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Editorial

The curious tale of how crocodiles farmed for designer leather handbags are helping to develop human anti-arboviral vaccines

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INTRODUCTION

Most residents of industrialised nations in temperate climatic zones know mosquitoes to be an irritant; those blood-sucking pests that can blight a summer barbeque or family picnic. However, for people living in tropical and subtropical regions of the world, mosquitoes carry the threat of transmission of pathogens responsible for debilitating and often life-threatening blood-borne infectious diseases (Randolph and Rogers, 2010). Chief among these is malaria, caused by the protozoan parasite *Plasmodium*, and transmitted between people by the infectious bite of mosquitoes belonging to the *Anopheles* genus (Satapathy and Taylor-Robinson, 2016). Also, noteworthy are so-called arthropod-borne viruses, arboviruses, which are defined as viruses that replicate in both vertebrate host and invertebrate vector and which are transmitted between vertebrate hosts by biting arthropods (such as mosquitoes, ticks, sandflies and midges). Among their number are included the aetiological agents of such global infections as dengue, yellow fever, chikungunya, Japanese encephalitis and Zika that present a significant public health risk worldwide (Wilder-Smith et al., 2017). These viruses are transmitted from person to person by various species of *Aedes* mosquito. These insects bite not only humans but a whole spectrum of animals – other mammals but also birds and reptiles, each of which acts as a reservoir host that enables the arbovirus to be sustained in the environment (Gyawali et al., 2017a).

MOSQUITOES BITE CROCODILES

Unlikely as it may at first appear, included on this mosquito menu is the estuarine or saltwater crocodile, *Crocodylus porosus*. This largest of all living reptiles (males reach up to 7 m in length) is a fearsome apex predator that is usually best left well alone. While it can live in marine environments, the 'saltie', as it is commonly known in Australia and as its name implies, is usually to be found in saline and brackish mangrove swamps, estuaries, deltas, lagoons and

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rivers mouths (Griffiths, 2017). It has the broadest distribution of any crocodylian, ranging from the eastern coast of India, throughout most of southeast Asia, to northern Australia. It is in this latter region, comprising the northernmost tropical parts of the Northern Territory, Queensland and Western Australia, that the saltwater crocodile is thriving. Once threatened by unregulated hunting for its meat, eggs and skin, and still persecuted across most of its range, the Australian wild population is currently estimated at over 200,000 adult specimens, boosted by the granting of full legal protected status since the early 1970s (Northern Territory Government, 2018).

CROCODILE FARMING FOR LEATHER

The saltwater crocodile has been farmed very successfully for both meat and skins in northern Australia for several decades. Crocodile meat is marketed as a bespoke, high protein, low cholesterol food while it is also popular in Chinese traditional medicine as a scientifically unsupported treatment for colds and cancer. Commercial farms have also provided a novel attraction for the tourist industry in the tropical north (AgriFutures Australia, 2017). First and foremost, however, farming has grown out of a demand for skins to manufacture into high quality leather goods, such as designer handbags and shoes. The skin of the Australian saltwater crocodile is considered one of the best leathers in the world. If purchased on behalf of a fashion house like Hermes or Louis Vuitton the wholesale price of an unmarked adult skin can reach several hundreds of dollars (AgriFutures Australia 2017; Caldwell, 2017).

VIRUS INFECTION DEVALUES SKINS

It is against this backdrop that the Australian crocodile industry has been hit recently by an arbovirus infection outbreak that threatens to affect the livelihood of farmers. The cause is the Kunjin strain of West Nile virus that is endemic in the Northern Territory and far north Queensland (Hall et al., 2001). Its primary host are birds – herons, finches and crows – but it also infects and causes clinical symptoms in other animals (in particular, horses, in which it can trigger a fatal neurological disease) and in people (in which it often goes undiagnosed as an undifferentiated febrile illness; UFI) (Wildlife Health Australia, 2016). Infection of saltwater crocodiles with Kunjin virus is largely asymptomatic but can often produce skin sores that are detrimental to the quality of the reptiles' lucrative leather hide. Although crocodiles may look impervious to a biting insect, the *Culex* mosquitoes that transmit the virus are able to take a blood meal from around the eyes and, when exposed, also the softer underbelly. Since unblemished skins are highly sought after to turn into high-end fashion leather accessories, even small lesions can destroy the quality of the skin and therefore diminish its value. As the trade in crocodile skins is estimated to be worth over \$100 million a year to the Australian economy, the financial impact of the current viral episode is significant (Simmons, 2017). Moreover, if the Kunjin virus were to be maintained in specimens acting as reservoir hosts, it may prove problematic to eradicate infection from crocodile farms, each of which may hold tens of thousands of animals.

VACCINE DESIGN TO PREVENT LEATHER DAMAGE

The good news is that a pair of novel vaccines is being developed with the aim of preventing Kunjin virus from affecting farmed saltwater crocodiles (Cluff, 2018). A research team led by the University of Queensland and the Australian Infectious Diseases Research Centre in Brisbane has applied to the national gene technology regulatory authority in Australia to conduct field trials on crocodile farms, initially in the Northern Territory and potentially in far north Queensland (Australian Government Department of Health, 2018). Each prototype vaccine to be tested is a hybrid constructed of arbovirus fragments. The specific details relating to the identity of the parent organisms, the design, construction and genetic modifications, including the virus genes, corresponding proteins and their function, are currently confidential commercial information. What is public knowledge is that the candidates are founded on either of two insect-specific arboviruses, termed ISFa and ISFb, carried by *Anopheles* mosquitoes in northern Australia. Both of these are very distantly related to Kunjin but do not infect humans or animals and are thus entirely safe to use as a vehicle for vaccine delivery (Colmant et al., 2017a, b). Into this platform have been inserted two genes from a naturally attenuated strain of Kunjin virus. These express the constituent proteins of the outer coat of the virus particle, precisely those antigens recognised by the host immune system in order for immunity to infection to develop. The chimeric virion is disabled such that it cannot replicate in vertebrate cells, so cannot be transmitted between animals, and thus represents a very low risk of spread.

ON-FARM VACCINE TRIAL

Preliminary studies on mice have demonstrated that administration of two doses of each genetically modified vaccine

is both safe, with no side-effects, and efficient. This regimen evokes a strong immune response that rapidly clears the virus from the body and will protect from subsequent Kunjin virus challenge (Cluff, 2018). Approval is now pending to conduct a trial – scheduled to start later this year – on saltwater crocodiles at two farms in Litchfield Council in the Northern Territory (Australian Government Department of Health, 2018). It will involve inoculation of up to 2,800 juvenile crocodiles with the two vaccines using around 70 juveniles per group (Smith, 2018). This is important in order to optimise the dosage and route of inoculation, so the effect of each vaccine can be optimised to achieve the best possible protection. Crocodiles would be harvested approximately 18-30 months after inoculation, and processed for leather products and meat on-site at the crocodile farms. The five-year trial aims to identify how to prevent Kunjin virus infection of farmed saltwater crocodiles and thereby to provide a solution to current problems of skin spoilage. The boon to the economy of the tropical north of Australia that this would accomplish is clear for all to see.

CROCODILE DISEASE CONTROL STRATEGY

The development of a vaccine to the point at which it is ready for on-farm trials forms part of a crocodile disease control project underpinned by AU\$ 4.8 million funding to an academic consortium – from the University of Queensland, James Cook University, the Australian National University and La Trobe University – from industrial partner Porosus Pty Ltd. (Australian Government Business, 2017), a major Northern Territory saltwater crocodile farming business based 40 km outside Darwin (University of Queensland, 2017). The Australian Federal Government has contributed an additional AU\$ 1.15 million grant to support this research through its ‘Developing Northern Australia’ program (Australian Government Department of Industry, Innovation and Science, 2017). In addition to the limited and controlled release of recombinant insect-specific viruses as non-infectious vaccines against Kunjin virus infection in farmed crocodiles the wider aims of the project are to determine the natural history, epidemiology, pathogenesis and clinical sequelae of viral infection. This will facilitate a better understanding of the risk that the Kunjin virus poses to the saltwater crocodile industry. Collectively, this knowledge will enable crocodile breeders in northern Australia to become more globally competitive. This is happening at a time when the international trade in crocodile leather is in a state of oversupply, conducive to a buyers’ market, and thereby compelling farmers to produce the best quality skins that they can. The investment is motivated by economic interests; the more affected a hide is the lower the price that it will fetch, with badly spoilt skins simply destroyed at a commercial loss that is estimated at more than AU\$ 11 million per annum to the northern Australia economy (Australian Government Department of Industry, Innovation and Science, 2018). A virus outbreak can infect – and therefore damage the skins of – between a third and half of a farm’s stock.

MODEL TO TEST VACCINES TO MAJOR HUMAN ARBOVIRUS PATHOGENS

Importantly, the potential benefits of this dual-pronged approach are not confined simply to leather handbag production. An inadvertent but arguably even more valuable spin-off is that trialling the technology in a crocodile model also provides a proof of principle for testing of candidate arbovirus vaccines. Hence, in a parallel research strategy, the Australian National Health and Medical Research Council has granted AU\$ 550,000 funding over three year to 2020 for the University of Queensland to harness these insect-specific arboviruses to develop a new generation of diagnostics and safe hybrid vaccines to protect humans against mosquito-borne viral disease (University of Queensland, 2018). It is planned to construct hybrid viruses with a similar safety profile in order to target immunisation against a number of different diseases including dengue, yellow fever, chikungunya and Zika – in fact, a whole spectrum of mosquito-borne human pathogens. This is a prime example of the way in which the use of novel and relevant animal models, synthetic vaccinology and platform manufacturing can accelerate the pre-clinical stages of immunogen design and vaccine development. By harnessing these important innovations, the time needed to start the process of production and initial regulatory approval for a vaccine in humans may be greatly truncated (Graham et al., 2018).

AUSTRALIA’S UNIQUE NATIVE ARBOVIRUSES

This derivative advancement is of immediate significance to Australia itself since more than 75 native arboviruses, including the two used in the vaccine formulations, have been identified (Centers for Disease Control and Prevention, 2018). While a few of these indigenous antipodean viruses are recognized to cause disease in humans, there are limited or, in most cases, no information on the potential human pathogenicity of the vast majority (Gyawali et al., 2017a). This is of particular interest since as part of the same ‘Developing Northern Australia’ program that is funding crocodile vaccine research, the Australian Federal Government is actively encouraging increased settlement and economic activity in currently less populated areas of northern Queensland and the Northern Territory (Australian Government, 2015).

This industrial, business and agricultural development is inadvertently leading to the accelerated mobilisation and settlement of an infection-naïve human population into hitherto un-encountered ecosystems inhabited by reservoir animals and vectors for neglected arboviruses (Gyawali and Taylor-Robinson, 2017). Together with an escalating number and increased impact of dramatic climate events, such as tropical cyclones and floods caused by torrential monsoonal rainfall that promote mosquitoes, this heightens the potential for viral transmission to humans (Taylor-Robinson, 2017). This multifactorial scenario creates a perfect storm for indigenous arboviruses to emerge as significant agents of human disease in the coming decades in the tropical north of Australia (Gyawali et al., 2017b).

VACCINES TO TARGET LOCAL ARBOVIRUS PATHOGENS

Today, well over half of so-called undifferentiated fevers (those with non-specific symptoms) in Australia still go undiagnosed (Gyawali et al., 2017a). In such cases, an association could be assumed but not proved between arboviruses and feverish illness. Ross River virus and Barmah Forest virus each trigger an often debilitating and sometimes chronic type of arthritis that affects several joints at once. Both Murray Valley encephalitis virus and the aforementioned Kunjin virus cause inflammation of the brain, also called encephalitis. Sporadic outbreaks of dengue in northern Queensland are typically associated with influenza-like symptoms or, less commonly, life-threatening manifestations of haemorrhagic fever (with internal bleeding) or shock syndrome (with circulatory collapse). All these human arboviral pathogens are worthy of vaccine development (Gyawali et al., 2016).

CONCLUSION

The Australian saltwater crocodile farming industry is currently blighted by the mosquito-transmitted Kunjin virus, infection with which blemishes, and therefore, devalues, the hides. Skins that might otherwise be turned into luxury leathers fashion items are now often deemed to be next to worthless. The economic impact to northern Australia has spurred research into a possible vaccine with which to immunise farmed crocodiles to prevent them from becoming infected and therefore limit damage to their skins. A trial of two candidate vaccines is scheduled to start later this year. Moreover, there are plans for this crocodile vaccination model to be exploited to develop similar vaccines against other arboviruses – including dengue, Zika and more obscure native species – that pose a health threat not to reptiles but to humans. Thus, the crocodile skin luxury handbag-driven vaccine technology may, as a by-product, find a niche in the public healthcare sector at home and overseas. While the feasibility of using crocodiles as ‘guinea pigs’ for vaccination is recognised, of course it takes a lot longer for human vaccines to receive approval, so clinical trials are still several years away.

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