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Genetically Engineered Mengo Virus Vaccination of Multiple Captive Wildlife Species

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ABSTRACT: Encephalomyocarditis virus (EMCV), has caused the deaths of many species of animals in zoological parks and research institutions. The Audubon Park Zoo, (New Orleans, Louisiana, USA) attempted vaccination of several species with a killed EMCV vaccine with mixed results. This paper reports an attempt at vaccination against EMCV using a genetically engineered, live attenuated Mengo virus (vMC0) at the Audubon Park Zoo and Miami Metro Zoo, (Miami, Florida, USA) from December 1996 to June 1997. Several species of animals were vaccinated with vMC0, which is serologically indistinguishable from the field strain of EMCV. Serum samples were taken at the time of vaccination and again 21 days later, then submitted for serum neutralization titers against EMCV. The vaccinate species included red capped mangebey (*Cercocebus torquatus*), colobus (*Colobus guereza*), angolan colobus (*Colobus angolensis*), ruffed lemur (*Lemur variegatus ruber* and *Lemur variegatus variegatus*), black lemur (*Lemur macaco*), ringtailed lemur (*Lemur catta*), siamang (*Hylobates syndactylus*), diana guenon (*Cercopithicus diana*), spider monkey (*Ateles geoffroyi*), common marmoset (*Callithrix jacchus*), talapoin monkey (*Cercopithecus talapoin*), Brazilian tapir (*Tapirus terrestris*), Baird's tapir (*Tapirus bairdii*), Malayan tapir (*Tapirus indicus*), dromedary camel (*Camelus dromedarius*), bactrian camel (*Camelus bactrianus*), gerenuk (*Litocranius walleri*), guanaco (*Lama glama guanicoe*), black duiker (*Cephalophus niger*), Vietnamese potbellied pig (*Sus scrofa*), babirusa (*Babyrousa babyrussa*), collard peccary (*Tayass tajacu*), and African crested porcupine (*Hystrix africaeaustralis*). The vaccine response was variable, with high virus neutralizing antibody titer responses in some primate species and mixed to poor responses for other species. No ill effects were seen with vaccination.

Key words: *Cardiovirus*, Encephalomyocarditis virus, Mengo virus, seroconversion, serum virus neutralizing antibody titer, vaccination.

Encephalomyocarditis virus (EMCV) is a member of the family Picornaviridae. The genus *Cardiovirus* contains EMCV and Mengo virus, which are serologically indistinguishable (Zimmerman, 1994), as well as other serologically dissimilar viruses. Transmission and maintenance of cardioviruses is thought to be through murine rodents, although arthropod vectors are occasionally implicated (Wells et al., 1989; Zimmerman, 1994). It is felt that both the original and recent outbreaks of EMCV at the Audubon Zoo (New Orleans, Louisiana, USA) were associated with high numbers of rodents.

Disease caused by EMCV is peracute, seen as sudden death or anorexia and depression observed 12 to 24 hr before death (Gutter, 1993). Gross lesions are typically limited to the cardiopulmonary system. Findings include hydropericardium, pale streaks throughout the myocardium, and pulmonary edema (Gutter, 1993). Histologically, lesions may show massive cardiac myocyte necrosis with edema and lymphocytic, plasmacytic, and histocytic infiltrates. Pulmonary lesions of edema and congestion are secondary to acute cardiac failure (Gutter, 1993). Most viruses of the genus *Cardiovirus* contain a unique homopolymeric polyribocytidylate sequence within their 5′ non-coding RNA segments, that may contain from 60 to 420 pyrimidine nucleotides in a row (Martin et al., 1996). These poly(C) tracts and their particular lengths have been directly correlated with the virus's pathogenicity (Osorio et al., 1996b). Genetically engineered live Mengo viruses with shortened poly(C) tracts have been shown to be dramatically attenuated in animals, and moreover to steadfastly maintain their artificially truncated sequences during serial tissue culture pas-

sage (Palmenberg and Osorio, 1994). Laboratory mice receiving the short-tract strains developed life-long protective immunity against normally lethal challenge with wildtype Mengo or EMCV (Osorio et al., 1996b). Vaccination with a variety short poly(C) Mengo viruses has been shown to protect macaques, baboons and domestic swine as well as mice when challenged with wildtype EMCV (Osorio et al., 1996a). The objective of the current project was to determine whether inoculated target species at the Audubon Park Zoo and Miami Metro Zoological Park. (Miami, Florida, USA) would seroconvert when inoculated with a genetically engineered shortened poly(C) Mengo virus.

Mengo virus (vMC0) was grown from a plaque that resulted after transfection of HeLa cells with T7 RNA transcripts of cDNA origin (Martin et al., 1996). For intramuscular inoculation studies, suspensions of HeLa cells infected with vMC0 were clarified by centrifugation (10,000 g for 30 min, 4C), followed by ultra centrifugation through a 30% sucrose cushion. The resuspended pellet was stored at 4 C (Osorio et al., 1996a). From December 1996 through June of 1997 the following species were vaccinated at the Audubon Park Zoo and Miami Metro Zoo: red capped mangebey (*Cercocebus torquatus*), colobus (*Colobus guereza*), angolan colobus (*Colobus angolensis*), ruffed lemur (*Lemur variegatus ruber* and *Lemur variegatus variegatus*), black lemur (*Lemur macaco*), ring-tailed lemur (*Lemur catta*), siamang (*Hylobates syndactylus*), diana guenon (*Cercopithicus diana*), spider monkey (*Ateles geoffroyi*), common marmoset (*Callithrix jacchus*), talapoin monkey (*Cercopithecus talapoin*), Brazilian tapir (*Tapirus terrestris*), Baird's tapir (*Tapirus bairdii*), Malayan tapir (*Tapirus indicus*), dromedary camel (*Camelus dromedarius*), bactrian camel (*Camelus bactrianus*), gerenuk (*Litocranius walleri*), guanaco (*Lama glama guanicoe*), black duiker (*Cephalophus niger*), Vietnamese potbellied pig (*Sus scrofa*), babirusa (*Babyrousa babyrussa*), collard peccary (*Tayass tajacu*), and African crested porcupine (*Hystrix africaeaustralis*). The animals were injected intramuscularly during annual physical examinations with 5.0×10^6 PFU (plaque-forming units) of the prepared supernatant. The concentration was achieved by serial dilution of the virus with phosphate buffered saline to a 0.5 ml dose volume. To minimize the number of times an animal was handled, no prescreening titers were performed. Blood samples were collected immediately prior to vaccination, and again in 21 days. The blood was allowed to clot for 20 min at room temperature and then centrifuged at 10,000 g for 5 min to separate the serum. Serum samples were stored at −20 C until analysis. Samples were tested by two independent laboratories for serum microneutralization titers to EMCV using 2 fold serial dilutions of serum samples (Hubbard et al., 1992; Osorio et al., 1996a). Seroconversion was considered to be a four fold increase in serum titer.

Table 1 summarizes the serum virus neutralizing antibody titer results for all tested animals. In many primates without pre-existing titers (1:<8) to EMCV there was greater than a four fold serum titer increase at day 21. Several animals had preexisting titers (>8) to EMCV which indicated exposure to a wildtype *Cardiovirus.* For some of these animals, their preexisting high titer may have interfered with vMC0 vaccine replication or acted as a booster by increasing their low pre-vaccination titers. Many animals, primarily primates, had at least a four fold increase in titer indicating seroconversion to vMC0 vaccination. However, in other species, such as the camels, common marmosets and lemurs, only minimum increases in serum virus neutralizing antibody titers were seen or there were none at all.

EMCV is a peracute disease that can cause devastating losses to a zoological collection. One of the most important components of EMCV mortality prevention is an effective and consistent rodent control

TABLE 1. EMCV titers by serum microneutralization to genetically engineered Mengo virus (vMCO) for multiple captive species.

Species	Animal number										
	1	2	3	4	5	6	7	8	9	10	11
Ateles geoffroyi	16/16[a]	<8/64									
Babyrousa babyrussa	<8/8	<8/128	<8/512								
Callithrix jacchus	16/16	64/64	16/16								
Camelus bactrianus	<16/32	16/16	<16/8								
Camelus dromedary	<8/128	<8/>32	8/32	<16/64	8/8	8/8	8/8				
Cephalophus niger	<8/64										
Cercocebus torquatus	<8/8	<8/64	<8/128	<8/>128	<8/>8	<8/>32					
Cercopithecus diana	<8/128	<8/>64									
Cercopithecus talapoin	<8/256	<8/256	<8/128	<8/256	<8/256	<8/256					
Colobus angolensis	<8/<8	<8/<8	<8/128	8/128	8/256	<8/256	<8/64	<8/32	<8/128	<8/64	<8/64
Colobus guereza	<8/256	<8/<8	<8/64	<8/128							
Hylobates syndactylus	128/256	128/512									
Hystrix africaeaustralis	<8/128	<8/64									
Lama glama guanicoe	>16/128										
Lemur catta	<8/<8	<8/<8	<8/<8	<8/16	<8/16	<8/32					
Lemur macaco	<8/<8	<8/<8									
Lemur variegatus ruber	<8/<8	<8/<8									
Lemur variegatus variegatus	32/512	<8/>32									
Litocranius walleri	<16/128	<8/256	8/128	32/512	<16/64	<8/64	<8/64	<16/64			
Sus scrofa	<8/32	<8/16	<16/32								
Tapirus bairdii	512/512	32/32	<8/256	<8/32							
Tapirus indicus	512/256	<8/<8									
Tapirus terrestris	512/512	256/512	256/512								
Tayass tajacu	32/256										

[a] Serum neutralization (SN) antibody titer at day 0/SN antibody titer at day 21 post vaccination.

program. However, based on responses seen in this study where seroconversion was considered to be a four fold increase in serum virus neutralizing antibody titers, vaccination with vMC0 may also be a weapon to prevent mortality due to EMCV. This live attenuated virus did not produce visible disease; change in attitude, appetite or activity levels in any tested animals. Vaccination did produce a four fold serum virus neutralizing antibody titer increases in many inoculated animals. In individuals that did not develop a measurable titer it is not known whether the vaccine was ineffective or if more time was needed to see a measurable titer increase. Those animals may have had an amemnestic response to a second "booster" vaccination. It is not known whether the titers achieved would be protective against wild-type EMCV challenge in the species vaccinated. Based on laboratory animal challenges, serum virus neutralizing antibody titers of >1:16 are known to be protective, and possibly confer lifelong immunity to baboons, domestic pigs and mice (Osorio et al., 1996a). A virulent challenge was deemed inappropriate in a zoological setting and was not performed. However, rising antibody responses without any observed vaccination related complications suggest possible successful vaccination in some cases. It is hoped that a commercially available modified live virus vaccine, based on vMC0 will become available to aid in the protection of captive wildlife from EMCV morbidity and mortality.

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