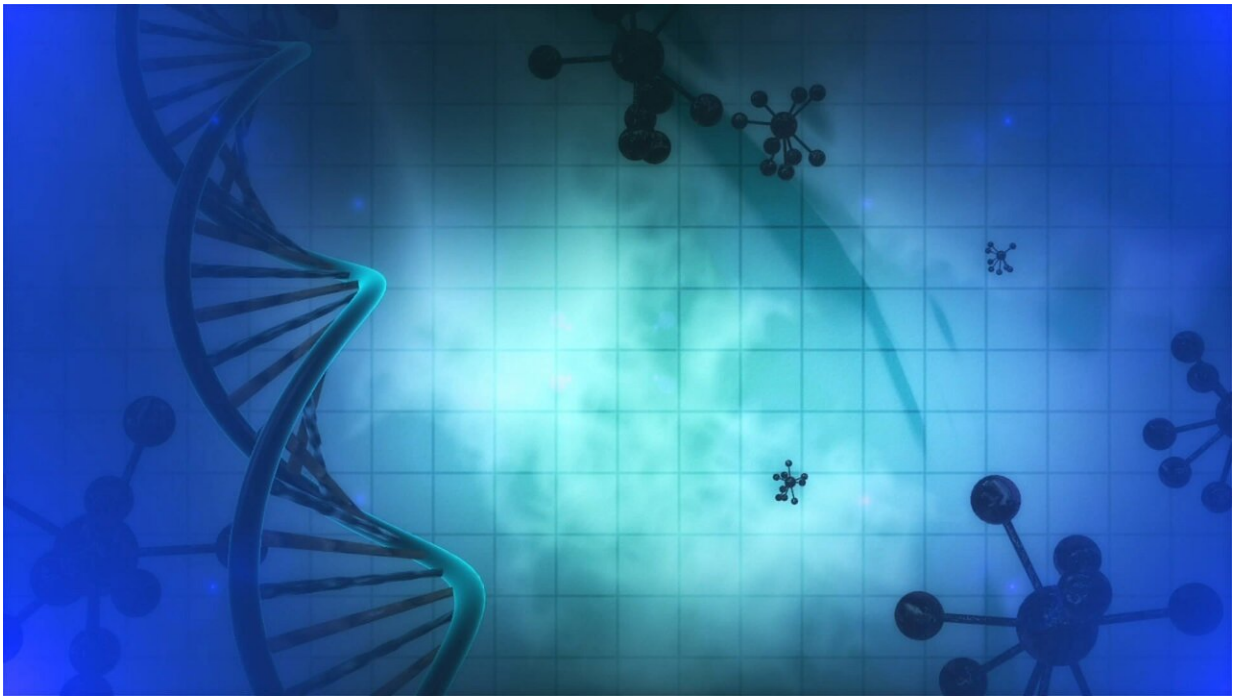
# Genetic engineering without unwanted side effects helps fight parasites

December 23 2020



Credit: CC0 Public Domain

Modified CRISPR-Cas9 gene editing scissors are enabling researchers at the University of Zurich to make alterations to the genetic material of single-cell organisms that are indistinguishable from natural mutations. This method makes it possible to develop a harmless experimental live vaccine for the widespread parasite Toxoplasma gondii.

Around a third of the world's population carries Toxoplasma gondii, a parasite that puts people with a weakened immune system at risk and can trigger malformations in the womb. The single-celled pathogen also leads to economic losses in agriculture, with toxoplasmosis increasing the risk of abortion among sheep, for example.

The parasite has a complex life cycle and infects virtually all warm-blooded creatures, including wild rodents and birds. It is introduced into livestock, and thus into humans, exclusively via cats. In cats, infectious stages form that are shed in feces into the environment as encapsulated oocysts, and from there, enter the food chain.

"If we succeed in preventing the production of these oocysts, we can reduce the occurrence of toxoplasmosis among humans and animals," says Adrian Hehl, professor of parasitology and Vice Dean of Research and Academic Career Development at the University of Zurich's Vetsuisse Faculty. He and his research group have developed methods making an intervention of this sort possible.

## Live vaccine protects cats from natural infection

In earlier research, the team had already identified various genes that are responsible for the formation of oocysts. This enabled them to develop a live vaccine for toxoplasmosis. The researchers used the CRISPR-Cas9 gene editing scissors to switch off these essential genes and inoculated cats with the modified parasites. These pathogens do not produce infectious oocysts, but still protect cats from natural infection with Toxoplasma in the wild.

While CRISPR-Cas9 enables precise modifications to the genetic material, the process can also have disadvantages depending on the protocol, as errors and unintended genetic alterations can creep in. Now, the research group around Hehl reports that they avoided such unwanted

side-effects using a modified technique.

During CRISPR-Cas9 gene editing, scientists usually insert a ring-shaped piece of DNA, a so-called plasmid, into the cell. This contains all the information necessary to create the gene scissors and the elements that recognize the desired site in the genetic material. The cell thus produces all the components of the CRISPR-Cas9 scissors itself. Afterward, however, the plasmid remains in the cell and can trigger additional, unplanned genetic changes.

## Gene scissors disappear without a trace

The method used by the Zurich team works differently. The researchers assemble the preprogrammed gene scissors outside the cell and then implant them directly into the parasites. After the genetic material has been manipulated, the components are very rapidly broken down, with only the desired edit remaining.

"Our approach isn't just quicker, cheaper and more efficient than conventional methods. It also enables the genomic sequence to be altered without leaving traces in the cell," explains Hehl. "This means we can now manufacture experimental live vaccines without plasmids or building in resistance genes."

## Genetic engineering legislation lags behind

Given these results, Hehl questions the federal government's plans to make CRISPR-Cas9 genome editing subject to the existing law on genetic engineering (and the moratorium, which has been extended to 2025): "Our method is good example of how this new technology differs from conventional approaches to genetic engineering," has says, because it is now possible to inactivate a gene without leaving unwanted traces in

the genetic material in a way that is indistinguishable from naturally occurring mutations. Unlike many other controversial applications of genetic engineering, this procedure does not affect the production of food, and thus does not constitute a direct intervention in the food chain.

**More information:** *Journal of Biological Methods*, DOI: 10.14440/jbm.2020.343

Provided by University of Zurich