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Using Hysterectomy Rederivation to Produce Guinea Pigs (*Cavia porcellus*) Free of Guinea Pig Cytomegalovirus

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Due to similarities in placentation, guinea pigs can be used as models of human cytomegalovirus infection, but they must be free of guinea pig cytomegalovirus. Many commercial guinea pig colonies are enzootically infected with guinea pig cytomegalovirus, which can be transmitted vertically as well as horizontally through saliva, vaginal secretions, and milk. These characteristics make its eradication in a commercial setting challenging. Because embryo transfer technology in guinea pigs is in its infancy, it is not generally a viable option for obtaining animals free of guinea pig cytomegalovirus. However, a combination of hysterectomy rederivation and testing by PCR assay and serology of both dams and offspring from an enzootically infected colony produced offspring free of guinea pig cytomegalovirus.

Abbreviations: CRL, Charles River Laboratories; GPCMV, guinea pig cytomegalovirus; CMV, cytomegalovirus;

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Guinea pigs, a caviomorph rodent native to the highlands of South America, have a long gestation period (average, 67 d) for rodents of their size, after which they typically birth 1 to 7 precocial young. Because guinea pigs have a discoidal, hemomonochorial placenta very similar to the human placenta, they are used as a model of human placentation.[3] In guinea pigs, a single trophoblast layer separates the fetal and maternal circulation, through which antibodies readily transfer from dam to offspring; consequently guinea pigs are not reliant on antibody transfer through milk. The guinea pig's precocial young can survive without maternal care, although the pups benefit from milk and socialization during their first 2 wk of life.

Many commercial guinea pig colonies test positive for guinea pig cytomegalovirus (GPCMV), an enveloped DNA virus in the family *Herpesviridae*, subfamily *Betaherpesvirinae*. After infection, CMV persist in animals, remaining latent in lymphocytes and endothelial cells for the life of the host. Virus generally is shed asymptomatically in bodily fluids, including saliva, urine, blood, semen, and milk. GPCMV can be transmitted vertically or horizontally through contact with infectious fluids. In Dunkin Hartley guinea pigs, infection is often asymptomatic, whereas infection of Strain 2 guinea pigs results in systemic illness with significant mortality.[1] Guinea pigs are commonly used as models of human CMV infection because the sequelae in congenitally infected guinea pigs, such as sensorineural hearing loss, mimic those of humans. In humans, the worst fetal outcomes are seen after initial infection of the mother during the second trimester of pregnancy. When a woman is positive for CMV antibody (IgM) prior to pregnancy, she is much less likely to transmit CMV to her fetus: the rate of CMV transmission to the fetuses of IgM-positive mothers is 1.4% compared with 32% in mothers experiencing a primary infection.[9] Vertical transmission rates in natural primary GPCMV infections have not been described.

Mouse or rat colonies positive for CMV frequently are rederived through embryo transfer because this method is least likely to result in transmission of vertically transmitted agents. However, embryo transfer in guinea pigs is only described twice in the literature.[6,12] Use of this method in guinea pigs is complicated by the species' long estrous cycle, the duration of which varies greatly between individual animals, the relatively few embryos produced per mating event, challenging oviduct anatomy, and lack of easily applied and validated hormone priming protocols (although progress is being made in this regard).[4] In addition, not all guinea pigs in our barrier room are positive for GPCMV. Because not all animals are freely exposed to each other and because GPCMV transmission requires relatively intimate contact, we theorized that transmission occurred both horizontally and vertically within the barrier room.

Management of potential horizontal transmission within a research guinea pig colony is relatively simple, because animals can be tested for the presence of virus or antibodies and then isolated from one another. Preventing vertical transmission of GPCMV is more difficult than preventing horizontal transmission, because pregnant females can acquire the virus from infected cage-mates. We hypothesized that the offspring of guinea pigs that were positive for GPCMV antibodies but negative by PCR analysis during pregnancy would likely be protected from in utero GPCMV infection because they would receive maternal antibodies through the hemochorial placenta during gestation. These pups could be removed from the uterus via terminal hysterectomy and then tested by using both serology and PCR assays to confirm that they were free of GPCMV.

Materials and Methods

All work was performed at Charles River Laboratory (CRL)'s AAALAC-accredited facility (Kingston, NY) and was approved

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by CRL's IACUC. Animals originated from Kingston room K61; at the time of the experiment, the colony was reported to be positive for GPCMV, *Staphylococcus aureus*, *Klebsiella oxytoca*, and intestinal protozoa. In the barrier room, all animals resided in solid-bottomed cages that were bedded with heat-treated hardwood shavings (NEPCO, Warrensburg, NY). Animals were group-housed in either breeding groups or age-matched same-sex cohorts. Food (Purina LabDiet 5KG0, LabDiet, St Louis, MO) and ultrafiltered, hyperchlorinated water (by automatic waterers) were provided without restriction. The room had a 12:12-h light:dark photocycle (lights on, 0630; lights off, 1830), humidity was maintained between 30% and 70%, and temperature ranged between 19 and 22 °C.

To begin the hysterectomy process, barrier-room staff identified candidate guinea pigs, that is, those that had the abdominal appearance and pubic symphyseal separation typical of late pregnancy, had given birth to pups 62 to 65 d prior, and had had at least 1 previous litter. We removed 5 guinea pigs meeting these criteria from the barrier room and allowed them to acclimate for 24 h in the surgical suite. The number of guinea pigs chosen reflected the number that could be removed from the colony without negatively affecting overall production.

Guinea pigs were anesthetized with 5% isoflurane (Henry Schein Animal Health, Dublin, OH) in 500 mL/min oxygen in an induction box and maintained on 2% to 3% isoflurane in oxygen through a nosecone. The abdomen was prepared aseptically. A ventral midline incision was made to expose the uterus. The uterus was clamped at the cervix and the uterine arteries, removed from the dam, and transferred to a second laminar flow hood for pup removal. Samples taken from the dam included whole blood, serum, kidney, spleen, mandibular salivary gland, uterus, and placenta. Dams were euthanized by isoflurane overdose after sample collection.

As each pup was removed from the uterus, the fetal membranes were stripped, the umbilical cord was clamped, and the pup was handed to a reviver who dried the animal and stimulated respiration. After the pup was pink, vocalizing, and moving, it was placed with its littermates in a recovery cage bedded with surgical toweling and sitting on a heating pad. Pups were monitored for 30 min, after which they were weighed, identified through injection of a small amount of tattoo ink (Stone Manufacturing and Supply, Kansas City, MO) into a toe pad, and transferred to a housing cage. The solid-bottomed housing cage was bedded with heat-treated hardwood shavings and contained a shelter and a food crock; access to a water bottle was provided though a long sipper tube. The cage was placed on a rack and, for the first week of life, half of the cage rested on a heating pad. Chow (Purina LabDiet 5KG0) was moistened and supplemented with gnotobiotic rat feces (CD Rat Foundation Colony, Charles River Laboratory, Wilmington, DE) for the first 5 d to establish gut flora. Animals were weighed every 2 to 3 d.

Samples from the dams were transferred to a diagnostic laboratory (360 Diagnostics, Wilmington, MA) for infectious agent screening. Serum was evaluated by multiplex fluorometric immunoassay to determine the presence of antibodies;[15] the screening panel included Sendai virus, pneumonia virus of mice, simian virus 5, reovirus, *Encephalitozoon cuniculi*, parainfluenza virus 3, lymphocytic choriomeningitis virus, C*lostridium piliforme*, *Mycoplasma pulmonis*, guinea pig adenovirus, and GPCMV. Sample suitability assessment, nucleic acid isolation methods, PCR inhibition controls, and negative template controls have been described.[8] A positive template control (that is, plasmid containing a PCR target; 100 copies per reaction) was used to confirm a functional master mix and PCR amplification. A proprietary real-time 5′ fluorogenic exonuclease (that is, TaqMan) PCR assay targeting the genome of GPCMV was used to evaluate tissues. Animals in the study were euthanized by using CO_2.

Results

Of the 5 dams identified as suitable for this experiment, pups survived from 4. The remaining dam was incorrectly identified as more advanced in gestation, and the pups did not survive surgical removal from the uterus; that dam was GPCMV negative by both PCR analysis and serology (Table 1). Of the 4 remaining dams, 3 were serologically positive for GPCMV, but all tissues sampled were PCR-negative for the virus. The final dam was serologically negative, but her mandibular salivary gland and uterus were PCR-positive for GPCMV. The survival rate of pups at the appropriate stage of gestation was 64% (14 of 22 pups); overall pup survival was 56% (14 of 25 pups). Litter size (mean ± 1 SD) was 5.0 ± 1.4 pups (Excel, Microsoft, Redmond, WA).

All surviving pups were necropsied and tested for the presence of GPCMV nucleic acid and antibodies. (Table 1) Litter sizes precluded sending animals from every surviving litter at every time point. Of the 14 surviving pups, 5 were antibody-positive according to multiplex fluorometric immunoassays. None of the tissues collected from pups were PCR-positive for GPCMV.

Discussion

Because guinea pigs are large rodents with a relatively placid disposition and precocial young, they are a logical target for hysterectomy rederivation to obtain germ-free animals. Guinea pig rederivation through hysterectomy is not a new idea.[2,5,10,11,14,16] However, previous work focused mainly on either the elimination of bacterial agents or the generation of germ-free animals for further experiments. Our work is novel in that it provided proof of principle that this method is suitable for rederiving guinea pigs free of a vertically transmitted viral agent that can reactivate during pregnancy and that was present enzootically in the colony. In addition, our goal was achieved by using natural mating.

In typical rodent hysterectomy rederivations, timed mating is used to allow for precise timing of offspring harvest to maximize survival chances. Timed mating in guinea pigs is not a well-developed technique, relying mainly on the postpartum estrus for mating and dating pregnancies. Even in the hands of experienced technicians, using cues such as past mating evidence in the form of a litter delivered 62 to 63 d prior, pubic symphyseal separation, and dam body size, we were unable to successfully predict pup gestational age for all dams selected for this experiment. Our pup survival rate is similar to that reported in other studies,[5,10,11,13] although direct comparison of survival rates is misleading due to differences in pup management. We did not attempt to maintain our animals germ-free, a practice that appears to decrease survival rates, nor did we provide milk or milk substitutes, which might increase survival rates. Guinea pigs with litters similar in age will readily accept fosterlings and typically communally nurse; consequently once a GPCMV-free colony has been established by using hand-reared guinea pigs, future fosterlings could be reared by guinea pig dams, thus likely increasing survival rates.

Of the 4 litters tested, 3 included pups that were positive for antibodies against GPCMV. This result likely reflects the presence of maternal antibodies, but this assumption should be

Table 1. Numbers of pups, pup survival, and results of GPCMV testing

Dam	Dam serology	Dam PCR	No. of pups born	No. of pups that survived	No. of PCR-positive surviving pups	No. of serology-positive surviving pups	Postnatal sampling day			
							27	37	41	48
1	—	—	3	0[a]	NT	NT				
2	+	—	5	2	0	2	+	NT	+	NT
3	+	—	4	4	0	1	±	—	—	—
4	+	—	7	5	0	0	—	—	—	—, —[b]
5	—	+[c]	6	3	0	2	+	—	+	NT

NT, not tested
[a]Pups younger than expected.
[b]The 2 pups remaining at this point were sent for sampling, and both were negative.
[c]Strongly positive in uterus, and salivary gland; ± positive in blood.

verified by serial blood sampling of pups derived in this way to document waning immunity. We did not follow these pups through serial serology, because the primary focus of the study was the potential detection of virus by using PCR analysis. The hemochorial placenta of guinea pigs enables the transmission of maternal antibody to fetuses. Authors studying the transmission of maternal antibodies in guinea pigs examined antibody transfer to pups in either seropositive or seronegative dams inoculated with GPCMV.[7] Animals that had received antibodies passively through the placenta were antibody-positive at birth, and some still had measurable antibody levels at 8 wk of age.[7] The animals that were only fostered onto seropositive dams did not receive protective levels of antibodies during 10 d of nursing.[7] The antibodies present in our pups must have been acquired through the placental route because there was no exposure to milk.

If these rederived pups were to be used to found a GPCMV-free colony, offspring from dam 5, although PCR-negative, should be excluded in light of the PCR results of the dam and the lack of clarity regarding the source of the antibodies in the pups. Antibody-negative but PCR-positive dams could be viremic and in the process of mounting an immune response to a primary infection, thus making their pups more susceptible to vertical infection. Had the pups from dam 1, the seronegative and PCR-negative dam, survived, they would have been the best candidates to establish a GPCMV-free colony. Testing guinea pigs from the enzootically infected colony and using only seronegative animals to establish a new colony would be another option. Potential drawbacks to this plan include that it might limit the genetics of the colony unacceptably and that animals might be tested during active infection but before antibodies are formed (as we saw in one guinea pig in this study), leading to the inadvertent incorporation of GPCMV-shedding animals into the colony. In addition, each seronegative animal would have to be singly housed and tested repeatedly to confirm its status, consequently resulting in some guinea pig dams aging past the point of safe reproduction and likely having negative effects on animal welfare. Past health monitoring results from this colony indicate that 40% to 70% of the colony is positive for GPCMV, but seronegative animals are not isolated from the rest of the colony. This study indicates that seropositive dams could serve as a source of GPCMV-negative offspring, providing that the offspring are confirmed either to lack antibodies to the virus or are sampled to ensure waning of maternal antibody.

In this proof-of-principle study, we used serology and PCR analysis to confirm the GPCMV status of several guinea pig dams prior to hysterectomy rederivation of their offspring. Combined serologic and PCR testing allowed us to produce guinea pig pups free of GPCMV. Further refinements of this procedure might include serial serologic sampling of pups to document the disappearance of maternal antibody to GPCMV and repeated PCR testing of bodily fluids to verify the continued absence of the virus.

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