

## **Primates and HIV Research - Infecting our closest relatives**

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Since the first recognised case in 1981, human immunodeficiency virus, known as HIV, has claimed millions of lives and wreaked havoc on survivors, leaving many children orphaned, people and families debilitated, and even the economic stability of communities disrupted. The virus damages the human immune system, leaving the body susceptible to a syndrome of different diseases. This is the condition known as acquired immunodeficiency syndrome or AIDS. Although HIV is preventable, it has run amok, particularly in developing countries. Two decades after the sexual liberation of the contraceptive pill in the sixties, the expressions 'HIV' and 'Aids' emerged into public awareness, and this was the first time that people began to realise that having sex without a condom could get you more than an unwanted pregnancy. Of course that was just the tip of the iceberg and fear set in en masse promoting the search for a cure or at least a vaccine.

Although preventative strategies have offered the greatest advances, these are difficult to implement in third world countries and research has focused on the development of a vaccine and drug treatment known as anti-retrovirals. Researchers have looked for answers by using animals as a model for this disease in humans.

Nonhuman primates (NHP) are the animal most often used for research because of their similarity to us. However, even our closest relative, the chimpanzee does not develop AIDS, when artificially infected with HIV. Researchers found that monkeys had their own variety of HIV known as the simian immunodeficiency virus (SIV) and they have studied the progression of this disease and tried to draw parallels between it and the human version. So not only are they studying different animals but they are also looking at a different virus. They have also tried altering HIV so they can infect monkeys with it, and they have genetically modified animals so that they will react more like humans, but still there has been no success.

When we watch primates we may feel in awe of the similarities between us particularly as we see their curiosity and intelligence. We can see how they interact with their families, a cuddle for a young one, a chide for an adolescent, but at no time can we say they are the same as us.

### The statistics:

The United Nations Programme on HIV/AIDS and the World Health Organisation estimate the number of people affected by the disease as 33.2 million and the death rate at nearly 20 million adults and children. Developing countries account for 95% of these cases. In Australia 26,268 cases up to 2007 have led to 6,685 deaths (WA AIDS Council).

What these statistics don't show is the amount of suffering that has been inflicted on animals who can reason, communicate, express empathy and who live in complex social groups with strong family bonds. They don't include the number of NHPs who, spend their life in solitary confinement in cages in labs, or those who get rented out to different laboratories for repeated bouts of research often involving intrarectal, intravaginal and intravenous infection until, after an infinite array of invasive tests, and a drawn out period of illness and wasting, scientists conclude the study and the monkeys get the only peace they have ever known. This is when they are killed, disposed of into a garbage skip and then incinerated. Not all NHPs get treated this way, some are killed so that their body can be dismembered and used in a tissue data base.

If the lack of empathy for another living animal is not of concern, then the questionable results should be, particularly when there is an endless supply of humans on which to conduct research. Most advances so far have been brought about by population studies and invitro studies which have shown that the virus is spread between humans via blood, semen, vaginal fluids and breast milk and that it dies quickly when outside of the body. Any control over this deadly disease is from behaviour modification and these changes are slow to make their way into all communities.

Not only is the current research practice involving NHPs not providing a cure, but it is also misleading scientists who are not following up many drug candidates which could prove successful. Treatments which may have shown up as ineffective or toxic in NHPs are not taken to clinical trials in humans.

**In over thirty years there is still no cure and no vaccine for this preventable disease.**

### What are HIV and AIDS

HIV infection is a retrovirus which causes destruction of the immune system in humans, by destroying blood cells known as CD4+ T cells, creating an inability to resist infections and cancer. The resulting diseases are termed AIDS and this syndrome may not show up until years after the initial infection. HIV is notorious for being able to change its form from a small viral population to a genetically complex one by planting its own code into cells. In this way it can change its form and replicate itself. It is this viral diversity that makes it difficult to eradicate. Treatment is based around antiretroviral drugs which interfere with

reproduction of the virus, but because of the side effects and expense of medicating people in developing countries, the medical world is looking to vaccines to provide a cure.

### Use of primates in research for HIV

Although exact numbers are elusive, the number of NHPs in research has increased over the last decade. It is estimated that around 100,000 are used worldwide and of these 26% are used in HIV research. Reviews have indicated that research using NHPs for HIV involves “substantial severity” (Carlsson, 2004).

Their use allows researchers to control genetic and environmental factors and to conduct invasive and terminal experiments (those that end in the death of the animal) that would not be tolerated in human populations. Most research involving NHPs is conducted in North America, Europe (mostly Britain and France) and Japan. Australia also has three NHP breeding colonies and conducts research.

Initially, scientists focused on chimpanzees, however, they are expensive and their endangered status, along with rising pressure from the public has made their use increasingly difficult to justify. The use of Great Apes (chimpanzees, orang-utans, gorillas, bonobos and man are in this family) for research has been banned in The European Union but not in America and is restricted in Australia. They have come under scrutiny because they have complex thought patterns showing insight, a sense of time and purpose, empathy and communication. Macaques are thought to be slightly less neurologically complex than the Great Apes and this has provided researchers with an alternative.

The study of SIV in NHPs has given rise to a multitude of vaccines, from live attenuated vaccines, DNA vaccines, viral and bacterial vectors and subunit proteins leading to limited degrees of protection. A critical review of HIV vaccines showed that after showing success in chimps none of the 8000 volunteers in a clinical trial were protected. Over 90% of drug candidates fail during human trials and not one of the 80 drug candidates for a HIV vaccine was successful (Bailey, J., 2005 )

### **Why is the use of NHPs unacceptable and unbeneficial?**

#### The science:

There are many differences in the progress of SIV in monkeys and HIV in humans:

- Infection by SIV/ SHIV (a hybrid of the two) move at a different speed to HIV.
- Proteins in the outer layer of the virus have vaccine-like effects on monkeys but not people.
- Humans’ lymph nodes undergo structural changes and deposits of the virus remain in the nodes in the presence of HIV, unlike monkeys.

- Humans and primates have a different infection processes, metabolisms, and therefore responses to drugs.
- Genetically, humans are 97-98% identical to chimpanzees leaving a significant difference of two to three percent. Differences in our DNA (deoxyribonucleic acid), which is a molecule responsible for carrying genetic information, can activate or deactivate genes. Thus a small variance can create an avalanche effect.
- Chimpanzees do not develop antibody activity after infection with HIV, and their immune system is structured differently to ours in that they have different ratios of T4 to T8 lymphocytes.
- Humans with AIDS produce antibodies against a portion of a glycoprotein on the outside coating of HIV but SIV infected monkeys do not. Even different strains of SIV yield different results.

Even subtle differences between immune responses in different species make major consequences (Kaufman). Many researchers themselves believe that NHPs are too different to us to be reliable. In a study by the Department of Pharmaceutics in Washington National Primate Research Centre, the author states that a clear map for HIV vaccine development has yet to emerge. This is partly because of the nature of the model (NHPs), and that it is improbable for any single model to fully capture the complex interactions of natural HIV infection in humans. Incomplete understanding of the immunobiology of NHPs contributes to the difficulty extrapolating findings from such models to HIV vaccine development. The author says that the predictive value of any NHP model remains unknown (Hu SL., 2005).

#### Environmental factors affect validity of results:

- Physical and social environments affect the validity of the results. For example, stressed NHPs react in the same way as humans with altered cortisol and adrenalin levels creating altered cardiovascular parameters. Stress that is sustained over a long period of time can create cardiovascular, gastro intestinal and, importantly for HIV studies, changes within the immune system.
- NHPs cost hundreds of thousands of dollars and have a lifespan of thirty years. A study on primate usage showed that in chronic or long term experiments over 63% had undergone previous procedures and even more in acute or short term experiments. They are often rented out to other labs "Primates who make it to, perhaps, ten years old, have a trail of horrible experiences behind them." (Leary, 2011). This reuse affects the validity of the research because changes which occurred in the previous study have an ongoing influence (Carlsson, 2004).
- When most research is reported, it does not include detailed descriptions of the way the NHPs were obtained and then cared for during the study, so replication of an experiment is difficult as the conditions are not consistent.

### The Ethics:

Monkey species have rich social lives taking place in diverse groups and mixed habitats. Their behaviour is complex and varied. Their mental abilities are developed to the point that they can reflect on what is happening to them. It is a widely held assumption that if an animal is similar to a human, then its ability to feel, or its sentience must be greater. This assumption equates intelligence with the capacity to suffer.

Any animal with a nervous system suffers from events such as: removal from its own kind, fear, being subjected to invasive and often painful tests with no knowledge of what is going to happen next. No animal testing is acceptable, but if the ability to suffer is the cut off point for animal experimentation then it is clear from all our knowledge of NHPs that these animals are supremely unacceptable for research.

As a result of greater public awareness and pressure there have been a multitude of investigations into the welfare of primates used in research and there have been vast improvements in their care and treatment. However, many recommended improvements are not implemented because they are costly, time consuming and because old habits die hard. For example, research which has not succeeded is often not reported, there is replication of research because data banks are not maintained and NHPs are not trained to become familiar and accepting of laboratory techniques, thereby increasing stress levels.

Guidelines which govern the use of animals within laboratories are in place as are ethics committees which assess the validity of a study. These committees are ineffectual and cloaked in secrecy and the guidelines indicate that the expression 'humane treatment' is a subjective term that not everyone sees the same way.

### For Example:

An experiment conducted "...According to the National Institutes of Health guidelines on the care and use of laboratory animals and...approved by the University of Melbourne Animal Experimentation and Ethics Committee", was conducted as follows:

This study compares the characteristics of modified HIV and SIV infections in 54 pigtail macaques. They were inoculated intravaginally, intrarectally, and intravenously with doses of SIV, SHIV (a hybrid of the two) and HIV-1. Viruses were obtained from infected macaque newborns.

All inoculations were conducted over a period of two days. The authors write that "Encouragingly, all naive pigtail macaques studied became infected, although the outcome of infection was markedly different". Monkeys infected with SHIV became ill within two weeks immediately post inoculation. They suffered immune deficiency respiratory illness, diarrhoea and weight loss, and by 11 to 12 weeks over half of the monkeys were euthanized. Those infected with HIV-1 were poorly predictive and "Despite attempts at

infecting pigtail macaques ...only lower non-sustained levels of viral replication were observed" (Batten, 2006).

Researchers and 'carers' interacted with, responded to and nursed these animals over several weeks. They created suffering and then killed more than half of the animals as they were of no further use. The remainder would either go on to other studies or would be killed, as Australia does not have the facility for retiring them. All of this was for information that was not substantially different to any other results gleaned over the last thirty years, and it all comes under the heading 'humane'.

Although the three Rs of replacement, refinement and reduction of animal use is bandied around in theory, researchers get locked into experimenting on primates because all previous work has been conducted on NHPs and to change would create expense and inconsistencies. Primates are often used for toxicology studies for reasons of bureaucracy and fear of litigation rather than valid scientific reasons.

### Alternatives:

#### Prevention

- UN Aids state that the epidemic can only be halted if preventative measures are intensified.
- The Burnet Institute works within communities and conducts research on groups vulnerable to HIV, promoting awareness, working on strategies to expand access to condoms, cooperating with developing countries to provide a response to HIV, use of social network sites to promote safe sex, encouraging HIV testing amongst high risk gay men. (Burnet Institute)
- Antiretroviral medications prevent HIV from multiplying and reduce the amount of virus in the blood. PLOS Medicine reported that early treatment with antiretroviral drugs curtail the effect on the body's immune system and decreases the chance of it spreading to other people by 96% (Erin Loury , 2012). To take full advantage of this treatment depends on easy access to HIV testing for at-risk population. However, an over the counter HIV testing kit that is available in America is not going to be available in Australia (Kretowicz, E., 2012).
- Repressive drug law enforcement has increased HIV transmission. Those countries with needle exchange programs and safe injecting facilities, such as Australia, have lower levels of HIV infection.

#### Alternatives to NHPs in drug development

- There is a range of relevant in vitro cell and tissue tests, and in silico tests. Examples of successful drugs developed with no reliance on animal derived data are anti HIV protease inhibitors and nucleoside-analogue drugs. These are both anti-retroviral drugs that interrupt viral replication.

- Research in the American Journal of Primatology showed that of all primate research, half of the studies analysed primate biological materials rather than whole animals. It would make sense that instead of using another species we use a database made up of the millions of people who have the disease, such as the Australian HIV Observational database, and study their ex vivo samples.
- Microarrays and other DNA technologies, mathematical computer modelling, human clinical research, microfluidics, and post marketing drug surveillance will all provide data that is more relevant to humans.
- Preclinical tests of HIV vaccines are currently done on NHPs but apart from being costly they are still questionable and new research has focused on screening the HIV-1 vaccine on human cells and tissue via an in vitro system that allows accurate predictions of in vivo immune responses (Malaspino, 2011). Microdosing is also a safe way to test minute amounts of a drug in humans as a clinical trial.

### Population and family studies

- The Australian HIV Observational Database is a cohort study of people infected with HIV. It has been in existence since 1999 and monitors patterns of treatment, success, and problems with medication (antiretroviral). The information gleaned from this database is published and widely circulated (University of New South Wales Australia, 2010).
- Human genomic variability:  
People differ in their susceptibility to the HIV-1 virus and this difference is encoded in the human genome. (Loeuillet, 2008). Through this study researchers have used family lines to identify the part of the genome responsible for susceptibility.

Humans are wonderfully intelligent and articulate creatures. They are capable of empathy that can stretch across boundaries of time and distance, crying over the vision of human suffering in a country they've only ever seen on the television. If they don't see, hear or read about the suffering though, it's almost as if it never happened and that is largely what is happening to NHPs in research. The numbers used, the types of procedures endured and the end results of studies are kept under wraps whilst the general public comforts itself by believing that the research is vital because it saves human lives. But it doesn't.

Vivisection or the use of animals in research is not a new concept as it began around 300BC when there weren't state of the art imaging facilities, global communication to ensure sharing of knowledge and there weren't data bases to provide a multitude of human material to study. There were animals and cadavers, and even then people complained that it was inhumane. Although many scientists will tout success stories using animals as research models, most health improvements have evolved as a result of population studies and epidemiology and through changes of human living conditions. Vivisection did not have to prove itself, it did not need to go through rigorous approval procedures, it's just something that has always been done...no justification required. The new non-animal technology does, however, have to justify its efficacy and is doing so superbly.

The time for non human primates to move out of the lab and back into their natural habitats is well and truly here. If their use and abuse has not provided a cure for HIV/Aids after 30 years of research it's because the research is misaligned and needs to be brought back to studying the human model of illness, and not continuing to experiment on a different species and using a different virus.

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### Glossary:

Antibodies—a substance produced by lymphocytes in response to infection.

Antigen—a substance that the body recognises as foreign and which can evoke an immune response.

Genome—a complete set of genes in the chromosomes of each cell of a specific species.

Human immunodeficiency virus—a virus(retrovirus) that is capable of splicing into the host cell's DNA. It disrupts the immune system via T-cells and has a long incubation period resulting in AIDS. Opportunistic infections such as Kaposi's sarcoma, pneumonia and tuberculosis are able to attack the body.

In vitro—Taking place within laboratory apparatus such as a test tube.

Lymphocytes – a small white blood cell which develops in the bone marrow and is a component of blood. There are two forms of lymphocytes known as B-cells and T-cells.

T-cells – lymphocytes that divide rapidly in response to an antigen. They secrete compounds to assist B-cells to destroy foreign protein.

(Harris, 2006)

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