## Abstract:

Drug resistance against first-line antimalarials warrants search for new lead compounds and repurposing of drugs such as methotrexate. Animal models are required for preclinical drug development before clinical testing. This study aimed to develop a preclinical drug development system in baboons infected with Plasmodium knowlesi. METHODS: Protocols for drug administration, pharmacokinetics, clinical chemistry and haematology were developed in the baboon model. Baboons were infected with P. knowlesi and methotrexate administered orally for 5 days. Clinical signs, parasitaemia, gross and histopathology examinations were conducted to determine effect of methotrexate in baboons. RESULTS: No major clinical chemistry, haematology and pathological changes attributable to methotrexate were observed. Parasitaemia suppression of 77.67% was achieved at a methotrexate dose of 3.0 mg/kg. CONCLUSIONS: A protocol for preclinical drug development in the baboon was optimized. Methotrexate suppressed P. knowlesi malaria in baboons. These findings warrant further characterization of methotrexate for use in combination therapy.