed antibodies, and it was shown that following MPXV challenge, the vaccinated animals developed antibodies faster than nonvaccinated controls. Following a 10^6-PFU dose, the challenged animals fared poorly, with one out of two animals dying on day 11 p.i. and the second experiencing severe illness, multiple lesions, severe nasal involvement and no recovery from weight-loss; this animal was euthanized. Both Dryvax and ACAM2000 protected against death and rash, and a single dose of IMVAMUNE also protected against death, but animals developed a modified rash (n = 8 was used in each vaccination group).

Prairie dogs: antiviral studies

The antiviral drug, ST-246 has also been evaluated using the in. CB strain (ROC-2003-358) model. Groups of animals were treated orally with 30 mg/kg of the antiviral drug ST-246 daily, for 14 days, to evaluate its efficacy in a lethal (10^5 PFU) in. model [72]. Treatment began at day 0 p.i., 3 p.i. or when clinical signs (rash) were observed. At day 8 p.i., three out of four vehicle-treated animals developed clinical signs similar to those previously observed [71]. At day 6–8 p.i., animals began to lose weight (2–10%), one animal developed a typical papular or pustular rash 2 days before death (on day 12 p.i.), whereas one out of four only developed a petechial rash just before death; a third animal did not develop a rash at all. Three out of four infected animals had died by day 10–12 p.i. Drug treatments at day 0 (prophylactic treatment) protected all animals, which lost minimal weight, and infectious virus could not be detected from swabs (except for oral swabs on day 12 p.i.). Animals treated with ST-246 on day 3 p.i. (postexposure treatment) also remained visibly asymptomatic and lost minimal weight. Following rash-onset, three out of four animals were treated with ST-246 from day 10–12 (therapeutic treatment) when a rash of five to 50 lesions appearing as macules developed on the chest, abdomen, back, groin and tongue. The fourth animal did not develop a rash until day 24 (2 weeks after the other animals in the group). Animals that developed a pustular rash (two out of four) experienced the most weight-loss, but ultimately all animals survived infection. These findings suggest that rash onset is a suitable trigger for the initiation of antiviral therapy, and this is neatly aligned with the US FDA requirement that the trigger for treatment of smallpox or human monkeypox be based on a visual manifestation [73].

Prairie dogs: summary

Findings from in. prairie dog challenges appear to be relatively consistent to what was found in the US 2003 strain outbreak [68,69]. One drawback to the in. model is that some animals fail to develop rash and that the time of rash onset is inconsistent; also the type of rash that the animals present with appears to be variable. Furthermore, mortality rates from various studies often fail to produce a 100% lethal model, although it seems clear that the CB strain is more pathogenic than WA strains [28,30,31,70,71]. That said, the prairie dog model appears to be an excellent system to study MPXV pathogenesis as well as the efficacy of antivirals and vaccines, and this is only diminished by the lack of prairie dog reagents and the inability to breed the animals in a vivarium.

Squirrels

A 1979 survey of monkeys, arboreal and terrestrial rodents, and bats in Zaire revealed MPXV-specific antibodies in the Thomas's rope squirrel (*F. anerythrus*). A second survey in 1985 focused on collecting samples from animals in the areas surrounding villages with human MPXV infections. A total of 172 terrestrial rodents, 22 squirrels, 120 sheep and goats and 67 domestic cats were sampled. One squirrel (*F. anerythrus*) in this group (found 50 m from a village) had round/oval, flat lesions of 2–3 mm in diameter on the abdomen and inguinal areas. MPXV was isolated from the skin, lungs, spleen and kidney and MPXV-specific antibodies were detected in the blood. This is the first and only isolation of MPXV

from a wild animal in nature [17]. Two out of a total of 18 *F. anerythrus* (18 out of 22 of the squirrels captured were *F. anerythrus*) appeared healthy, but also had MPXV antibodies [17,74].

A third survey in Zaire in 1986 examined the surrounding areas of villages that had experienced human MPXV infections suspected of coming from infected squirrels. A total of 233 rodents (including small terrestrial rodents and Gambian rats) were negative for MPXV-specific antibodies. A total of 25% (79 out of 320) of *F. anerythrus* and 16% (six out of 37) of red-legged sun squirrels (*H. rufobrachium*) were found to have MPXV-specific antibodies [19]. These squirrels commonly inhabit the forests around human settlements in the rural areas of Zaire [18]. Further west in the country, a survey was conducted where no human MPXV cases had been reported; MPXV-specific antibodies were reported in 49% (58 out of 119) of ribboned rope squirrels (*F. lemniscatus*) and 19% (six out of 31) of Gambian sun squirrels (*H. gambianus*) [16]. These findings over four widely separated areas indicate that MPXV is circulating in squirrels of the genera *Funisciurus* and *Heliosciurus*. The results of the CDC testing of 800 small animals imported prior to the 2003 US MPXV outbreak were supportive of these findings. These animals consisted of nine different species and six genera of African rodents. Testing revealed that three out of six *Funisciurus* sp. were positive by MXPV-specific PCR and virus isolation; however, *H. gambianus* squirrels were not positive by PCR (n = 27) and were not tested for virus isolation [21]. Testing of two field sites where the squirrels imported to the USA were captured revealed that 40 and 14% of *Funisciurus* and *Heliosciurus*, respectively, were positive for OPV-specific antibodies; however, the studies did not evaluate if these were MPXV-specific antibodies [75].

MPXV isolated from an infected *F. anerythrus* squirrel (from the African survey described above) was used at 10^6 PFU to infect Eurasian red squirrels (*Sciurus vulgaris*) via the in., *per os* (PO) and SCR routes. At day 1 p.i. an elevated temperature was observed and at day 3–5 p.i. animals became limp and stopped moving; dyspnea followed in in.- and PO-infected animals. Disease was noted to develop most rapidly in animals infected by PO or in. routes. Skin lesions were not observed and death occurred by days 7–8 p.i. Virus titers of 10^6 PFU/ml (on chorioallantoic membranes) were reported in lungs, livers, kidneys, spleens and the esophagus of dead animals, regardless of route. Nasal discharge was also reported for all animals, regardless of route [76].

Shelukhina *et al.* evaluated the response of *Funisciurus* sp., *Protoxerus* sp., and *Heliosciurus* sp. to in., sc. and PO challenges with a CB strain. Squirrels were infected with 10^5–10^6 PFU and developed an acute, generalized infection with 100% lethality. Susceptibility varied between species at lower challenge doses. sc. challenge resulted in a thick, red papule at the inoculation site. Skin lesions (in non-fur areas, or the borders of the skin and mucous membranes) occurred in only a few animals challenged in. or PO with nonlethal doses. Transmission studies found that infected animals were able to transmit the disease to naive animals by airborne or direct contact [Shelukhina EM *et al.*, Unpublished Data].

The 13-lined ground squirrel (*Spermophilus tridecemlineatus*) has been extensively evaluated as a model for MPXV infections. Following a $10^{5.1}$-PFU challenge via the in. and ip. routes with a WA strain (US03), a fulminant disease with lethargy and anorexia was reported. No skin lesions, respiratory distress or other obvious clinical signs were reported. Death occurred at days 6–7 p.i. for ip.-infected animals and at days 8–9 p.i. for in.-infected animals. Virus cultures were positive from throat swabs at day 4 p.i. for both routes; however, cultures for blood were positive at days 5 and 3 p.i. for in. and ip., respectively. High levels of virus were detected in the kidney, lungs, heart and brain, but the highest levels were in the liver and spleen. Virus levels in organs appeared not to be related to route of infection. At death, the ip.-infected animals presented with liver centrilobular

hepatocellular necrosis, steatosis and basophilic inclusion bodies, and the spleen had necrosis characterized by lymphocytic karyorrhexis in the white pulp, and fibrinoid necrosis, congestion and endothelial cell swelling in the red pulp. The lungs had thickening of the alveolar septa by inflammation with focal consolidation. in.-infected animals exhibited lesions in the liver, with multifocal steatosis and diffuse hepatocellular necrosis. Lungs of the in.-infected animals also had variable consolidations and interstitial inflammation with some necrosis in the peribronchial lymphoid tissue. Cytoplasmic inclusion bodies were present in all livers. Splenic necrosis was similar to that observed in ip.-infected animals, but in.-infected animals also had necrosis in the lymph nodes [77].

S. tridecemlineatus was also used in the development of a sc. model using CB (Z79) and WA (US03) strains. Both viruses produced a similar fulminant disease with 100% lethality at a 100 PFU dose (LD_{50} = 0.35 and 0.46 for Z79 and US03, respectively) by days 6–11 for both strains. Respiratory distress was uniform and rapid, with bleeding diatheses, anorexia and lethargy from day 3 in CB, and days 4–5 in WA-infected animals. Clinical signs were more severe for CB-infected animals, animals experienced epistaxis and tissue damage in the lung was increased in CB-infected animals, and pulmonary edema and hemorrhage occurred earlier in the disease course. From day 5, CB-infected animals experienced respiratory distress, labored breathing and wheezing; however, similar clinical signs were only observed in WA-infected animals within the 24 h preceding death. Viral loads in the blood, lungs, liver, spleen and skin lesions increased until death, with loads 1–2 logs higher in CB-infected animals. MPXV antigen was detected in the lungs, liver, lymphoid organs, esophagus and intestines, again with higher intensity and diffusion of IHC staining occurring in the respiratory tract of CB-infected animals [29]. Clinical signs and disease course in the sc. WA-challenged animals appeared to be very similar to those reported for in. and ip. WA-challenged animals [77]. Although both viral strains produced lethal disease, an earlier day of death, more severe clinical signs, and higher viral and tissue loads indicate the increased virulence of the CB strain.

The antiviral drug, ST-246, has been tested in the sc. *S. tridecemlineatus* model. Squirrels were infected with 100 PFU of CB (Zaire 1979–005) and treated with 100 mg/kg/day of ST-246 for 14 days, with treatment commencing at 0, 24, 48, 72 and 96 h p.i. Placebo treated animals developed signs of illness by day 4 and experienced 100% mortality by days 6–9 p.i. Groups treated with ST-246 at 0, 24, 48 and 72 h p.i. had 100% survival, and the 96-h groups had 33% mortality, with two out of six animals euthanized on day 7 p.i. No weight change was observed in any surviving animals. Animals with treatment initiated at 0, 24, 48, and 72 h p.i. developed no detectable viremia or viral titers in the liver, spleen and lung at day 7. Interestingly, these groups did not develop an antibody response, suggesting that insufficient antigen was generated in the presence of ST-246 to trigger an immune response [78].

The serological surveys, and experiments performed by Shelukhina are strongly supportive of the hypothesis that *Funisciurus* and *Heliosciurus* are a major reservoir of MPXV. Consideration should be given to developing these species as models to further understand the biology of the virus. Experiments in the 13-lined ground squirrel were also informative with mortality rates of 100% following low-dose sc. challenges; thus, highlighting the sensitivity of squirrels to MPXV. The only drawback of the 13-lined ground squirrel model is the lack of rash that is observed in *Funisciurus* and *Heliosciurus* in an experimental setting and was observed in *Funisciurus* in nature. In summary, the squirrel is a good model of MPX and should be considered for further evaluation.

Rabbits

Rabbits were evaluated in several older (circa 1970s) reports and generally adults were resistant to challenge (except intracranial [ic.] and SCR in albinos); however, animals less than 10 days old were sensitive to SCR, ic., PO and in. challenges – presumably the disparity exists due to the absence of a fully functioning immune system. Two reports give differing accounts of the outcome following an id. challenge; one reports a severe hemorrhagic reaction followed by pustular lesion development at various sites on the body, but no deaths [34,79-81]. Adult albino rabbits used in the second study developed swelling at the inoculation site, a rash over the body on day 7 and death by days 9–12 [82]. ic. inoculation was fatal to adults and 2-day-old rabbits; in adults, a fatal meningoencephalitis was reported, with death at 6–9 days p.i. [38]. SCR resulted in fever, and on approximately day 3 the development of a localized papulopustular lesion in adults and albino adults was followed by a full recovery [81,82]; however, it was fatal to 2-day-old animals [34]. Interestingly, SCR and id. serial passages were successful in many animals [36,38,41,79] and generated red, indurated lesions with black-brown necrotic centers [79]. sc. administration resulted in a generalized infection with widespread pox eruptions followed by a full recovery in adults [34,41]. iv. challenge of adults was reportedly followed by acute disease, an explosive rash (papules that later developed into pustules followed by scabbing) on the skin and mucous membranes observed on days 5–6 p.i. and full recovery [34,37]. PO challenge resulted in generalized disease and pox eruptions on the skin, ears and lips and death by days 4–14 in 10-day-old animals; however, no reaction was observed in adults [81]. in. challenge was particularly effective in 10-day-old rabbits and caused weight-loss and death by days 4–5 with no rash development [81]. More recently, following the 2003 US MPX outbreak, a rabbit was reported to have become ill after exposure to an ill prairie dog at a veterinary clinic [40]; however, no rabbits could be identified that were MPXV-specific PCR positive or positive for virus isolation [21]. The failure to observe severe disease in adult animals infected by these routes of infection limits the utility of this model.

Mice

Mice have been evaluated as models since the discovery of MPXV. Adult non immunocompromised mice are generally resistant to challenge (except via ic. route and sometimes ip. route), with adult BALB/c and C57BL/6 mice experiencing some weight-loss when infected via the in. or FP routes with 10^5 PFU of the CB (Z79) strain but not with the WA (US03) strain [28].

ic. challenge of adults results in death in all mouse strains and ages tested [36-38,41,46]. in. inoculation results in 100% mortality by day 15 and by day 17 in adult SCID mice [26,34,37,41,81]. Following a 10^2–10^3 PFU CB (Z79) challenge, 129 *stat1*$^{-/-}$ and C57BL/6 *stat1*$^{-/-}$ mutants also experienced weight loss with 40 and ≥90% mortality by day 10, respectively; however, the antiviral drugs, CMX001 and ST-246, were shown to protect the *stat1*$^{-/-}$ mutants from lethal challenges [26]. White mice at 8-days-old are partially sensitive to PO and id. inoculations (40 and 50%, respectively) and fully susceptible to in. inoculations. Mice at 12-days-old experience 24% mortality following a PO challenge (dose not given) [37,81].

Following an ip. challenge, adult BALB/c mice became sick but survived [83]; other adult strains have been reported to be both resistant and sensitive to ip. challenge [36,41], with SCID (~10^2 PFU) and newborn Swiss Webster (dose not given) mice all becoming sick and experiencing 100% mortality by days 11, 8–16 and 4, respectively [26,46].

Robust disease in adult nonimmuno compromised mice has only been achieved in the CAST/EiJ strain, which experienced 25% mortality following a 10^4-PFU FP CB (Z79)

challenge with swelling and edema presenting on the foot, but no mortality when virus was administered by SCR in. challenges resulted in 100% mortality by day 8 for both CB (Z79; LD_{50} = 680 PFU) and WA (US-C1) challenges ($LD_{50} = 7.6 \times 10^3$ PFU); however, these mice could be protected by Dryvax vaccination or treatment with CDV. Following a 10^4-PFU CB (Z79) ip. challenge, the CAST/EiJ strain experienced 100% mortality by day 8 with an LD_{50} of 12 PFU; in contrast to the other challenge routes, these mice experienced little to no weight loss. Two other nonimmunocompromised strains are also sensitive to MPXV, but to a lesser extent than the CAST/EiJ strain; following in. challenges the MOLF/EiJ and PERA/EiJ strains experience 75 and 40% mortality, respectively [27,84].

Other small animals

The multistate US MPX outbreak revealed that multiple species of animals had been infected with MPXV from approximately 800 small animals imported from Ghana: two out of 21 Gambian-pouched rats (*Cricetomys* sp.) tested positive for MPXV-specific PCR and one out of 15 tested positive by virus isolation [66]. One out of six southern opossum (*Didelphis marsupialis*) tested positive by PCR and virus isolation. Several other species also tested positive for MPXV-specific PCR: one out of 22 African hedgehogs (*Atelerix* sp.), one out of four Jerboas (*Jaculus* sp.), one out of four gray short-tailed opossums (*Monodelphis domestica*) and one out of four woodchucks (*Marmota monax*) [21]. In addition, a 1986 survey in Zaire found that one out of four porcupines (*Atherurus africanus*) were seropositive for MPXV-specific antibodies [16].

Various small animals have been experimentally tested for their susceptibility to MPXV. Guinea pigs failed to show signs of disease following iv., ip., id., sc., PO or ic. inoculation, although animals inoculated via the in. and ic. routes developed antibody titers by day 14 p.i. FP-infected guinea pigs also presented with no systemic disease, but did experience swelling and edema at the injection site after 7–10 days, development of a granulomatous lesion/edema confined to the injection site, and antibody titers by day 14; however, the lung, liver and spleen of these animals had no viral titers on days 6, 11, 16 and 22 p.i. [82]. SCR resulted in a slight inflammatory reaction on day 2 and the development of discrete papules on day 4, followed by scabbing and healing [34,37,41,81,82]. The multimammate mouse (*Mastomys natalensis*) was reported to be highly susceptible to ip. and in. challenge; however, details of the disease have not been forthcoming [16]. Adult chickens (*Gallus gallus*) failed to present with clinical signs following inoculation via iv., ip., id., and sc. routes [34,82]; however, when inoculated id., 3–4-day-olds and 4–8-week-olds developed vesicles that dried-up without leaving a scar by day 10 [82]. White rats (*Rattus* sp.) had no clinical signs following in., iv. or SCR. Newborn rats (1–3 days old) experienced 100% mortality by days 5–6, with virus replication in the lungs and liver following an in. challenge [81]. Adult hamsters (genus not reported) were resistant when infected via the PO, in., ic. or SCR routes; however, during the first 7 days p.i., virus was detected in the lungs, liver and spleen of animals infected by the ic. route [81]. In another study, hamsters were infected with 10^6-10^7 PFU via intracardiac injection. No visible disease was observed. Serial sacrifices were performed and virus was detected in blood and internal organs (not specified) during the first 7 days p.i. After 7 days the virus was no longer detected in most animals. At 14 days p.i. NA were found; however, seroconversion only occurred in 50% of animals. A generalized pathology was noted primarily in the liver, kidney and brain; all of which had focal lesions. The main manifestations were perivascular lymphohistiocytic infiltrate and damage to the endothelial structure of blood vessels [81,85].

Two animal species have been shown to be highly susceptible to severe disease: in. inoculation of cotton rats (*Sigmodon* sp.) induced an acute generalized illness with labored breathing, coughing, rhinitis, conjunctivitis, progressive emaciation and 50% mortality (time to death not given). High concentrations of virus were detected in the blood and internal

organs (unspecified) and antibodies were produced in survivors. iv. inoculation caused a more severe disease course with 100% mortality (time to death not given) [86]. Following the US MPXV outbreak, nine African dormice tested positive by PCR and virus isolation [21,66]. Vivarium-bred *Graphiurus kelleni* has subsequently been challenged with the CB (Z79) strain and experienced 100% mortality following a 10^4-PFU FP infection, and was observed to have an in. LD_{50} of 12 PFU. The average day of death at a 200-PFU in. inoculum was 9 days, with 14% weight loss (similar mortality was observed with the COP-58 strain following in. challenges). Following a 10^4-PFU in. challenge, virus was detected in nasal lavages by day 2, the spleen by day 3 and in the liver, lungs and blood by day 4 p.i. The highest viral titers were observed in the liver at day 8. From day 3–4 p.i., dehydration, conjunctivitis, upper GI tract hemorrhage, hepatomegaly, and lymphadenopathy was reported. By day 4–5 p.i., rhinitis was observed, along with lymphoid necrosis in the submandibular lymph nodes, spleen and thymus. Hepatocellular necrosis and hemorrhage in the lungs, stomach and small intestine were observed. Necrosis and hemorrhage was noted to affect many organs [87]. Vaccination with Dryvax 4 weeks prior to a 10^4-PFU in. challenge resulted in 100% protection. Treatment with cidofovir at 4 h p.i. with a 10^3-PFU in. challenge resulted in 81% protection [87].

In summary, many small animals appear to have the basic characteristics that could allow them to be developed into useful models, excluding guinea pigs, rats, chickens and hamsters, which do not develop robust disease and should not be developed further. Of the animals involved in the US 2003 outbreak, the Gambian-pouched rat and southern opossum were positive for both vDNA and virus isolation. A subsequent serological survey in the area of Ghana where the US 2003 rodents were imported from, found that approximately 5 and 2% of Gambian-pouched rats were positive for OPV DNA and antibodies, respectively [75]; one drawback to the Gambian pouched rat is that it is a relatively unused model, although some rudimentary studies have been performed [88,89]. The southern opossum, although native to South America and therefore not a natural host of MPXV, could also be utilized for studies, and has previously been used as a model of other viral infections [90]. The multimammate mouse, which has been utilized in at least one virus study (minute virus), was also reported to be highly susceptible to MPXV, and this species should also be evaluated further [91]. The main drawbacks to all of the potential models are the lack of available reagents to monitor immune responses, disease progression, pathology and transmission. Furthermore, the already-available models of MPXV disease are fairly comprehensive, thus making the development of more models somewhat counterintuitive. The disease presentation of cotton rats is reminiscent of that of prairie dogs (although it is not clear if cotton rats develop a rash). Cotton rats should be considered for further development – particularly in light of the fact that this species is relatively well used in various other disease models [92]. The dormouse proved susceptible to low-dose inoculums of MPXV and responded well to both vaccine and antiviral therapy. Lack of reagents for cotton rats and dormice limit their use as small-animal models of MPXV.

Experimental & natural MPX in large animals other than *M. fascicularis* & *M. mulatta*

During the Rotterdam Zoo outbreak of 1964, chimpanzees were noted to present with pox lesions on the skin, but experienced no other signs of general illness [39]. Chimpanzees were challenged with MPXV (U65–32) via the iv. route ($10^{6.8}$ $TCID_{50}$) with an isolate derived from one of the infected orangutans from the 1964 Rotterdam zoo outbreak [38,39]. An animal previously vaccinated with the smallpox vaccine developed no signs of disease; however, a nonvaccinated animal developed a mild infection with a few scattered eruptions on the face and hands. Previously splenectomized and nonvaccinated chimpanzees were also

challenged via the iv. route and two out of three animals initially developed a few lesions, but lesion development continued until most of the body was covered. Exanthema was severe, with confluent lesions on the face, arms, legs and upper-body. Crusts formed and appeared to be healing at the time of necropsy (day = 22). One out of three animals developed severe lymphadenopathy and CNS depression; however, only a few lesions were reported at the time of death (day 10) [93].

Two adults baboons (*Papio cynocephalus*) were inoculated im. with $10^{7.5}$ $TCID_{50}$ (COP strain [34]) and were housed with six immature (6–7 months) sentinels. By day 8, inflammation was observed at the injection site and by day 11 pox lesions appeared on the face and extremities of both animals. One animal also had truncal lesions. Lesions increased in number and developed into pustules, which crusted over and healed by day 18. HAI antibodies were detected by day 8, and peaked by day 14 p.i. Both animals fully recovered. Two out of six sentinel animals developed lesions on the face and extremities at day 28; however, fewer lesions were observed compared with the inoculated animals, although the disease course was similar and the animals were resistant to re-challenge with $10^{8.5}$ $TCID_{50}$. The remaining four out of six sentinels did not develop illness and were HAI antibody-negative when challenged at 91 days. Inflammation was observed at the inoculation site and exanthema was observed by day 7 p.i. Vesicular lesions were observed on the face and extremities by day 10 p.i. One animal also developed exanthema on the trunk, face, mucous membranes, buccal cavity and nasopharyngeal area and died on day 12 p.i. Maximum HAI antibody levels were reported at day 16–24 p.i. and MPXV could be isolated from the throat and stool of all animals during the second week after appearance of lesions [94].

In a second baboon experiment, two gr oups of ani mals were inoculated with 10^{7} $TCID_{50}$ via the SCR route (WA, COP strain). Animals in the first group were approximately 1 year old and those in the second group were approximately 3 months old. Initially, one animal was inoculated with virus and subsequent animals were inoculated with serial passages from lesions from the previous animal. The animals from the first group experienced erythematous and pustular lesions at the SCR site and an increase in rectal temperature until day 6 p.i and enlargement of the axillary and inguinal lymph nodes. Secondary lesions appeared on the trunk, extremities, face and mouth. Lesions became vesicular, pustular and hemorrhagic. Subsequent animals infected by serial passage experienced similar clinical signs with decreasing levels of severity. No animals died. The initially infected animal and the first two serial-passage-infected animals in the second (younger) group followed a disease course similar to those in the first group; however, with each passage the pustular lesions at the site of SCR contained increasing amounts of fluid that became increasingly hemorrhagic, with subcutaneously edematous SCR sites extending to the hips, base of the tail, abdomen and chest. All baboons from passage 4 died by day 17 and virus was constantly isolated from the throat, stools and blood samples as well as the heart, lung, thymus, adrenal and lymph nodes from dead animals. These data suggest that younger baboons are more susceptible to MPXV compared with older animals and/or that MPXV was adapting to the host [95].

Experimental inoculations via the in. routes have been performed on tufted capuchins (*Cebus apella*) and red-faced spider monkeys (*Ateles paniscus*), which failed to become sick; however, in the case of the capuchins, a coinfection via the ip. and ic. route did result in the development of skin lesions and HAI antibodies [60]. Inoculation of pigs (*Sus* sp.) by rubbing virus into the skin did not result in disease; however, MPXV could be detected in the skin from days 0–5 with gradual lowering of titers. NA were not detected in heat-inactivated sera, but there were significant levels of heat-labile neutralizing antibody activity [96].

Conclusion

The broad host-range of MPXV is a cause for concern, as it may facilitate the adaptation of MPXV to new hosts in new regions. Inspection of the host-range reveals that MPXV can infect animals from pan-geographical locales; namely, North America, South America, Asia, and Africa. Data from the Rotterdam Zoo outbreak indicates that MPXV is a highly transmissible disease and that there is no correlation between severity and geographical location of the diseased host [39]. It is interesting that MPXV has not been reported in nature outside of Africa, suggesting that species from other continents either cannot support the long-term lifecycle of MPXV, or that the virus has not been introduced to those locations. The broad host-range of MPXV suggests that it would have the capacity to expand out of Africa if given the opportunity, much like VACV becoming established in water buffalo in India and in cattle in Brazil [97-99]. Introduction of MPXV to locales outside of Africa could be disastrous. Of note is that nearly all recent NHP experiments have utilized the highly sensitive *M. fascicularis* and *M. mulatta* – both of which are restricted to Asia and are not natural hosts of MPXV, as serological surveys of these, and other primates, in India and the Malaysian peninsula were all negative [11,100]. The origins of the monkeys that caused outbreaks in various institutions were mostly of Asian origin [12]; this suggests that the animals were either cohoused at some point with infected African animals, or that specialized shipping containers contained fomites from previously transported, infected African animals; or, that MPXV does circulate in Asia and has thus far been undetected. Interestingly, the 1959 US outbreak of *M. fascicularis* and *M. mulatta* could be attributed to the cohousing of these animals with African *C. aethiops*, which made up approximately 5% of the population; however, these monkeys apparently presented with no observable clinical signs of disease [35,37]. Assuming that MPXV does not circulate in Asia, that specialized shipping containers were used to import animals from Africa or Asia, and that squirrels – the proposed reservoir – and/or other potential reservoir rodents were unlikely to be cotransported, MPXV could have more than one reservoir host in Africa, which is likely to be a NHP. Supporting this argument is the finding of MPXV-specific antibodies in *Cercopithecus* sp. and *Colobus* sp. captured in Africa [14,15,19]. African primates, such as *Cercopithecus*, inhabit a similar strata of the rainforest to squirrels, and it has been observed that these monkeys mix with various rodents [14], giving a basis for interspecies transmission.

Future perspective

MPXV was first discovered over 53 years ago in a disease outbreak in an animal facility in Denmark [34]. Subsequent outbreaks, field surveys and experimental studies have revealed that the virus has the capacity to infect and cause high levels of mortality and morbidity in a broad spectrum of hosts from across the globe. Despite these experiences, definitive proof as to the natural host of MPXV remains elusive – although prime candidates are squirrels. Indeed, a natural MPXV infection has still only been recorded in one animal – the squirrel *F. anerythrus* [17]. Future field studies utilizing modern experimental assays should be considered in Central and Western Africa. The CDC is likely to be the only US-based organization with the facilities and infrastructure to complete such a task.

Respiratory (in., ib., aerosol and BMAD) NHP challenge models of MPXV have been developed with high levels of success as determined by the presentation of disease; however, these routes of challenge do not induce the reproducible level of 100% mortality that is characteristic of the iv. models [23,24,51,53,55,58,61]. This in itself would not be a problem, since in nature MPXV is likely to not cause 100% mortality; however, the primary purpose of NHP studies is to develop models of smallpox and human monkeypox for the development of disease countermeasures, and to date the FDA encourages lethal models for

these studies; thus, the iv. route is the obvious choice. Two alternatives should be considered to the iv. route: the it. route has been shown to also induce 100% mortality with a longer disease course and later symptom onset than that of the iv. route. The later disease onset and the respiratory challenge recapitulate classical smallpox [55]; the sc. route also leads to high mortality and morbidity levels and should be considered as a nonrespiratory model of human OPV infections. Several studies have found that MPXV may transmit to humans via routes other than respiratory; namely, by handling infected animals and/or by consuming infected meats. For these reasons, a nonrespiratory challenge model may be required in the future, once the exact mechanism of human-to-human spread is elucidated – the sc. model would be an obvious choice [32].

One drawback to the NHP studies is the high virus inoculum that is required for mortality to be achieved. The prairie dog model has the advantage of being a small animal model that has an extended disease course, presents with skin lesions and has lesion onset that has been used as a trigger for therapeutic intervention. It has also been extensively tested in vaccine and antiviral studies with good success [71,72]. One drawback to the model is that, like the NHP models, an artificially high inoculum is required for mortality [30,70]. This finding is not always observed in small animal models; the 13-lined ground squirrel, dormouse and CAST/EiJ mice all have relatively low LD_{50} values. The two former species are not well-described and limited reagents are available; however, antiviral efficacy has been demonstrated in both [78,87]. The CAST/EiJ strain is arguably the best small animal model of MPXV for the following reasons: the strain is sensitive to a low-dose respiratory (in.) challenge; the strain is immunocompetent; the strain can be easily bred in a research vivarium, facilitating the execution of studies requiring a large number of animals; a multitude of reagents, assays and kits are available for the mouse; and many vaccines and antivirals have already shown efficacy against OPV challenges in the mouse model [27,84]. For these reasons, it is likely that the CAST/EiJ strain will be the primary small animal choice for the future, with the prairie dog also providing useful data. Both models should also be considered for synergy studies with the two lead anti-OPV antivirals, CXM001 and ST-246 [101].

The next 10 years will reveal interesting developments in our understanding of MPXV in many animal systems. The majority of these findings will be driven by the need to develop and approve countermeasures to protect humans against MPXV from both natural outbreaks and from the potential release of the virus as a biological weapon.

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■ of interest

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Executive summary

Monkeypox virus: basic facts

- First isolated from cynomolgus monkeys in Denmark, 1958.
- Responsible for several outbreaks in monkey colonies and zoos.
- Monkeypox virus (MPXV) belongs to the family *Poxviridae* subfamily *Chordopoxvirinae* and genus *Orthopoxvirus*. Other members include variola (smallpox virus), vaccinia, cowpox and ectromelia (mousepox virus).
- The virion is enveloped, brick-shaped and 200–250 nm in size.
- *Orthopoxvirus* have varying host specificities, ranging from narrow to broad. MPXV has a broad host-range.
- Highly susceptible monkeys experimentally infected with MPXV present with almost identical clinical signs to humans infected with variola.
- The natural lifecycle probably involves a complex interaction between reservoir and incidental hosts.

MPXV can be separated into distinct groups

- At least two separate groups, or ‘clades’, of MPXV exist: West African and Congo basin.
- MPXV isolates from Central Africa (Congo basin) are more virulent than those from West Africa.
- Congo basin and West African isolates are approximately 95% identical. This value approaches 99% when comparing isolates from within the Congo basin or West African regions.

MPXV host-range

- MPXV likely circulates in many species in Africa; however, squirrels appear to be the most likely reservoir of the virus.
- Monkeys of the *Cercopithecus* genus also appear to have a propensity for MPXV infection.
- Over 40 species have been documented to have been naturally or experimentally infected with MPXV.
- Infected species have originated from North America, South America, Africa and Asia.
- Cynomolgus monkeys (*M. fascicularis*) and Rhesus monkeys (*M. mulatta*) have been utilized for the majority of experimental infections in NHPs. These species are susceptible to intravenous, aerosol, intradermal, intramuscular, intrabronchial, intratracheal, intranasal and subcutaneous routes of infection. The disease appears more severe in *M. fascicularis* than in *M. mulatta.*
- Various small-animal models have been developed, of which the prairie dog, squirrel, dormouse, and CAST/EiJ mouse are the most robust.

Use of MPXV models in evaluation of therapeutics & prophylactics

- Traditional, attenuated, subunit and DNA vaccines have been shown to protect many species against MPXV challenge. Some studies have shown

that post-exposure vaccination has also provided protection. Attenuated vaccines were safe in immunocompromised *M. mulatta* but did not provide protection against subsequent challenge.

- The antiviral drug ST-246 protected *M. fascicularis*, 13-lined ground squirrels and prairie dogs against a lethal MPXV challenge. The antiviral drug CMX001 protected *stat1*$^{-/-}$ mice against a lethal MPXV challenge and cidofovir treatment protected better than post-exposure vaccination in an intratracheal *M. fascicularis* model.

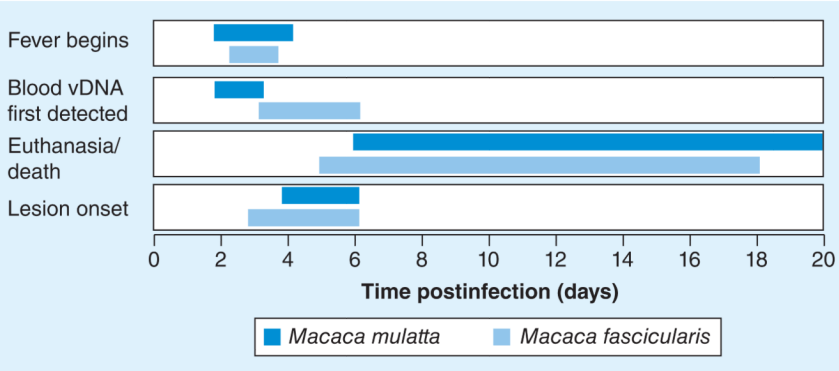


Figure 1. Four disease biomarkers are shown following an intravenous 10^7 PFU Congo Basin (Z79) challenge in *Macaca mulatta* and *Macaca fascicularis*

Initial detection of fever, vDNA in blood and lesion appearance are shown. The ranges of euthanasia/death are also shown (see Tables 1 & 3 for more details).

vDNA: Viral DNA.

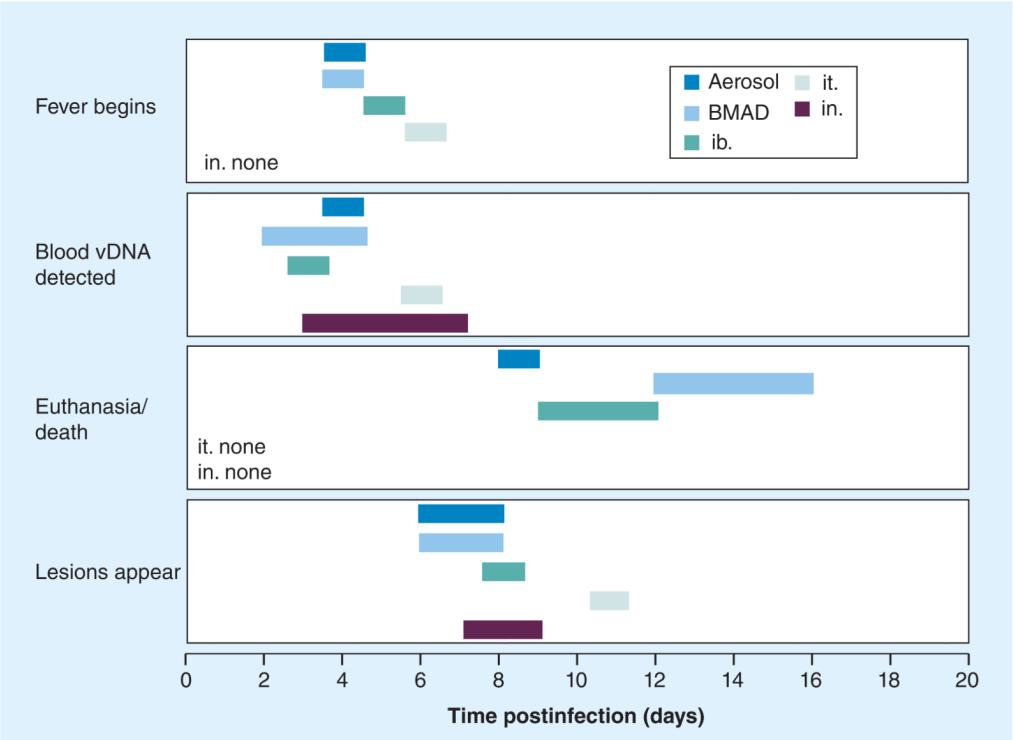


Figure 2. Four disease biomarkers are shown following five different respiratory challenges with 10^6 PFU of monkeypox virus (Congo Basin) in *Macaca fascicularis*

Initial detection of fever, vDNA in blood and the lesion appearance are shown. The ranges of euthanasia/death are also shown. Data is taken from Table 4.

BMAD: Bronchoscope-microsprayer aerosol delivery; ib.: Intrabronchial; in.: Intranasal; it.: Intratracheal; vDNA: Viral DNA.

Table 1

Intravenous *Macaca fascicularis* challenges with Congo Basin strain (Zaire 79)

Dose	Fever onset (duration)	Weight loss (%)	Lesion onset (maximum lesion count)	Mortality/ euthanasia (days of death)	Blood vDNA detected† (duration of detection)	Infectious virus from swabs (duration of detection)	Ref.
10^7	Day 3	4–10	Day 3–6 (>1000)	2/6 (by day 18)	Day 6 (to day 27)		[23]
10^7		2–6	Day 5–6 (>2500)	4/4 (5, 7, 7, 7)	Day 5–7 (to day 7)		[48]
10^6		4–8	Day 6 (>1200)	4/6 (12, 12, 12, 14)	Day 6–9 (to day 14)		[48]
10^7	Day 3 (to day 7)	1–10	(>250)	8/8 (by day 9)		Throat from day 2–6 Blood from day 5	[102]
10^7		6–11	Day 4 (>1000)	4/6 (9, 9, 11, 14)	Day 4 (to day 28)		[103]
10^7			Day 3–4 (>1500)	3/3 (11, 12, 13)	Day 4 (to day 13)		[104]
10^7			Day 5–8 (>1400)	3/3 (9, 13, 13)	Day 4 (to day 12)		[105]
10^7	Day 3 (to day 9)	7–10	By day 6 (>750)	3/4 by day 15	Day 4 (to day 15)		[106]
10^7			By day 6 (TNTC)	5/5 (11, 11, 11, 13, 15)	Day 4 (to day 15)		[107]
10^7	Day 3 (to day 12)	>4	Day 6 (>200)	3/3 (9, 11, 15)	Day 3 (to day 15)		[49]

TNTC: Too numerous to count; vDNA: Viral DNA.

†Does not include detection at day 0 following intravenous challenge.

Table 2

Evaluation of therapeutics and prophylactics against intravenous inoculation of *Macaca fascicularis* and *Macaca mulatta*[†]

Vaccine/treatment/therapy (administration days prechallenge)	MPXV strain (PFU)	End point		Outcome	Ref.
		Primary	*Secondary*		
M. fascicularis *vaccination*					
MVA (120 and 60)	CB Z79 (10^7)	Death	Blood vDNA/lesions (onset, description)/ weight change	Survival/low vDNA levels/1–36 lesions (day 9–15, small atypical lesions)/no weight loss	[23]
MVA (120) + Dryvax (60)				Survival/low vDNA levels/no lesions/no weight loss	
Dryvax (60)				Survival/low vDNA levels/no lesions/no weight loss	
MVA (30) or MVA (10)	CB Z79 (10^7)	Death	Blood vDNA/lesions (onset, description)/ weight change	Survival/reduced vDNA/2 to >996 lesions (day 6–9)/no weight loss	[48]
Dryvax (30) or Dryvax (10)				Survival/low-level vDNA/no lesions at day 30 vaccination; 0–29 lesions at day 10 vaccination (day 6–9)/no weight loss	
MVA (10) or Dryvax (10)	CB Z79 (10^6)	Death	Blood vDNA/lesions (onset, description)/ weight change	Survival/reduced vDNA/MVA lesions (day 6–9); Dryvax no lesions/ no weight loss	[48]
MVA (6)				1 of 4 mortality/reduced vDNA/lesions (day 6–9)/6% weight loss in nonsurvivor	
Dryvax (6)				Survival/reduced vDNA/lesions (day 6)/no weight loss	
MVA (4)				Survival/reduced vDNA/lesions (day 6)/no weight loss	
Dryvax (4)				3 of 4 mortality/reduced vDNA/lesions (day 6)/15% weight loss	
ACAM2000 (60) or Dryvax (60)	CB Z79 (10^7)	Death	Temperature/virus in blood and throat swabs/ lesions/weight change	Survival/normal temperature/virus by day 5/no lesions/1% weight gain	[102]
NYCBH (49 and 28)	CB Z79 (10^7)	Death	Temperature/weight change/blood vDNA	Survival/normal temperature/weight gain/low vDNA	[108]
NYCBH with *E3L* deletion (49 and 28)				2 of 8 mortality/slight temperature increase/4% weight loss/ increased vDNA	
Dryvax (119)	CB Z79 (10^7)	Death	Lesions/blood vDNA	Survival/0–7 lesions/low vDNA levels	[107]
A33+B5+L1+A27 subunit proteins (119, 91, 35)				Survival/0–32 lesions/reduced vDNA	
A27+F9+H3+L1+A33+A56+B5 DNA vaccine (0, 28, 56)[‡]	CB NR-523 (10^7)	Death	Lesions/blood vDNA/ temperature/weight change	Survival/133–175 lesions/reduced vDNA/normal temperature/no weight change	[106]
Wyeth[§] or Wyeth-IL15[§,¶] or Wyeth-IL12[§,¶] or MVA[§] or MVA- IL15[§,¶] (all at 1000)	CB Z79 (10^7)	Death	Lesions (onset)/blood vDNA/temperature/ weight	Survival/>200 lesions (day 6)/low vDNA levels/normal temperature/ some weight loss >4%	[49]
MVA-IL2[§,¶] (1000)				2 of 3 survival/>200 lesions (day 6)/low vDNA levels/normal temperature/some weight loss >4%	

Vaccine/treatment/therapy (administration days prechallenge)	MPXV strain (PFU)	End point		Outcome	Ref.
		Primary	*Secondary*		
M. fascicularis *vaccination + antiviral therapy*					
Dryvax (59)	CB Z79 (10^7)	Death	Lesions (onset)/weight change	Similar to above Dryvax outcomes	[103]
Dryvax (59) + cidofovir (59)				1 of 6 survival/600–1800 lesions (day 7)/4–11% weight loss	
ST-246[#] (day 0 or 3 p.i.)	CB Z79 (10^7)	Death	Lesions/blood vDNA	Survival/no lesions/low vDNA levels	[104,105]
M. mulatta *vaccination*					
Dryvax (>365)	CB Z79 (10^7)	Death	Lesions (onset)/blood vDNA/throat swab/ temperature	Survival/no clinical changes	[51]
L1R+A27L+A33R+B5R DNA vaccine (multiple over ~365)[††]				Survival/<50 lesions (day 6)/low vDNA levels/throat swab from day 4/mild temperature increase	
Dryvax (26)/control AB	CB Z79 (10^7)	Death	Lesions/blood vDNA	Survival/no lesions/no vDNA	[109]
Dryvax (26)/antibody CD8 depletion (1, 0, +1, +2, +6)					
Dryvax (29)/antibody CD4 depletion (4, 0, +5, +10)					
Dryvax (26)/antibody CD20 depletion (34, 27, 26, 19, 11, 1, 0, +1, +3, +6)				3 of 4 mortality/lesions TNTC/high vDNA levels	
VIG (3, 0)				Survival/12–330 lesions/ reduced vDNA levels	
L1R+A27L+A33R+B5R DNA (35, 31, 25)[‡‡]	CB Z79 (10^7)	Death	Lesions (onset)/blood vDNA	2 of 3 mortality/lesions TNTC/high vDNA levels	[110]
L1R+A27L+A33R+B5R DNA (35, 31, 25) [‡‡] followed by L1R+A27L+A33R+B5R proteins (16, 12, 5)[§§]				Survival/3–15 lesions (day 7)/low vDNA levels	
Dryvax (896)	CB Z79 (10^7)	Death	Lesions/vDNA in blood/ temperature	Survival/0–200 lesions/low vDNA levels/no temperature change	[111]
MVA-HIV recombinant (1092)				Survival/0–200 lesions/reduced vDNA levels/no temperature change	
MVA-HIV recombinant (912)[¶¶]	CB Z79 (10^7)	Death	Lesions (onset)/blood vDNA	Survival/7–38 lesions (days 7–12)/low vDNA levels	[112]
***Vaccination of immunodeficient* M. mulatta**[##]					
MVA (388, 360)/Dryvax (180)	CB Z79 (10^7)	Death	Lesions/throat swab/ blood swab/blood vDNA	3 of 4 mortality/lesions TNTC/high levels blood swab/high levels blood vDNA	[113]
NYVAC (388, 360)/Dryvax (180)					
Dryvax (180)				2 of 4 mortality/lesions TNTC/high levels blood swab/high levels blood vDNA	

CB: Congo Basin; MPXV: Monkeypox virus; p.i.: Postinfection; TNTC: Too numerous to count; vDNA: Viral DNA; VIG: Vaccinia immunoglobulin.

[†] Vaccines administered by the following routes: MVA (intramuscular); ACAM2000 (scarification); Dryvax (scarification).

[‡] Administered intramuscularly or intradermally with similar results.

[§] Administered intradermally.

[¶] Recombinant viruses.

[#] Administered for 14 days.

[††] Administered via gene gun.

[‡‡] Administered intramuscularly and intradermally.

[§§] Administered intramuscularly.

[¶¶] Administered intramuscularly or intradermally using a needle-free Biojectior or in the palatine tonsils via a SyriJet MkII; with similar results regardless of route.

[##] Immunodeficent due to infection with SIV-induced AIDS.

Table 3

Intravenous Macaca mulatta challenges with Congo Basin strain (Z79)

Dose	Fever onset (duration)	Lesion onset (maximum lesion count)	Mortality/euthanasia (days of death)	Blood vDNA detected[†] (duration of detection)	Infectious virus from swabs (duration of detection)	Ref.
10^7	Day 2 (to day 8)		2/2 by day 13	Day 2 (to day 8)		[111]
10^7		Day 6 (734)	0/0 (but severe disease)	By day 3 (to day 15)		[112]
10^7		Day 4 (TNTC)	3/3 (11, 17, 21)	By day 3 (to day 9[‡])		[110]
10^7	Day 2–4 (to day 14)	Day 6 (>100)	3/3 (7, 10, 14)	Day 2–6 (to day 14)	Throat from day 6 (to day 14)	[51]
10^8	Only on day 2 in 1/2	No lesions	2/2 (6, 6)			[51]
10^6	Day 2 (to day 8)	Day 6 (>100)	0/2 (but severe disease)			[51]

TNTC: Too numerous to count; vDNA: Viral DNA.

[†]Does not include detection at day 0 following intravenous challenge.

[‡]No samples taken from day 9.

Table 4

Respiratory challenges with Congo Basin strains in *Macaca fascicularis* and *Macaca mulatta*

Route	Species	Dose (PFU)	Fever onset (duration)	Weight loss	Lesion onset (maximum lesion count)	Mortality (days of death or euthanasia)	Blood vDNA detected (duration of detection)	Oral vDNA detected (duration of detection)	Infectious virus from oral swabs (duration of detection)	Ref.
Aerosol	MF	10^5 EIU	Day 5–8		Day 9–10	2/4				[52]
Aerosol MF		10^4–10^5	Day 6–7		Day 6–7	15/15 (9–17)				[53]
Aerosol	MF	10^4	Day 5 (4 days)	No sig†	Day 6–8	2/3 (10, 11)	Day 6 (to day 22)	Day 6 (to day 23)		[54]
Aerosol	MF	10^5	Day 4 (5 days)	No sig†	Day 6–8	4/6 (8, 9, 10, 10)	Day 6 (to day 22)	Day 8 (to >day 26)		[54]
Aerosol	MF	4×10^5	Day 3 (5 days)	No sig†	Day 6–8	5/6 (11, 10, 10, 9, 8)	Day 4 (to day 20)	Day 6 (to >day 26)		[54]
Aerosol	MF	10^6	Day 4 (5 days)	No sig†	Day 6–8	2/3 (8, 9)	Day 4 (to day 14)	Day 6 (to day 18)		[54]
BMAD	MF	3×10^6	Day 4 (4–8 days)	10–15%	Day 8 (304)	1/3 (12)	Day 4 (to day 20)			[24]
BMAD	MF	8×10^6	Day 4 (until death)	>10%	Day 6 (550)	2/2 (12, 16‡)	Day 2 (until death)			[24]
BMAD	MF	3×10^7	Day 4–6 (<2 days)	~10%	Day 4–10 (350)	2/3 (8, 8)	Day 2–4 (8–17 days)			[24]
ib.	MF	10^4	Day 6 (10 days)		Day 8 (170)	0/3	Day 5 (17 days)			[55]
	MF	10^5	Day 7 (7 days)		Day 9 (237)	1/6 (9)	Day 5 (12 days)		Day 7	[55]
	MF	10^6	Day 5 (9 days)		Day 8 (288)	2/3 (9, 12)	Day 3 (17 days)		Day 7	[55]
ib.	MM	10^5			Day 7–12	0/4	Day 4–14 (~10 days)			[56]
it.	MF	10^6	Day 6 (5 days)		Day 11 (>100)	0/3	Day 6 (13 days)	Day 4 (23 days)	Day 4 (23 days)	[58]
it.		10^7	Day 5 (until death)		Day 8–10 (>100)	3/3 (15, 19, 19)	Day 4 (until death)	Day 4 (until death)	Day 4 (until death)	[58]
it.	MF	10^7			Day 9 (>100)	6/6 (9, 9, 11, 11, 15, 15)	Day 5 (until death)			[59]
in.	MF	10^6	None	~10%	Day 7–9 (178)§	0/2	Day 3–7 (10–20 days)			[32]

BMAD: Bronchoscope-microsprayer aerosol delivery; EIU: Egg infectious unit; ib.: Intrabronchial; in: Intranasal; it: Intratracheal; MF: Macaca fascicularis; MM: Macaca mulatta; Sig: Significant; vDNA: Viral DNA.

†No significant changes over time; however, survivors were noted to be approximately 20% heavier than nonsurvivors at the start of the study.

‡A third animal died on day 22 but is thought to have died from secondary bacterial infection and has therefore been excluded.

[§]One animal had 178 lesions and the other had one lesion.

Viral Shedding from Vaccines

Mass immunizations in schools and communities
May actually endanger the immune deficient via vaccine shedding.

Shedding is when the live virus that is injected via vaccine, moves through the human body and comes back out in the feces, droplets from the nose, or saliva from the mouth. Anyone who takes care of the child could potentially contract the disease for some time after that child has received certain live vaccines. This was a huge problem with the oral polio vaccine (OPV), and was one of the reasons why it was taken off the market in the US. The OPV is still used in developing counties.

Secondary transmission happens fairly often with some of the live virus vaccines. Influenza, Varicella, and Oral Polio Vaccine (OPV) are the most common. On the other hand it may happen very seldom or not ever with the measles and mumps vaccine viruses. Here are the vaccines that shed or have been known to result in secondary transmission:

Measles Vaccine - Although secondary transmission of the vaccine virus has never been documented, measles virus RNA has been detected in the urine of the vaccinees as early as 1 day or as late as 14 days after vaccination. (1)
In France, measles virus was isolated in a throat swab of a recently vaccinated child 4 days after fever onset. The virus was then further genetically characterized as a vaccine-type virus. (2)

Rubella Vaccine - Excretion of small amounts of live attenuated rubella virus from the nose and throat has occurred in the majority of susceptible individuals 7-28 days after vaccination. Transmission of the vaccine virus via breast milk has been documented. (3)

Chicken Pox Vaccine - Vaccine-strain chickenpox has been found replicating in the lung (4) and documented as transmitting via zoster (shingles sores) (5) as well as “classic” chickenpox (6) rash post-vaccination.

References:
(1) Detection of measles virus RNA in urine specimens of vaccinated persons - Rota et al., Journal of Clinical Microbiology, 1995 can be accessed at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC228449/
(2) Detection of measles vaccine in the throat of a vaccinated child - Morefin, et al., Vaccine 2002, can be accessed at http://www.ncbi.nlm.nih.gov/pubmed/11858860?dopt=AbstractPlus
3) Prescribing Information, MMRII vaccine, can be accessed at http://www.merck.com/product/usa/pi_circulars/m/mmr_ii/mmr_ii_pi.pdf
(4) Quinlivan et al, J Infect Dis. 2006, Vaccine Oka Varicella-Zoster Virus can be accessed at http://www.journals.uchicago.edu/doi/full/10.1086/500835
(5) Brunell, et al., J. of Pediatrics 2000, Chickenpox Attributable to a Vaccine Virus can be accessed at http://pediatrics.aappublications.org/cgi/content/full/106/2/e28
(6) Sauerbrei et al., J Clin Microbiol. 2004, Genetic Profile of an Oka Varicella Vaccine Virus can be accessed at http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=15583288

The Johns Hopkins Hospital Patient Information

Original Date

Oncology
Revised Reviewed

Care at Home for the Immunocompromised Patient

What can I do to prevent infection?

- Hand washing is the **best way** to prevent infection.
- Carry hand sanitizer with you at all times.
- Wash with soap and water or hand sanitizer
  - -before and after you use the bathroom
  - -before and after preparing or eating food
  - -after touching pets or animals
  - -after contact with someone who has an infection such as a cold or the flu
  - -after touching surfaces in public areas (such as elevator buttons, handrails and gas pumps)

Do I need to wear a mask?

- Wear an N95 respirator mask when you travel to and from the hospital, when you are in the hospital, within two football fields of construction or digging, and in any public place.
- Close all car windows and turn on the re-circulate button of your ventilation system.
- Avoid crowds if possible. An area is crowded if you are within an arm's length of other people.
- Avoid closed spaces if possible.

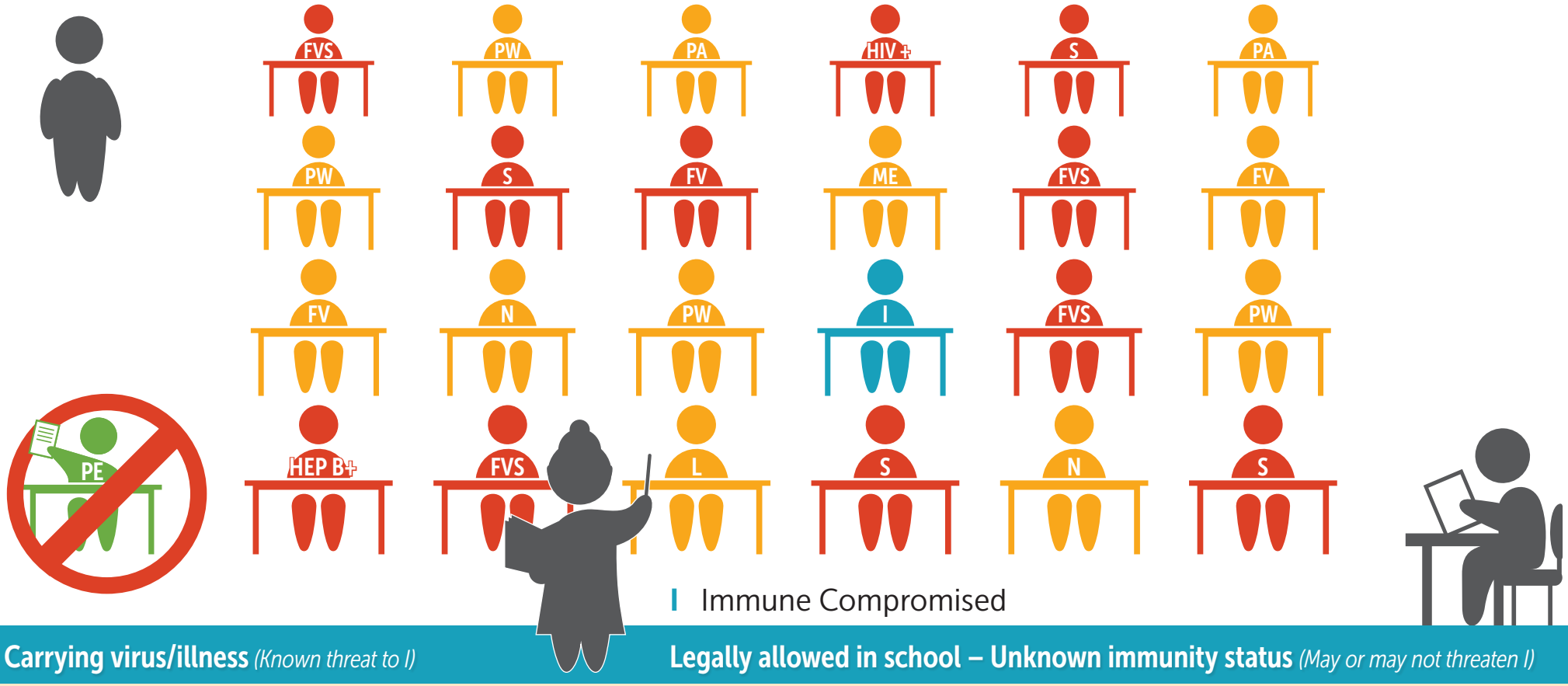
Can I have visitors?

- Tell friends and family who are sick, or have recently had a live vaccine (such as chicken pox, measles, rubella, intranasal influenza, polio or smallpox) not to visit.
- It may be a good idea to have visitors call first.
- Avoid contact with children who were recently vaccinated.

Are there any precautions I should follow about my medicine?

- Do not take aspirin or aspirin-like products (such as Advil™, Motrin™ or Excedrin™) unless told by your doctor.
- You should wear a medical alert bracelet that identifies you as a cancer patient or bone marrow transplant patient at risk for bleeding or infection.
- **Keep a current medication list with you at all times**.
- Do not take any herbal products.
- Avoid grapefruit juice, which interacts with many medications.

Eliminating students with Philosophical Exemption does NOT protect the immune compromised



Carrying virus/illness *(Known threat to I)*

- **S** Shedder, recent MMR, Varicella, or FluMist recipient – up to 28 days post vaccination via viral shedding – **FluMist is administered in schools!**
- **HIV +** Legally allowed in school and medical privacy protected
- **HepB +** Legally allowed in school and medical privacy protected
- **FVS** Fully vaccinated child sick with common cold, strep, bronchitis, etc.
- **FV** Fully vaccinated – immune status unknown

Legally allowed in school – Unknown immunity status *(May or may not threaten I)*

- **PA** Provisional admittance, not fully vaccinated *(7.9% is > than PE rate)*
- **PW** Vaccinated for pertussis but immunity has waned
- **L** Low responder *(vaccinated but antibody response low, not immune)*
- **N** Non-responder *(vaccinated but no antibody response, not immune, 7% of MMR recipients)*
- **ME** Medically exempt – not fully vaccinated
- **PE** Philosophical exemption, could be fully vaccinated but missing only 1 dose
  (First grade PE rates: DTaP 2.6%, Polio 2.9%, MMR 3.1%, HepB 3.3%, Chicken Pox 4.3%)

Removing students with PEs does NOT protect the immune compromised, is discriminatory and denies healthy children the right to a free public education.

§100.3 Vaccine injury table.

(a) In accordance with section 312(b) of the National Childhood Vaccine Injury Act of 1986, title III of Pub. L. 99-660, 100 Stat. 3779 (42 U.S.C. 300aa-1 note) and section 2114(c) of the Public Health Service Act (42 U.S.C. 300aa-14(c)), the following is a table of vaccines, the injuries, disabilities, illnesses, conditions, and deaths resulting from the administration of such vaccines, and the time period in which the first symptom or manifestation of onset or of the significant aggravation of such injuries, disabilities, illnesses, conditions, and deaths is to occur after vaccine administration for purposes of receiving compensation under the Program:

VACCINE INJURY TABLE

Vaccine	Illness, disability, injury or condition covered	Time period for first symptom or manifestation of onset or of significant aggravation after vaccine administration
I. Vaccines containing tetanus toxoid (e.g., DTaP, DTP, DT, Td, or TT)	A. Anaphylaxis or anaphylactic shock	4 hours.
	B. Brachial Neuritis	2-28 days.
	C. Any acute complication or sequela (including death) of an illness, disability, injury, or condition referred to above which illness, disability, injury, or condition arose within the time period prescribed	Not applicable.
II. Vaccines containing whole cell pertussis bacteria, extracted or partial cell pertussis bacteria, or specific pertussis antigen(s) (e.g., DTP, DTaP, P, DTP-Hib)	A. Anaphylaxis or anaphylactic shock	4 hours.
	B. Encephalopathy (or encephalitis)	72 hours.
	C. Any acute complication or sequela (including death) of an illness, disability, injury, or condition referred to above which illness, disability, injury, or condition arose within the time period prescribed	Not applicable.
III. Measles, mumps, and rubella vaccine or any of its components (e.g., MMR, MR, M, R)	A. Anaphylaxis or anaphylactic shock	4 hours.
	B. Encephalopathy (or encephalitis)	5-15 days (not less than 5 days and not more than 15 days).
	C. Any acute complication or sequela (including death) of an illness, disability, injury, or condition referred to above	Not applicable.

	which illness, disability, injury, or condition arose within the time period prescribed	
IV. Vaccines containing rubella virus (e.g., MMR, MR, R)	A. Chronic arthritis	7-42 days.
	B. Any acute complication or sequela (including death) of an illness, disability, injury, or condition referred to above which illness, disability, injury, or condition arose within the time period prescribed	Not applicable.
V. Vaccines containing measles virus (e.g., MMR, MR, M)	A. Thrombocytopenic purpura	7-30 days.
	B. Vaccine-Strain Measles Viral Infection in an immunodeficient recipient	6 months.
	C. Any acute complication or sequela (including death) of an illness, disability, injury, or condition referred to above which illness, disability, injury, or condition arose within the time period prescribed	Not applicable.
VI. Vaccines containing polio live virus (OPV)	A. Paralytic Polio	
	—in a non-immunodeficient recipient	30 days.
	—in an immunodeficient recipient	6 months.
	—in a vaccine associated community case	Not applicable.
	B. Vaccine-Strain Polio Viral Infection	
	—in a non-immunodeficient recipient	30 days.
	—in an immunodeficient recipient	6 months.
	—in a vaccine associated community case	Not applicable.
	C. Any acute complication or sequela (including death) of an illness, disability, injury, or condition referred to above which illness, disability, injury, or condition arose within the time period prescribed	Not applicable.
VII. Vaccines containing polio inactivated virus (e.g., IPV)	A. Anaphylaxis or anaphylactic shock	4 hours
	B. Any acute complication or sequela (including death of an illness, disability, injury, or condition referred to above which illness, disability, injury, or condition arose within the time period	Not applicable.

	prescribed.	
VIII. Hepatitis B. vaccines	A. Anaphylaxis or anaphylactic shock	4 hours.
	B. Any acute complication or sequela (including death) of an illness, disability, injury, or condition referred to above which illness, disability, injury, or condition arose within the time period prescribed	Not applicable.
IX. Hemophilus influenzae type b polysaccharide conjugate vaccines	No Condition Specified	Not applicable.
X. Varicella vaccine	No Condition Specified	Not applicable.
XI. Rotavirus vaccine	No Condition Specified	Not applicable.
XII. Pneumococcal conjugate vaccines	No Condition Specified	Not applicable.
XIII. Hepatitis A vaccines	No Condition Specified	Not applicable.
XIV. Trivalent influenza vaccines	No Condition Specified	Not applicable.
XV. Meningococcal vaccines	No Condition Specified	Not applicable.
XVI. Human papillomavirus (HPV) vaccines	No Condition Specified	Not applicable.
XVII. Any new vaccine recommended by the Centers for Disease Control and Prevention for routine administration to children, after publication by the Secretary of a notice of coverage *	No Condition Specified	Not applicable.

*Now includes all vaccines against seasonal influenza (except trivalent influenza vaccines, which are already covered), effective November 12, 2013.

(b) *Qualifications and aids to interpretation.* The following qualifications and aids to interpretation shall apply to the Vaccine Injury Table to paragraph (a) of this section:

(1) *Anaphylaxis and anaphylactic shock.* For purposes of paragraph (a) of this section, Anaphylaxis and anaphylactic shock mean an acute, severe, and potentially lethal systemic allergic reaction. Most cases resolve without sequelae. Signs and symptoms begin minutes to a few hours after exposure. Death, if it occurs, usually results from airway obstruction caused by laryngeal edema or bronchospasm and may be associated with cardiovascular collapse. Other significant clinical signs and symptoms may include the following: Cyanosis, hypotension, bradycardia, tachycardia, arrhythmia, edema of the pharynx and/or trachea and/or larynx with stridor and dyspnea. Autopsy findings may include acute emphysema which results from lower respiratory tract obstruction, edema of the hypopharynx, epiglottis, larynx, or trchea and minimal findings of eosinophilia in the liver, spleen and lungs. When death occurs within minutes of exposure and without signs of respiratory distress, there may not be significant pathologic findings.

(2) *Encephalopathy.* For purposes of paragraph (a) of this section, a vaccine recipient shall be considered to have suffered an encephalopathy only if such recipient manifests, within the applicable period, an injury meeting the description below of an acute encephalopathy, and then a chronic encephalopathy persists in such person for more than 6 months beyond the date of vaccination.

(i) An acute encephalopathy is one that is sufficiently severe so as to require hospitalization (whether or not hospitalization occurred).

(A) *For children less than 18 months of age* who present without an associated seizure event, an acute encephalopathy is indicated by a significantly decreased level of consciousness lasting for at least 24 hours. Those children less than 18 months of age who present following a seizure shall be viewed as having an acute encephalopathy if their significantly decreased level of consciousness persists beyond 24 hours and cannot be attributed to a postictal state (seizure) or medication.

(B) *For adults and children 18 months of age or older,* an acute encephalopathy is one that persists for at least 24 hours and characterized by at least two of the following:

(*1*) A significant change in mental status that is not medication related; specifically a confusional state, or a delirium, or a psychosis;

(*2*) A significantly decreased level of consciousness, which is independent of a seizure and cannot be attributed to the effects of medication; and

(*3*) A seizure associated with loss of consciousness.

(C) Increased intracranial pressure may be a clinical feature of acute encephalopathy in any age group.

(D) A “significantly decreased level of consciousness” is indicated by the presence of at least one of the following clinical signs for at least 24 hours or greater (see paragraphs (b)(2)(i)(A) and (b)(2)(i)(B) of this section for applicable timeframes):

(*1*) Decreased or absent response to environment (responds, if at all, only to loud voice or painful stimuli);

(*2*) Decreased or absent eye contact (does not fix gaze upon family members or other individuals); or

(*3*) Inconsistent or absent responses to external stimuli (does not recognize familiar people or things).

(E) The following clinical features alone, or in combination, do not demonstrate an acute encephalopathy or a significant change in either mental status or level of consciousness as described above: Sleepiness, irritability (fussiness), high-pitched and unusual screaming, persistent inconsolable crying, and bulging fontanelle. Seizures in themselves are not sufficient to constitute a diagnosis of encephalopathy. In the absence of other evidence of an acute encephalopathy, seizures shall not be viewed as the first symptom or manifestation of the onset of an acute encephalopathy.

(ii) *Chronic Encephalopathy* occurs when a change in mental or neurologic status, first manifested during the applicable time period, persists for a period of at least 6 months from the date of vaccination. Individuals who return to a normal neurologic state after the acute encephalopathy shall not be presumed to have suffered residual neurologic damage from that event; any subsequent chronic encephalopathy shall not be presumed to be a sequela of the acute encephalopathy. If a preponderance of the evidence indicates that a child's chronic encephalopathy is secondary to genetic, prenatal or perinatal factors, that chronic encephalopathy shall not be considered to be a condition set forth in the Table.

(iii) An encephalopathy shall not be considered to be a condition set forth in the Table if in a proceeding on a petition, it is shown by a preponderance of the evidence that the encephalopathy was

caused by an infection, a toxin, a metabolic disturbance, a structural lesion, a genetic disorder or trauma (without regard to whether the cause of the infection, toxin, trauma, metabolic disturbance, structural lesion or genetic disorder is known). If at the time a decision is made on a petition filed under section 2111(b) of the Act for a vaccine-related injury or death, it is not possible to determine the cause by a preponderance of the evidence of an encephalopathy, the encephalopathy shall be considered to be a condition set forth in the Table.

(iv) In determining whether or not an encephalopathy is a condition set forth in the Table, the Court shall consider the entire medical record.

(3) [Reserved]

(4) *Seizure and convulsion.* For purposes of paragraphs (b) (2) of this section, the terms, “seizure” and “convulsion” include myoclonic, generalized tonic-clonic (grand mal), and simple and complex partial seizures. Absence (petit mal) seizures shall not be considered to be a condition set forth in the Table. Jerking movements or staring episodes alone are not necessarily an indication of seizure activity.

(5) *Sequela.* The term “sequela” means a condition or event which was actually caused by a condition listed in the Vaccine Injury Table.

(6) *Chronic Arthritis.* (i) For purposes of paragraph (a) of this section, chronic arthritis may be found in a person with no history in the 3 years prior to vaccination of arthropathy (joint disease) on the basis of:

(A) Medical documentation, recorded within 30 days after the onset, of objective signs of acute arthritis (joint swelling) that occurred between 7 and 42 days after a rubella vaccination;

(B) Medical documentation (recorded within 3 years after the onset of acute arthritis) of the persistence of objective signs of intermittent or continuous arthritis for more than 6 months following vaccination; and

(C) Medical documentation of an antibody response to the rubella virus.

(ii) For purposes of paragraph (a) of this section, the following shall not be considered as chronic arthritis: Musculoskeletal disorders such as diffuse connective tissue diseases (including but not limited to rheumatoid arthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, mixed connective tissue disease, polymyositis/determatomyositis, fibromyalgia, necrotizing vascultitis and vasculopathies and Sjögren's Syndrome), degenerative joint disease, infectious agents other than rubella (whether by direct invasion or as an immune reaction) metabolic and endocrine diseases, trauma, neoplasms, neuropathic disorders, bone and cartilage disorders and arthritis associated with ankylosing spondylitis, psoriasis, inflammatory bowel disease, Reiter's syndrome, or blood disorders.

(iii) Arthralgia (joint pain) or stiffness without joint swelling shall not be viewed as chronic arthritis for purposes of paragraph (a) of this section.

(7) *Brachial neuritis.* (i) This term is defined as dysfunction limited to the upper extremity nerve plexus (i.e., its trunks, divisions, or cords) without involvement of other peripheral (e.g., nerve roots or a single peripheral nerve) or central (e.g., spinal cord) nervous system structures. A deep, steady, often severe aching pain in the shoulder and upper arm usually heralds onset of the condition. The pain is followed in days or weeks by weakness and atrophy in upper extremity muscle groups. Sensory loss may accompany the motor deficits, but is generally a less notable clinical feature. The neuritis, or plexopathy, may be present on the same side as or the opposite side of the injection; it is sometimes bilateral, affecting both upper extremities.

(ii) Weakness is required before the diagnosis can be made. Motor, sensory, and reflex findings on physical examination and the results of nerve conduction and electromyographic studies must be consistent in confirming that dysfunction is attributable to the brachial plexus. The condition should thereby be distinguishable from conditions that may give rise to dysfunction of nerve roots (i.e., radiculopathies) and peripheral nerves (i.e., including multiple monoeuropathies), as well as other peripheral and central nervous system structures (e.g., cranial neuropathies and myelopathies).

(8) *Thrombocytopenic purpura.* This term is defined by a serum platelet count less than 50,000/mm^3. Thrombocytopenic purpura does not include cases of thrombocytopenia associated with other causes such as hypersplenism, autoimmune disorders (including alloantibodies from previous transfusions) myelodysplasias, lymphoproliferative disorders, congenital thrombocytopenia or hemolytic uremic syndrome. This does not include cases of immune (formerly called idiopathic) thrombocytopenic purpura (ITP) that are mediated, for example, by viral or fungal infections, toxins or drugs. Thrombocytopenic purpura does not include cases of thrombocytopenia associated with disseminated intravascular coagulation, as observed with bacterial and viral infections. Viral infections include, for example, those infections secondary to Epstein Barr virus, cytomegalovirus, hepatitis A and B, rhinovirus, human immunodeficiency virus (HIV), adenovirus, and dengue virus. An antecedent viral infection may be demonstrated by clinical signs and symptoms and need not be confirmed by culture or serologic testing. Bone marrow examination, if performed, must reveal a normal or an increased number of megakaryocytes in an otherwise normal marrow.

(9) *Vaccine-strain measles viral infection.* This term is defined as a disease caused by the vaccine-strain that should be determined by vaccine-specific monoclonal antibody or polymerase chain reaction tests.

(10) *Vaccine-strain polio viral infection.* This term is defined as a disease caused by poliovirus that is isolated from the affected tissue and should be determined to be the vaccine-strain by oligonucleotide or polymerase chain reaction. Isolation of poliovirus from the stool is not sufficient to establish a tissue specific infection or disease caused by vaccine-strain poliovirus.

(c) *Coverage provisions.* (1) Except as provided in paragraph (c)(2), (3), (4), (5), (6), or (7) of this section, the revised Table of Injuries set forth in paragraph (a) of this section and the Qualifications and Aids to Interpretation set forth in paragraph (b) of this section apply to petitions for compensation under the Program filed with the United States Court of Federal Claims on or after March 24, 1997. Petitions for compensation filed before such date shall be governed by section 2114(a) and (b) of the Public Health Service Act as in effect on January 1, 1995, or by §100.3 as in effect on March 10, 1995 (see 60 FR 7678, *et seq.,* February 8, 1995), as applicable.

(2) Hepatitis B, Hib, and varicella vaccines (Items VIII, IX, and X of the Table) are included in the Table as of August 6, 1997.

(3) Rotavirus vaccines (Item XI of the Table) are included in the Table as of October 22, 1998.

(4) Pneumococcal conjugate vaccines (Item XII of the Table) are included in the Table as of December 18, 1999.

(5) Hepatitis A vaccines (Item XIII of the Table) are included on the Table as of December 1, 2004.

(6) Trivalent influenza vaccines (Item XIV of the Table) are included on the Table as of July 1, 2005.

(7) Meningococcal vaccines and human papillomavirus vaccines (Items XV and XVI of the Table) are included on the Table as of February 1, 2007.

(8) Other new vaccines (Item XVII of the Table) will be included in the Table as of the effective date of a tax enacted to provide funds for compensation paid with respect to such vaccines. An amendment to this section will be published in the FEDERAL REGISTER to announce the effective date of such a tax.

[60 FR 7694, Feb. 8, 1995, as amended at 62 FR 7688, Feb. 20, 1997; 62 FR 10626, Mar. 7, 1997; 63 FR 25778, May 11, 1998; 64 FR 40518, July 27, 1999; 67 FR 48559, July 25, 2002; 73 FR 59530, Oct. 9, 2008; 76 FR 36368, June 22, 2011]

Recent Scientific References

MEASLES / MMR VACCINE | Recent science

Response of Viral Specific CD4 T Cells to in vitro Stimulation with Vaccine and Wild Measles Virus Strains in Vaccinated and Naturally Infected Subjects: "…it is increasingly being considered that antibody-based definitions of vaccine success or failure may be incomplete."— Czescik et al, Polish Journal of Microbiology, 2014

Outbreak of measles among persons with prior evidence of immunity, New York City, 2011: In the NYC outbreak of 2011, "The index patient had 2 doses of measles-containing vaccine; of 88 contacts, 4 secondary patients were confirmed who had either 2 doses of measles-containing vaccine or a past positive measles IgG antibody." — Rosen et al., Clinical Infectious Disease, 2014

Largest Measles Epidemic in North America in a Decade—Quebec, Canada, 2011: Contribution of Susceptibility, Serendipity, and Super spreading Events: Detailed analysis of Quebec outbreak revealed under-diagnosis and under-reporting of measles in fully vaccinated persons. The mean age of case patients was 15 years and incidence was highest in adolescents and 20% of them had received 2-doses of vaccine as recommended. — De Serres et al., Journal of Infectious Disease, 2013

Waning of Maternal Antibodies Against Measles, Mumps*, Rubella, and Varicella in Communities With Contrasting Vaccination Coverage:* "Children of mothers vaccinated against measles and, possibly, rubella have lower concentrations of maternal antibodies and lose protection by maternal antibodies at an earlier age than children of mothers in communities that oppose vaccination. This increases the risk of disease transmission in highly vaccinated populations."— Waaijenborg et al, Journal of Infectious Diseases, 2013

The Re-Emergence of Measles in Developed Countries: Time to Develop the Next-Generation Measles Vaccines?: "Receiving less attention, however, is the issue of vaccine failure...At the same time, measles vaccine has a failure rate measured in a variety of studies at 2–10%...As a result, measles is re-emerging as a public health threat, and our current tool for prevention has limitations that increasingly look to be significant enough that sustained elimination, much less eradication, are unlikely."—Poland et al, Vaccine

Loss of maternal protection as a consequence of the vaccination program was well documented in the literature as recently as 2009.

Implications of vaccination and waning immunity: Implications of vaccination and waning immunity: "In the absence of vaccination, lifelong immunity is maintained through frequent encounters with infection, which act to boost the waning immune memory (this agrees with the findings of Whittle et al. 1999). However, when vaccination is introduced the prevalence of infection declines, which in turn reduces the amount of boosting and hence the level of immunity (in agreement with Muller 2001). What is more

Recent Scientific References

surprising is that the interaction between vaccination and waning immunity can lead to pronounced epidemic cycles in which the peak levels of infection can be of the orders of magnitude greater than the mean."—Heffernan and Keeling, Proceedings of the Royal Society B, 2009

Modeling the Impact of Subclinical Measles Transmission in Vaccinated Populations with Waning Immunity: "Several studies have shown that measles epidemics can occur even in highly vaccinated populations (1-4). A variety of factors are likely to be contributory to this observation including failure to seroconvert and waning of vaccine-induced immunity (5). It is well documented from outbreak investigations that current measles vaccines protect between 90-95 percent of vaccinees from typical measles (3, 6-8). However, evidence is accumulating which suggests that vaccine derived immunity might be less protective than previously assumed. There is a growing concern that among individuals who respond to vaccine, a substantial proportion are or will become susceptible to clinical (symptomatic) or subclinical (asymptomatic) infection."—Mossing, et al, Americal Journal of Epidemiology, 1999,

The future of measles in highly immunized populations. A modeling approach:"The results of this study suggest that measles elimination in the United States has been achieved by an effective immunization program aimed at young susceptibles combined with a highly, naturally immunized adult population. However, despite short-term success in eliminating the disease, long-range projections demonstrate that the proportion of susceptibles in the year 2050 may be greater than in the pre-vaccine era. Present vaccine technology and public health policy must be altered to deal with this eventuality." —Levy, Am J Epidemiol (1984)

WHOOPING COUGH/ DTap VACCINE | Recent science

"The advantages and disadvantages of routine immunization of infants against whooping cough have been debated since 1933" British Medical Journal Editorial, 1974.

- Martin et al, Clin Infect Diseases, 2015: Patients who had received at least one dose of vaccine had a significantly higher odds of having PRN- B pertussis compared with unvaccinated patients.

Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model: In this study, whooping cough vaccine failed to prevent infection & transmission in animal testing. Vaccinated animals asymptomatically carried the infectious bacteria for 42 days, longer than any of the other groups studied (including infected but unvaccinated animals). The infected but unvaccinated animals did not carry the bacteria upon re-infection. — Warfel et al., Proceedings of the National Academy of Science, 2014:

Rapid Increase in Pertactin-deficient *Bordetella pertussis* Isolates, Australia: Rapid Increase in Pertactin-deficient Bordetella pertussis Isolates explains that evolution of B. pertussis may be occurring in response to "vaccine selection pressure."—CDC http://wwwnc.cdc.gov/eid/article/20/4/13-1478_article.htm CDC April 2014

Recent Scientific References

> "Clearly it is a red light in terms of how well the vaccination works," said Peter McIntyre, study author and director of the National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases. “The fact that they have arisen independently in different countries suggests it's a response to the vaccine,” said Ms Lam, of the University of NSW school of biotechnology and biomolecular sciences.

Summer 2014 Statement from Professor Arthur Reingold, Head of Epidemiology at UC Berkeley’s School of Public Health:

> "You can be immunized and protected against getting the disease, pertussis, but still have the organism in your nose and throat and spread it to others. Or you can have a very mild illness that is caused by pertussis that causes you to cough, and thereby infect others. So the immunity is not 100 percent from the pertussis vaccine. And what it means is any kind of herd immunity—the way we see, for example, much more powerfully with measles—really can’t be relied upon."

- **Resurgence of Pertussis.** As reported at the May 2013 BSC meeting, the recent resurgence in pertussis cases has been associated with waning immunity over time in persons who received the acellular pertussis vaccine (which is administered as the pertussis component of DTaP vaccine). However, a recent study suggests another explanation for decreased vaccine effectiveness: an increase in *Bordetella pertussis* isolates that lack pertactin (PRN)--a key antigen component of the acellular pertussis vaccine. A study that screened *B. pertussis* strains isolated between 1935 and 2012 for gene insertions that prevent production of PRN found significant increases in PRN-deficient isolates throughout the United States.[2] The earliest PRN-deficient strain was isolated in 1994; by 2012, the percentage of PRN-deficient isolates was more than 50%.

  To assess the clinical significance of these findings, CDC used an IgG anti-PRN ELISA and other assays (PCR amplification, sequencing, and Western blots) to characterize 752 *B. pertussis* strains isolated in 2012 from six Enhanced Pertussis Surveillance Sites[3] and from epidemics in Washington and Vermont. Findings indicated that 85% of the isolates were PRN-deficient and vaccinated patients had significantly higher odds than unvaccinated patients of being infected with PRN-deficient strains. Moreover, when patients with up-to-date DTaP vaccinations were compared to unvaccinated patients, the odds of being infected with PRN-deficient strains increased, suggesting that PRN-bacteria may have a selective advantage in infecting DTaP-vaccinated persons.

Above is from: Meeting of the Board of Scientific Counselors, Office of Infectious Diseases, Centers for Disease Control and Prevention. Tom Harkins Global Communication Center, Atlanta, Georgia: February 2013 Statement made at the National Vaccine Advisory Committee meeting from Pertussis Epidemiology and Vaccination in the United States (Thomas Clark, M.D., M.P.H. of the CDC) http://www.hhs.gov/nvpo/nvac/meetings/pastmeetings/2013/feb2013_certified_minutes.pdf

> "Dr. Clark also did not believe the problem is related to unvaccinated children, because it occurred nationally and is widespread, and because the majority of those affected were vaccinated. CDC is discussing whether a single repeat Tdap dose would be effective. There is potential for developing new or improved vaccines to better control pertussis in the long term, Dr. Clark concluded."

Pertussis: Challenges Today and for the Future, 2013: According to expert Dr. James Cherry, the universal use of pertussis vaccines has been associated with genetic changes

Recent Scientific References

in circulating B. pertussis strains. —Cherry et al., http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1003418

> "There are five possible reasons for the resurgence: 1) genetic changes in B. pertussis; 2) a decrease in vaccine efficacy; 3) a more rapid occurrence of waning immunity; 4) increased recognition and reporting of pertussis; and 5) newer laboratory diagnostic tests."

- Klein, et al, N Eng J Med 2012, Waning Protection after Fifth Dose of Acellular Pertussis Vaccine in Children

The following additional information was already shared with Vermont Legislators in 2012:

USA/Washington

"Early waning of immunity might be contributing to increasing population-level susceptibility." http://jama.jamanetwork.com/article.aspx?articleid=1362036

USA/California

"In early 2010, a spike in cases appeared at Kaiser Permanente in San Rafael, and it was soon determined to be an outbreak of whooping cough -- the largest seen in California in more than 50 years. Witt had expected to see the illnesses center around unvaccinated kids, knowing they are more vulnerable to the disease. "We started dissecting the data. What was very surprising was the majority of cases were in fully vaccinated children. That's what started catching our attention," said Witt. http://www.reuters.com/article/2012/04/03/us-whoopingcough-idUSBRE8320TM20120403

Israel

"Pertussis is considered an endemic disease, characterized by an epidemic every 2–5 years. This rate of exacerbations has not changed, even after the introduction of mass vaccination – a fact that indicates the efficacy of the vaccine in preventing the disease but not the transmission of the causative agent (B. pertussis) within the population."http://www.ima.org.il/imaj/ar06may-2.pdf

Netherlands

"An important issue is whether vaccination has selected for the *ptxP3* strains. Several lines of evidence support this contention." "Based on mathematical modeling, vaccines designed to reduce pathogen growth rate and/or toxicity may result in the evolution of pathogens with higher levels of virulence" The authors "propose that waning immunity and pathogen adaptation have contributed to the resurgence of pertussis, although other factors such as increased awareness and improved diagnostics have also played a role."http://wwwnc.cdc.gov/eid/article/15/8/08-1511_article.htm

Finland

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“Pertussis is an infectious disease of the respiratory tract caused by *Bordetella pertussis*. Despite the introduction of mass vaccination against pertussis in Finland in 1952, pertussis has remained an endemic disease with regular epidemics.” and “During the last decade, the number of pertussis cases has increased in countries with high vaccination coverage rates including Finland.”http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1233997/

“Reemergence of pertussis has been observed in many countries with high vaccination coverage. In the United States, reported cases of pertussis in adolescents and adults have increased since the 1980s, despite increasingly high rates of vaccination in infants and children. At the same time, clinical *B. pertussis* isolates have become antigenically divergent from vaccine strains. This observation has raised the question of whether vaccination has caused selection for the variant strains, and whether the reemergence of pertussis in vaccinated populations is due to vaccination not protecting against these antigenic variants as effectively as it protects against vaccine type strains. On the other hand, vaccine-induced immunity wanes over time, and pertussis is not only a childhood disease but also a frequent cause of prolonged illness in adults and adolescents today.”http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3294326/

From: BLAKE BEAN
To: Supervisor Serna; Kennedy. Supervisor; Rich Desmond; Frost. Supervisor; Nottoli. Don
Subject: Monkeypox State of Emergency
Date: Sunday, August 7, 2022 2:23:53 PM

To the Honorable Sacramento County Board of Supervisors,

I am Supervisor Frost's constituent, and I live in the Orangevale area.

I am writing today in opposition to the ratification of the two proclamations surrounding the Monkeypox emergency. In Sacramento County, there are 65 cases with NO deaths and the vaccine is ready for those who want it.

Historically, we have never declared an emergency for annual seasonal flu every year that actually has killed people. An overreaction to this public health issue will ensure that the "cure" is worse than the disease.

We need to stop the incessant cycle of fear and bring the population back to reality. We can easily educate the public on the risks and treatments of Monkeypox without declaring an emergency, much like we have seen in the case of smoking tobacco or drinking and driving.

Thank you for all the work you do to try and help keep Sacramento County safe.

Thank you for your consideration,
Blake A. Bean

From: April Bean
To: Supervisor Serna; Kennedy. Supervisor; Rich Desmond; Nottoli. Don; Frost. Supervisor
Subject: Monkeypox State of Emergency
Date: Sunday, August 7, 2022 12:39:50 PM

To the Honorable Sacramento County Board of Supervisors,

I am Supervisor Frost's constituent, and I live in the Orangevale area.

I am writing today in opposition to the ratification of the two proclamations surrounding the Monkeypox emergency. In Sacramento County, there are 65 cases with NO deaths and the vaccine is ready for those who want it.

Historically, we have never declared an emergency for annual seasonal flu every year that actually has killed people. An overreaction to this public health issue will ensure that the "cure" is worse than the disease.

We need to stop the incessant cycle of fear and bring the population back to reality. We can easily educate the public on the risks and treatments of Monkeypox without declaring an emergency, much like we have seen in the case of smoking tobacco or drinking and driving.

Thank you for all the work you do to try and help keep Sacramento County safe.

In defense of our republic,
April Bean

From: Dena Da Prato
To: Clerk of the Board Public Email
Cc: Frost. Supervisor; Kennedy. Supervisor; Supervisor Serna; Nottoli. Don; Rich Desmond
Subject: Opposed: ratification of Monkeypox proclamations
Date: Monday, August 8, 2022 12:09:36 PM

I am Supervisor Frost's constituent and I live in the Folsom area. I am opposed to the ratification of the two proclamations surrounding the Monkeypox emergency.

In Sacramento County, there are 65 cases with NO deaths. The CDC has confirmed that 99% of all cases are male to male sexual contact.

There is low to no risk and this is not a fatal disease, there is no emergency.

Please stop the incessant attempt to control us and stop allowing special interest groups from being able to buy our freedoms.

Thank you for all the work you do to try and help keep Sacramento County safe.

Thank you for your consideration,
Dena Da Prato