CDC RESEARCH PLAN FOR INFECTIOUS VARIOLA VIRUS:

Determination of whether variola infection of *Cynomys ludovicanus* is a suitable animal model for human smallpox

This protocol has been approved by WHO through 2009.

Proposal for original research

CDC Investigators: Darin Carroll Ph.D, Kevin L. Karem Ph.D, Victoria A. Olson Ph.D., Scott K. Smith M.S., Zachary H. Braden B.S., Paul Hudson Ph.D., Jason Abel B.S., Inger K. Damon M.D., Ph.D. WHO Collaborating Center for Smallpox and other Poxvirus Infections Atlanta, GA

In 2009 an experimental challenge of *Cynomys ludovicianus* (black-tailed prairie dog) with Variola virus was performed as described in the proposal for original research. All work with live variola was conducted within the maximum containment laboratory (biosafety level 4) under the Terms of Reference of the WHO Collaborating Center for Smallpox and Other Poxvirus Infections at the WHO collaborating center in Atlanta, GA USA. The facility is reviewed for safety and biosecurity practices by independent U.S. and WHO teams on a frequent basis. All animal manipulations were performed on animals which are completely sedated using 5% inhalant isoflurane. Control animals (n=2) were sham infected for each route of infection and observed for possible non-specific signs (e.g., inoculation site trauma). Intranasal infection was performed in (n=4) animals with approximately 6.6×10^6 pfu of Variola strain Solamain in total volume of 10ul (5ul per nostril) administered with a pipette. Scarification was performed in (n=4) animals by administration of 6.6×10^6 pfu in total volume of 10ul to the skin on the back between the shoulder blades followed by ten intradermal sticks with a tuberculin syringe needle. Animals were bled for serum pre infection (day 0) and again at day 21 (termination of study). Oral swab and weight of animals were taken pre infection (day 0), day 3, day 7, day 10, day 14, and day 21. Animals were monitored for 21 days for any signs of overt illness. At the conclusion of the study (day 21), all animals were euthanized and necropsies were performed to collect lung, liver, gonads and spleen tissues for immunopathology analysis. Animal carcasses were autoclaved and incinerated for disposal.

Animals showed no weight loss and no signs of overt illness for the duration of the study. No gross lesions were observed in the nares. Scarification sites were unremarkable with the exception of some erythema from day 1 to day 3 post inoculations. However, no vesicular or pustular lesions were observed at any time during the study at inoculation sites or elsewhere on the animals. No viral DNA was detected from oral swabs taken post challenge by real time PCR. Testing of serum for Variola specific antibody indicates that 2/4 animals challenged i.n. and 4/4 animals challenged s.c. developed immune responses. Despite lack of overt illness, the detection of immune induction indicates subclinical infection in this species.