Letter

Dear Editor,

In a recent publication in *Lab Animal*,[1] a non-human primate (NHP) researcher at the California National Primate Research Center, USA, argued for the continued importance of experiments on macaques for better understanding HIV and AIDS, and for tackling the virus and the disease with therapies and vaccines. Though the stance of the author is far from surprising, given his background and current employer (an organisation which houses around 5000 monkeys for research), the paper, even as comprehensive as it is, is not sufficiently critical of NHP experiments and overlooks a number of their deficiencies and established problems with them. Given the harm caused to the macaques in HIV/AIDS research, it is essential that we adopt an ethical and critical view, and that we seek ways of avoiding such research at all costs, rather than non-critically seeking to support and excuse it, often superficially.

What needs to be done

Attempts at conveying an ostensibly balanced view are not enough. How "closely" do SIV-infected or SHIV-infected macaques resemble HIV-infected people? Do the differences between the model and reality matter, and to what degree? Is it acceptable to consider the NHP models as merely not being "perfect", when some would regard the evidence against them to indicate that they are very poor indeed, and of insufficient human relevance for their use to be scientifically justifiable? Is it fair, at the same time, to make the sweeping statement that "there are currently no good [alternatives]"? Shouldn't the prevailing attitude be *vice versa*: that the NHP models are 'no good', while the alternatives are merely imperfect? Have NHP data really provided essential data on HIV pathogenesis, which could not have been obtained without NHP use, by using a combination of *in vitro*, *in silico*, *ex vivo* and clinical studies? How reliable is the extrapolation of NHP data to humans, when the failures are factored in? It is not sufficient to claim that NHPs were essential, simply because they were used in some area of HIV research: to have been justifiable, the data they provided must have been reliable, predictive of human HIV infection and AIDS, not obtainable by other means, and crucial to a breakthrough that resulted in human benefit. Even then, anything from the past that meets these criteria has little or no bearing on the current and future need for NHP use, as alternatives continue to be developed and improved and are increasingly comprehensive and capable. The onus is on NHP researchers to make a robust case addressing all the above, and more — yet none has been offered to date.

Shortcomings of NHPs

There are, in fact, myriad criticisms and shortcomings of using SIV-infected and SHIV-infected NHPs that were not acknowledged — or at least not sufficiently appreciated and discussed — in the *Lab Animal* review, which is therefore, I believe, more of an opinion piece than a critical analysis. For example, my own review, at the time of writing in 2008, showed that close to 100 varied preventive and therapeutic HIV/AIDS vaccines had proceeded into clinical trials based on encouraging safety and efficacy data from animal tests, most (if not all) involving NHPs including SIV/SHIV-infected macaques and HIV-infected chimpanzees. In greater than 200 clinical trials, none had shown sufficient safety and efficacy — had 'worked' in other words — to be approved for human use.[2] This represented more than two decades of disappointment in HIV vaccine clinical trials, since the first in 1987. Almost another decade further on, these numbers will be greater still, and we continue to wait for a vaccine to work in people, while so many have worked in NHPs. Some of these have been high-profile failures after widespread and vociferous claims of promise and success: VaxGen's AIDSVAX vaccines, which failed to protect 7500 people in large trials;[3] Merck's V520 vaccine, "One of the most promising [vaccines] to be tested on people so far"[4] whose 'STEP study' trials were terminated after not only failing to protect thousands of people from HIV infection, but actually *increasing* their risk of infection;[5,6] the failed PAVE 100 and Phambili trials,[7] and the RV144 trial, which failed to protect almost 16,500 trial participants.[8] This can only be viewed as a staggering level of failure, yet attempts are still being made by many in the field to dress it all up as a learning experience — a series of well-intentioned dead ends that will eventually lead to science going down a path that will lead to success. Indeed, the author of the *Lab Animal* article suggests that it merely indicates the need to aim for "balance" in the immune responses induced by vaccines, "circular feedback" between NHP and human trials, and "optimisation of the input variables of *in vivo* NHP vaccine studies, to enhance their predictability". To be frank: to advocate the continued manipulation of demonstrably and unequivocally poor approaches to a problem in the hope of solving it, is not scientific. To use an old metaphor, the time has come for these advocates to stop looking for their keys under the

street lamp, just because they can see where they're looking, and to start looking elsewhere, where they may actually find them. In other words: to stop using monkeys, and move where the science demands — to alternative methods, which are human relevant, as well as humane.

In fact, misgivings about the NHP-oriented direction of HIV/AIDS research in general have been voiced for some time, within the field.[e.g. 9–19] For example: "…efficacy of HIV-1 based vaccines cannot be directly evaluated in the SIV model";[13] "Despite the similarity with infection of monkeys by SIV, this has not proven a practical animal model for studying vaccines";[9] "When it comes to testing HIV vaccines, only humans will do";[10] "the persistent view held by many that there is no predictive animal model for HIV infection in humans";[15] "No animal models faithfully reproduce... HIV-1 infection and disease in humans, and the studies of experimental vaccines in animal models... have yielded disparate results";[17] "…the crucial role of human testing in the development of any vaccine… human immune system variability or virus diversity can't really be mimicked by any of the currently used laboratory animal models";[18] "...current SHIV models do not reflect HIV-1 variants circulating globally and thus do not fully recapitulate the viral factors that contribute to the infection dynamics being studied".[19] In a 2008 essay in *Nature*, Dr Anthony Fauci, NIAID Director and an AIDS expert, when reflecting on the era of HIV/AIDS, noted that: "We must learn from our missteps, build on our successes in treatment and prevention, and renew our commitment to developing the truly transforming tools that will one day put this scourge behind us."[20] If such an epic scale of vaccine failures is not a misstep, then what is? As recently opined in a review in the leading journal, *Science*, we know that a preventive vaccine for HIV is *in principle* possible: the problem is translating benefit seen in NHPs to benefit for humans. As the authors stated, "What is not known is how studies in monkeys will translate into humans."[21] This honesty is refreshing, given the passive acceptance of NHP data and their relevance to humans so often seen in NHP HIV literature; but, given the available evidence of how NHP data from SIV/SHIV/HIV research have translated to humans over the years, this could be more accurately conveyed as "It is very unlikely that studies in monkeys will translate to humans."

General flaws with NHPs and lack of human relevance

Part of the problem is that macaques are poorly predictive of human drug responses generally, as well as being poor models of HIV/AIDS — the *Lab Animal* paper's superficial claims of broad "similar physiology and metabolism" do not withstand scrutiny. It is acknowledged that macaque toxicology data "can differ from humans as much as other species".[22] In developmental toxicity tests, data correlate with human data just 50% of the time, less even than results from more evolutionarily-distant species such as rats, hamsters and ferrets.[23] There remains "no statistically credible evidence" that NHP toxicology data "contribute any predictive value, either separately or in combination" (for example, with dog data) to human toxicology;[24] and for the prediction of drug-induced liver injury, NHPs are less predictive than rodents, which have failed to predict up to 51% of effects in humans.[25] Single-dose toxicity tests, to which 34% of all non-human primates in regulatory safety tests are subjected, have been scientifically discredited.[26] And my own, recent work shows that macaque tests for drug toxicity are not fit for purpose: if toxicity is absent in animal tests, indicating that a new drug may be 'safe' (or free from adverse effects) in humans, the animal test provides almost no evidential weight to the probability that this will be the case in people, too. In fact, the data indicated that NHPs were even poorer than other commonly-used animal species, such as rodents and dogs.[27]

Why NHPs are poor models of human HIV/AIDS

Importantly, we now know not only *how* NHP HIV/AIDS research is poorly relevant to human HIV/AIDS, but also *why*. Fundamental, widespread, significant genetic differences are at the root of the poor translation of data from NHPs to humans in HIV/AIDS research, and mean that the use of NHPs can never be judged to be scientifically valid or necessary. Differences between both humans and macaques, and between HIV and SIV, have clear and serious consequences for NHP-based research. In the course of my work, I have published two papers on known genetic differences — in terms of gene complement, expression and regulation — between humans and chimpanzees,[28] and between humans and monkeys, notably macaques.[29] Both papers list differences that impact the immune system in general, and also the pathogenesis of HIV/SIV in particular. Examples include: genomic rearrangements, and differences in gene complement and regulation, including differences in HLA/MHC alleles, which affect susceptibility to, and the outcome of, HIV infection; differences in F_c receptors that have implications for the development and testing of vaccines and antibody therapies; and differences in the *TRIM5* gene — even minor ones, resulting in single amino acid differences of the TRIM5 protein — which are responsible for restricting and altering the host range of HIV

and SIV viruses between different primates. Indeed, macaques from different origins and geographical regions differ in their responses to SIV. Rhesus macaques of Indian origin were used most often, at one time, simply because this was the species supplied to laboratories by breeding facilities in the USA.[30] Then, a shortage of these monkeys prompted a search for other sources, and attention switched to the Chinese macaque, as it was "more readily obtainable". However, differences in susceptibility to SIV infection and disease were noted, mirroring differences also seen among species of macaque (rhesus, cynomolgus and pigtailed). It was concluded that "...even subtle genetic differences between two subspecies (races) of primate may promote significant differences in the pathogenicity of the same virus". With regard to the viruses themselves: SIV and HIV-1 are only around 50% homologous by nucleic acid sequence, and critical genetic differences are acknowledged (and should not be overlooked for convenience). For example, SIV from sooty mangabeys and macaques don't contain the HIV *vpu* gene, but instead have the *vpx* gene, which differs functionally.[31–33]

The end of chimpanzee use

These misgivings and caveats, and the biological and genetic species differences underpinning them, have translated into some significant changes in practice. For example, in spite of continued, vociferous assertions from chimpanzee researchers that chimpanzees had a "critical role in the testing of potential [HIV] vaccines" and that they "are still important for testing vaccines aimed at preventing HIV-1 infection or reducing the virus load in infected individuals",[34] funding for AIDS research in chimpanzees reduced to vanishing point due to its lack of human relevance (chimpanzees can be infected with HIV, but don't get AIDS). Indeed, so sceptical was the US Food and Drug Administration (FDA) of chimpanzee data, that it permitted clinical trials of two HIV/AIDS vaccines despite data showing them to be ineffective in chimpanzees.[35] All of this was even prior to the US Institute of Medicine's 'coup de grace' inquiry into chimpanzee research concluding in 2011 that these (and other) claims had no substance.[36] Chimpanzee research, in HIV/AIDS and other areas, is no more. I believe evidence shows it must be a matter of time for monkey research, too. This is not only due to scientific evidence, and ethical considerations: the public simply don't want it. A UK poll conducted on behalf of the government in 2014 found that just 37% of people agreed with the use of animals in "all types of research", with only 16% accepting experiments on monkeys, even if they clearly benefitted people.[37] Across Europe, a 2009 poll revealed that 79% of people supported a new law to "prohibit all experiments on animals which do not relate to serious or life-threatening human conditions", with 84% agreeing this "should prohibit all experiments causing severe pain or suffering to any animal";[38] and in 2010, 56% of Europeans agreed that scientists should not experiment on larger animals like dogs and monkeys, for the improvement of human health and well-being.[39]

It must, of course, be acknowledged that delivering an HIV/AIDS vaccine is a monumentally difficult challenge. That is not in dispute. Yet, it must also be acknowledged that billions of dollars and many thousands of NHP lives have now delivered around 100 vaccines of many types that work in NHPs, but no vaccine that works in humans; and that the insistence of NHP researchers that they be allowed to press on with their 'models' regardless, is unscientific and borne of desperation rather than logic and scientific reflection and evaluation. In the light of a second article on NHPs in the same issue of *Lab Animal*, citing the fact that a staggering 62,000 NHPs (approx.) had been used in research in the USA alone in 2015,[40] the onus is heavily on science to use better human-relevant technologies that already exist and will deliver — if given a fair chance. Studies of HIV-infected people who are able to control and suppress the virus ('elite controllers') in the absence of therapy, and who do not develop AIDS, as well as of those who respond to short-term treatment and who continue to control the virus when treatment is halted ('post-treatment controllers'), are identifying the immunologic factors involved and responsible, and are pointing toward means of targeting and eradicating reservoirs of HIV as part of a cure.[41–43] *In vitro* efforts continue to be immensely productive. Recent examples include: elucidating the role of HIV-infected Langerhans cells in inducing T-cell immune responses;[44] showing how follicular CD8 T-cells kill HIV-infected cells, suggesting they could be a component of a cure;[45] unravelling the mechanism of NK-cell mediated control of HIV infection;[46] the discovery and development of HIV entry (attachment and fusion) inhibitors[47,48] and integrase inhibitors[49] as therapies; continued 'omic' analyses to identify host factors in HIV infection, and therefore new targets for interventions;[50] the characterisation of HIV protein domains to facilitate rational drug design;[51] and, in combination with computer-based approaches, creating, identifying and characterising a variety of nanobodies (in effect, tiny antibodies) as new therapies.[52–56]

Instead of embracing these superior techniques and approaches, and appreciating the need to move away from needlessly causing pain and suffering to monkeys to generate poor data, the second *Lab Animal* article reports on the NIH workshop on NHPs held in September 2016, which, instead of "conducting a larger review of its non-human pri-

mate research" as requested by members of the US Congress, focused on "Ensuring responsible oversight". It quoted attendees, far from asking sorely-needed questions of the human relevance of NHP research, as seeking to "help the public understand why we do research and why it helps human health". Until the attitude of NHP researchers changes to a truly scientific one, reflecting on their chosen field and asking critical questions of it, they will continue to make excuses for it — and tens of thousands of monkeys each year, as well as many millions of humans relying on science to provide cures and treatments for human diseases of many kinds, will continue to suffer and to wait in vain.

Sincerely,

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