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Popular Article

Patentable invention of intestinal kitten cell lines a legal remedy for kitten killing tragedy in toxoplasma research

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KITTEN Act and scientific fact

Since 1982 the agricultural research services (ARS) division of the US Department of Agriculture (USDA) had been conducting experiments on toxoplasma involving cats as primary hosts. Toxoplasma is a zoonotic disease that affects all warm-blooded animals including human beings. In the experimental protocol, cats are infected with toxoplasma, those infected cats are ethically sacrificed and incinerated to restrict the spread of infection to man and animals. Surprisingly in 2018 when the project reached 36 years of its completion all the research documents were collected through the Freedom of Information Act (FOIA) by the watchdog group expert panel for investigation under the "White Coat Waste Project" and made public. The expert panel detected serious issues regarding killing healthy kittens after experimental infection. Lawmakers in both the House and Senate of the US raised their voices against the kitten killing abuse. The 14-page document, titled "USDA Kitten Cannibalism", argued that such research is "irrelevant to American public health and the USDA's mission". Therefore, legislators introduced an act known as KITTEN Act "Kittens in Traumatic Testing Ends Now" (Keen 2019). Eventually, on 2nd April 2019, (USDA) announced the closure of all kinds of research on toxoplasmosis involving kitten scarification (Kaplan 2019). With this tough decision, a well-acclaimed lab in Beltsville, Maryland involved in Toxoplasma research under leading scientist Professor Jitender Dubey fell victim to wind up all their research activity. Toxoplasma is a tinny unicellular microscopic creature distantly related to the malarial parasite known as *Toxoplasma gondii*; a cyst-forming apicomplexan parasite infecting virtually all warm-blooded species, with all true cats (Felidae) as definitive hosts. More than 60 million, people are infected with *T. gondii* and most of the infected cases are asymptomatic. In humans, mother-to-child transmission has been reported with *T.gondii* (Al-Malki 2021).Toxoplasma colloquially as Toxo can survive in several

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animal species but completes its sexual reproductive cycle only in cats (*Felis catus*) and produce environmentally resistant oocysts released in feces (Dubey *et al*., 1970). The life cycle of Toxoplasma involves both an asexual phase and a sexual phase of reproduction. Once the parasite gets entry into the stomach of a cat the pepsin and acid digestion facilitate the release of bradyzoites, subsequently, bradyzoites invade the intestinal epithelial cells and differentiate into morphologically distinct five different pre-gamete stages A to E, designated merozoites prior to gamete formation (Dubey & Frenkel 1972). Within the feline intestine, merozoites are known to differentiate into micro and macrogametes that fuse to become diploid oocysts. Oocysts represent the final product of sexual reproduction which occurs in the intestinal lining of the cat family. Diploid oocysts have thick impermeable walls and are relatively resistant to several chemical disinfectants and remain viable for up to 18 months in adverse environments (Dabritz *et al*., 2010). Sporulation is required for oocysts to become infectious and occurs within 1–5 days in the environment. Under a normal environment, the oocysts undergo mitotic as well as meiotic cell division to produce haploid sporozoites enclosed with an oocyst wall. Humans are accidental hosts but once ingested the parasite transforms into a tissue-infective stage and then walks through the intestinal wall to enter the bloodstream, for home to other tissues and the central nervous system (Jones *et al*., 2014). Once Toxo gets into mice and rats the parasite can alter the behaviour of the rodents so they become fatally attracted to the scent of feline urine (Vyas *et al.*, 2007). Once the infected mice get eaten, the cat picks up the infection, and Toxo makes more Toxo due to the completion of its sexual reproductive cycle. Research reports suggest about 40% of cats in the United States are infected with Toxoplasma; remain asymptomatic, but they can develop jaundice or blindness and experience nervous symptoms. In the domestic environment, cats and rodents are considered the most significant reservoirs of human infection.

Cat Killing Practice Is an Obsolete Device

As of now for in vivo study the kittens are fed in the lab with meat contaminated with toxoplasma in order to harvest parasite oocysts shed in the animals' feces. Abiding by the animal ethics guidelines those infected cats must be incinerated to avoid spreading the infection to humans for which there is no vaccine and no cure as antibiotic treatments may not completely expel the parasites. Scientists have observed that this widespread parasite is also most difficult to study without sacrificing kittens. It is arguably true that killing kittens is cruelty to animals, therefore such unethical practice has been debated and drawn criticism from animal welfare activists. The ban on kitten scarification by USDA, although caused a blow to Toxoplasma research labs throughout the world, yet the scientific community never remained silent, rather went back to their working bench to search for an alternative to replace the kitten with a modified in-vitro cell system.

The cellular mat can replace the live cat

Some ill-defined molecular determinants present in the intestinal cells of cats may be considered as a specific marker that categorizes these species as the definitive host for *T. gondii* . The

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role of the delta-6-desaturase enzyme (D6D) in the conversion of linoleic acid to arachidonic acid in a rate-limiting manner has been probed as one of the contributing factors in this aspect. Felines are the only mammalian species, lacking D6D activity in their intestines (Rivers *et al*., 1975), resulting in higher levels (25%- 40%) of the linoleic acid detected in cat serum (Trevizan *et al*., 2012) whereas rodents serum contains merely 3%–10% linoleic acid (Jelińska *et al*., 2017). It has been hypothesized that the absence of delta-6-desaturase enzyme in the intestinal cells allows the accumulation of linoleic acid from the dietary source, which acts as a positive signal for *T. gondii* sexual development. In order to break the species barrier a few years ago, Laura Knoll and her team at the University of Wisconsin, treated mice with drugs (SC-26196) to inhibit murine D6D activity, and dietary supplementation of their diet with linoleic acid (Di Genova *et al*., 2019). In support of it, an earlier report has shown that in vitro cultured feline intestinal cells supplemented with linolenic acid and a chemical SC-26196, which specifically inhibit the enzyme delta-6-desaturase, established high levels of linoleic acid in blood serum (Obukowicz *et al*., 1998). This has permitted animals' intestines more like those of felines that successfully pushed Toxoplasma from its asexual phase into the sexual phase of development (Offord 2023). As mentioned in the international patent submitted by patent holders Laura Knoll and her group has disclosed an in-vitro method to generate cell monolayer derived from foetal intestinal crypts of kittens that can sustain the sexual life cycle of *T. gondi*. The crypts comprising of enterocytes, goblet cells, paneth cells, endocrine cells, and stem cells villi (Knoll *et al*., 2019). As per the report published by this group, the cells generated from the intestinal crypt displayed polarization and tight junction bridging as naturally occur in animal intestines (Di Genova *et al*., 2019). Supplementation of those cells with linoleic acid at a desired concentration (200 μM) for 24 hours prior to *T. gondii* infection supported the development of bradyzoites to enter the pre-sexual stage called merozoites. Merozoites are known to differentiate into micro and macrogametes that fuse to become diploid oocysts. The development of merozoites in cell culture was confirmed by the detection of merozoite-specific antibody staining against GRA11B marker protein. Further induction of linoleic acid on 7th day of protozoal infection the infected cells expressed amine oxidase and copper-containing protein 2(AO2) considered to be a positive indicator of intracellular gamete formation. Confirmation of gamete formation through PCR and detection of intracellular oocyst wall biogenesis in this linoleic acid–supplemented cat cells was established by gamete-specific staining with monoclonal antibody(3G4). They have frozen down the cells and later expanded those to confirm the immortality character like that of the established cell line (Di Genova *et al*., 2019). Similarly, mouse intestinal monolayers supplemented with both linoleic acid and D6D enzyme inhibitor (SC-26196), approximately 26% of the *T. gondii* vacuoles expressing both BRP1 and GRA11B as marker for merozoites (Fig-1) have been recorded in their report (Di Genova *et al*., 2019). Further, inhibition of delta-6-desaturase can cause sexual development in live mice has also been confirmed by these groups. On the 6th day of the post-infection oocyst-like structure was detected in mouse feces and those

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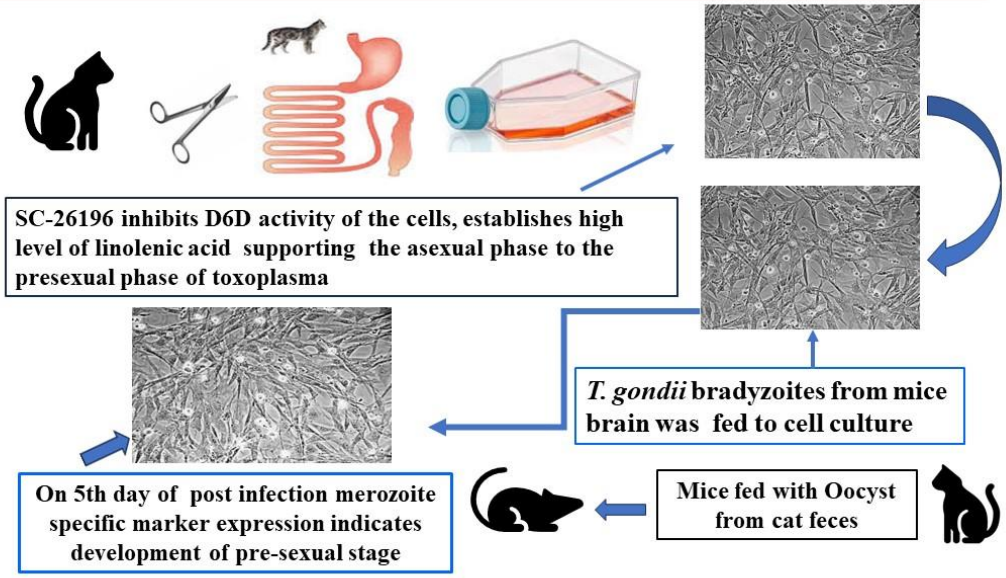
oocysts sporulate and were found to be infectious. In summary, we may conclude that the activity of delta-6-desaturase enzyme must be inhibited so that linoleic acid from extraneous sources should be sufficiently accumulated (without being converted to arachidonic acid) to induce development of pre-sexual stage of Toxoplasma gondii in nonfeline intestinal cells.

Fig-1: In-vitro culture of Cat intestinal cells with Linolenic acid with chemical SC-26196 that supports development of pre-sexual stages of Toxoplasma gondii : Ref: Di Genova *et al.*, 2019



Conclusion

Hopefully, the preserved cell lines derived from the kitten/mouse intestine and the epigenetic programming of *T. gondii* will pave the way to study the sexual life cycle of the parasite, the future reality without animal cruelty.

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