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Modified Dose Efficacy Trial of a Canine Distemper–Measles Vaccine for Use in Rhesus Macaques (*Macaca mulatta*)

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Measles virus causes a highly infectious disease in NHP. Clinical signs range from asymptomatic to fatal, although measles virus is most well-known for its characteristic generalized maculopapular rash. Along with appropriate quarantine practices, restricted human access, and appropriate personal protective equipment, vaccines are used to combat the risk of infection. The canine distemper–measles vaccine (CDMV), administered at the manufacturer's standard dose (1.0 mL IM), has been shown to be effective against clinical measles disease in rhesus macaques (*Macaca mulatta*). The goal of the current study was to test whether doses smaller than the manufacturer's recommended dose stimulated adequate antibody production to protect against infection. We hypothesized that either 0.25 or 0.5 mL IM of CDMV would stimulate antibody production comparable to the manufacturer's recommended dose. We found that the 0.25-mL dose was less effective at inducing antibodies than either the standard (1.0 mL) or 0.5-mL dose, which both yielded similar titers. The primary implication of this study informs balancing resource allocation and providing efficacious immunity. By using half the manufacturer-recommended dose, the 50% cost reduction may provide sufficient monetary incentive to implement, maintain, or modify measles vaccination programs at NHP facilities.

Abbreviations: CDMV, canine distemper–measles vaccine; MV, measles virus

Measles virus (MV) is one of the most infectious diseases in humans,[22,50,55] and NHP in contact with humans are at constant risk.[6,43] MV is the prototypic member of the genus *Morbillivirus* and family Paramyxoviridae.[24] All Morbilliviruses are highly infectious, closely related, and most likely have evolved from a common ancestor.[24] These viruses generally spread through the respiratory route, initially infecting and replicating in the immune system and inducing profound immune suppression; consequently, morbilliviruses have the potential to cause acute large-scale outbreaks with high morbidity and mortality in naive populations.[25,54] Morbilliviruses include canine distemper virus (infects dogs, coyotes, wolves, and seals), rinderpest (cattle), peste des petits ruminants (goats and sheep), phocine distemper virus (seals and otters), dolphin morbillivirus, pilot whale morbillivirus, Longman beaked whale morbillivirus, feline morbillivirus, and unclassified morbillivirus-related viruses (rodents, moles, shrews, and bats).[24,25,60,61,74,80,82]

MV is spread by direct contact, aerosols, and fomites.[6,26] Measles is typically characterized by a generalized maculopapular rash, although clinical signs can range from asymptomatic to fatal. Clinical illness demonstrates a prodromal period of 2 to 3 d consisting of a fever, malaise, and anorexia, followed by coryza, keratoconjunctivitis, and a dry cough; generalized lymphadenopathy and splenomegaly are commonly noted also. Pathognomonic 'Koplik spots' on the buccal mucosa are rare. The measles rash usually appears from 3 to 5 d after the onset of clinical signs. The rash often is first noticed on the head, especially the face, and rapidly spreads down the neck, trunk, and extremities over the next several days. In the late stages, the rash darkens, the fever decreases, and systemic manifestations resolve. The rash fades in the same top-down sequence as it appeared and may be associated with desquamation.[12-14,47,50] In addition, MV induces a transient yet profound immunosuppression that can last for weeks to months,[23,24,39,40,53] causing dysfunction of both the humoral (antibody) and cell-mediated immune systems for as long as 6 mo, resulting in increased susceptibility to pneumonia, which is the most common cause of death associated with measles infection,[22] as well as enteropathy, abortion, encephalitis, and even as a direct cause of death.[23,43,49,53] Stressed or immunosuppressed animals are even more likely to experience severe opportunistic sequelae, likely from disruption of the mucosal barrier,[39,40,43] and transient immunosuppression can interfere with delayed-type hypersensitivity reactions, such as skin testing for *Mycobacterium tuberculosis*,[15,32,75,79,83] further complicating preventative health measures.

Due to the rapid exhaustion of susceptible hosts, MV cannot be maintained below a minimum density threshold.[48] Wild NHP typically do not live in populations that are sufficiently large and dense to maintain a transmission cycle.[40,59] Herd immunity exceeding 90% to 94% is essential to prevent transmission.[30,34,55,85] MV is endemic only in high-density human populations, and humans are the only natural reservoir host.[18,22,59] Therefore,

exposure to humans remains the major risk factor for measles infection in NHP.[43] Human-to-NHP transmission of MV has been reported to produce marked morbidity and mortality in NHP populations.[43] Historically, measles was a common infectious disease in NHP, and numerous outbreaks, involving several species, have been reported in the past.[43,67,85] New World NHP species are especially susceptible to MV; they may present with respiratory or gastrointestinal symptoms and may develop a more severe form of disease with increased mortality.[2,27,45,46]

Prior to the introduction of the measles vaccine in humans, measles was nearly a universal disease,[58] and as many as 50% of all childhood deaths from infectious disease were associated with MV.[53] Safe and effective attenuated modified live measles vaccines, first introduced in 1963, have led to a substantial reduction in morbidity and mortality. In humans, these vaccines are 90% to 95% effective and provide protection for more than 20 y.[22] However, research shows that declines in measles vaccination rates are associated with resurgences of clinical cases.[30,34,53,63] These outbreaks pose a renewed health risk for NHP having contact with humans.

Effective occupational health and vaccination programs for animal care staff, donning personal protective equipment (for example, clothing, gloves, masks, and face shields), and restricting human access have limited and reduced MV exposure at most NHP facilities. However, immunity to measles is not all or nothing, but rather a continuum of clinical conditions. Humans with subclinical infections and suboptimal immunity may serve as a source of virus introduction.[16,84] In addition, focusing only on occupational and vaccination programs for the animal care staff may result in large, susceptible NHP populations.[19,71] The potential consequences of MV infection and disease in NHP can be devastating and far-reaching and include health, research, and occupational effects, as well as financial implications. Therefore, prevention of MV NHP infection and transmission is contingent on concerted quarantine practices and animal vaccination protocols.[75] MV is a vaccine-preventable disease in both humans and NHP.

We and others have evaluated various measles vaccines for use in NHP.[18,85] Although formulations previously investigated have been proven to provide adequate immunity to MV, they are impractical to implement in large-scale NHP settings for various reasons, including feasibility, availability, and financial considerations. In addition, for achieving the best immunity while still providing optimal individual and herd immunity, a 2-dose regime is recommended, which would be more economical if half or a quarter of the manufacturer's recommended dose were used. Moreover, a reduced dose volume could have the additional advantage of reducing potential local muscle damage and pain,[10,37] furthering the animal welfare benefits.

After previously investigating all of the commercially available measles vaccines currently on the market, we have found the canine distemper–measles vaccine (CDMV) formulation to be a safe, efficacious, and economical measles vaccine for use in NHP, as of the time when the current study was conducted.[18,85] In addition, the renewed availability of CDMV, as well as the unavailability of alternatives, were factors in initiating the current dose study. Therefore, we hypothesized that although the full dose of CDMV recommended by the manufacturer (1.0 mL IM) will be the most effective and yield the highest average measles antibody titer, smaller doses (0.25 or 0.5 mL IM) will also have high average titers and prove to provide adequate immunity for clinical and research use. The objective of the current study was to determine the minimal appropriate dose of CDMV that can be administered to NHP to promote and enhance animal welfare as well as to potentially save resources for reallocation to other aspects of the research and facility. Therefore, we performed a modified dose volume trial of CDMV in juvenile rhesus macaques.

Materials and Methods

Animals. The 65 animals (full dose group, $n = 22$; half dose, $n = 21$; quarter dose, $n = 22$) used in this study were previously unvaccinated, juvenile rhesus macaques (*Macaca mulatta*) of both sexes (age, 11 to 17 mo) that were housed outdoors in half-acre breeding corrals composed of large multimale, multifemale social groups of all ages at the California National Primate Research Center (University of California, Davis, Davis, CA). Animals were fed chow twice daily (LabDiet Monkey Diet 5047, Purina Laboratory, St Louis, MO), offered water freechoice through automatic watering devices, supplemented with fruits and vegetables biweekly, and provided with speciesappropriate environmental enrichment, manipulanda, and foraging opportunities. Daily health checks were performed by trained personnel according to standard operating procedures. This study was approved by the IACUC of the University of California, Davis. Animals were maintained in accordance with the USDA Animal Welfare Act and Regulations and the *Guide for the Care and Use of Laboratory Animals*.[4,5,42] The animal care and use program of the University of California, Davis, is fully AAALAC-accredited, is USDA-registered, and maintains a Public Health Services Assurance.[57]

Study design and sample collection. For this study, all unvaccinated juveniles older than 6 mo undergoing routine biannual health screening in 3 separate corrals were selected and assigned to individual groups to receive 1 of 3 CDMV vaccine regimens as follows: the juveniles from the first corral received the fulldose (1.0 mL IM) vaccination routinely used at the facility, those from the second corral received the half dose (0.5 mL IM), and those from the third corral received the quarter dose (0.25 mL IM). Each corral was vaccinated with a separate dose to reduce any potential, although unlikely, for viral shedding that might affect the results and to decrease variability. All subjects received a physical examination at the time of vaccination, and 1 mL whole blood was collected from all subjects for assessments of measles and neutralizing antibody titers at 0, 6, and 12 mo after vaccination. The blood was allowed to clot at ambient temperature to prevent hemolysis, which can potentially interfere with test results. The clotted blood was centrifuged at $800 \times g$ for 15 min within 6 h of collection and the serum was separated from the clot. Serum samples were stored at –70 °C prior to analysis.

Vaccine. The measles vaccine used in this study was Vanguard DM (Zoetis, Parsippany, NJ), as previously investigated.[18] It is an attenuated modified live bivalent vaccine in which the MV derives from the Enders attenuated Edmonston strain; this vaccine is propagated in chick embryo cell culture, has a 10-fold higher MV titer per dose than Attenuvax (Merck, Kenilworth, NJ),[52] and is administered to healthy puppies 6 to 12 wk in age to aid in the prevention of canine distemper disease. The MV component is designed to provide puppies with temporary cross-protection from canine distemper virus regardless of maternal antibody levels.[77] The safety and efficacy of this vaccine was previously validated in rhesus macaques at the manufacturer's recommended dose of 1.0 mL IM[18] and is currently in use at the facility. The freeze-dried vaccine was reconstituted according to the manufacturer's recommendations by using the sterile diluent provided and aseptic technique and was stored at 2 to 7 °C until administered.[88]

Measles antibody. Total IgG antibody, reported as the highest dilution of test serum showing antibodies against measles, was determined by using a multiplex microbead immunoassay (Luminex 100/200 system), with microbead-coupled recombinant measles nucleocapsid antigen and controls (Charles River Labs, Wilmington, MA). The instrument is an analyzer that uses the principles of flow cytometry to simultaneously measure multiple antibody–antigen analytes in a single well of a microtiter plate. For this protocol, 2-fold dilutions (starting at 1:50) of individual samples were made to determine the titer. The dilutions were added to antigen-coated beads diluted in Prionex (Millipore Sigma, Burlington, MA)–PBS–BSA; 50 µL each of the bead solution and diluted sample were added to a filter plate and left to mix on a shaker for 1 h at room temperature. After 1 h, the wells were washed and subsequently incubated on the shaker for 30 min with 100 µL of biotinylated goat antihuman antibodies diluted in BSA–PBS buffer solution. Next, the wells were washed with buffer before the addition of 100 µL of streptavidin–R-phycoerythrin in BSA–PBS and incubation on the shaker for 30 min. Finally, the wells were washed once more to remove unbound material, after which the beads were analyzed spectrophotometrically by using 2 lasers which identify the antibody–antigen complexes bound to the uniquely dyed xMAP microspheres. Digital signal processors and the xPONENT software (Millipore Sigma) were used to acquire and analyze the data. By measuring the spectral properties of the beads, the median fluorescence index for each antigen was determined. The median fluorescence index of the sample was compared with a positive–negative cutoff value of 3000 median fluorescent units to determine the presence of antibody.

Neutralization titers. The samples were tested for neutralizing antibodies in a plaque reduction–microneutralization assay as previously described.[85] Briefly, 50 TCID units of MVvac2GPF, a molecular clone of the Moraten vaccine strain expressing green fluorescent protein,[38,85] were incubated with 4-fold dilutions of the test serum, starting at 1:20. The neutralization media was DME with 2% FCS and antibiotics, and the cell line was low-passage (less than 6 mo) Vero cells.[3] Rhesus measles immune globulin was used as the positive control, and media in place of test serum was used as the negative control. After an incubation period of 3 d, the plates were examined for green plaques under a UV microscope at 10× magnification, and each well was marked as either positive (green fluorescence) or negative for virus. Any fluorescence at all was considered positive. Data were analyzed by using the Reed Muench calculation template, which measured a reciprocal titer for each sample.[65]

Statistical analysis. Statistical analysis was performed in JMP version 14 (SAS Institute, Cary, NC). Comparisons between the geometric mean antibody titers of the various doses at each time point for each assay were evaluated by ANOVA followed by Tukey–Kramer post hoc analysis.[18,41] Results were considered statistically significant when they had a P value less than 0.05.

Results

Measles antibody. The median, mode, and range of the IgG binding antibody titers for rhesus macaques comprising each CDMV dose group (full, half, quarter) at 6 and 12 mo after vaccination are shown in Tables 1 and 2. From the 6-mo stage to the 12-mo stage, the average titer for the full, half, and quarter doses decreased by 2.06 (84%), 2.45 (82%), and 3.03, respectively (88%; Tables 1 and 2). Our data suggest that total IgG binding antibody titers against measles at 6 and 12 mo after vaccination were measurable and decreased longitudinally in all 3 vaccine regimens (Figure 1 A). In addition, we found no statistically

Table 1. Binding antibody titers, reported as the highest dilution of test serum showing antibodies against measles, for all 3 dose groups at 6 mo after vaccination

	Median titer	Mode titer	Titer range	% negative (no antibody)
Full dose	48	32	16–512	0%
Half dose	128	32	8–256	0%
Quarter dose	32	32	4–512	0%

Data regarding titers are given as reciprocal values.

Table 2. Binding antibody titers, reported as the highest dilution of test serum showing antibodies against measles, for all 3 dose groups at 12 mo after vaccination

	Median titer	Mode titer	Titer range	% negative (no antibody)
Full dose	16	8	8–64	0%
Half dose	16	8	8–64	0%
Quarter dose	8	8	8–64	0%

Data regarding titers are given as reciprocal values.

significant differences in the measured binding antibody titer between doses at 6 mo. However, at 12 mo after vaccination, there was a marginally significant (P = 0.05) difference between the titers for the half and quarter doses but not between the half and full doses of CDMV (Figure 1 B).

Neutralizing antibody. Tables 3 and 4 summarize the 6- and 12-mo post-vaccination neutralizing antibody titers for the full-, half-, and quarter-dose groups. From the 6-mo stage to the 12-mo stage, the average titer for the full, half, and quarter doses decreased by 0.69 (38%), 1.39 (62%), and 1.38 (61%), respectively (Tables 2 A and B). Our data suggest that although neutralizing titers decreased over time after vaccination (Figure 2 A), similarly to the IgG antibody levels, no statistically significant differences between the 3 vaccine regimens were found, except for a marginally lower (P = 0.06) titer in the half-dose group compared with the quarter-dose group only at 12 mo after vaccination (Figure 2 B). These results indicate that, at both 6 and 12 mo, a full dose of CDMV has a similar effect to the half dose but not the quarter dose in rhesus macaques.

Discussion

NHP have contributed a vast amount to biomedical research by serving as an important preclinical and translational research model.[28,29,35,36,72] In 2010, approximately 70,000 NHP were housed in United States research facilities for biomedical research.[44] In particular, NHP are a valuable animal model for the study of MV from pathogenesis to long-term immunologic memory and for the development of new vaccines.[23,87] MV continues to kill more than 89,000 human infants and children each year because currently licensed vaccines cannot be administered in infants younger than 9 mo, despite the vaccine-preventable nature of the disease in adult populations.[20]

MV is highly infectious for many species of NHP. Despite restricted visitation policies, use of personal protective equipment for staff handling animals, and maintenance of closed breeding colonies, the potential for humans with suboptimal immunity and subclinical infections to serve as sources of virus introduction into susceptible NHP colonies remains a possibility, especially with current MV vaccination in most human populations below the threshold level required for herd immunity. In
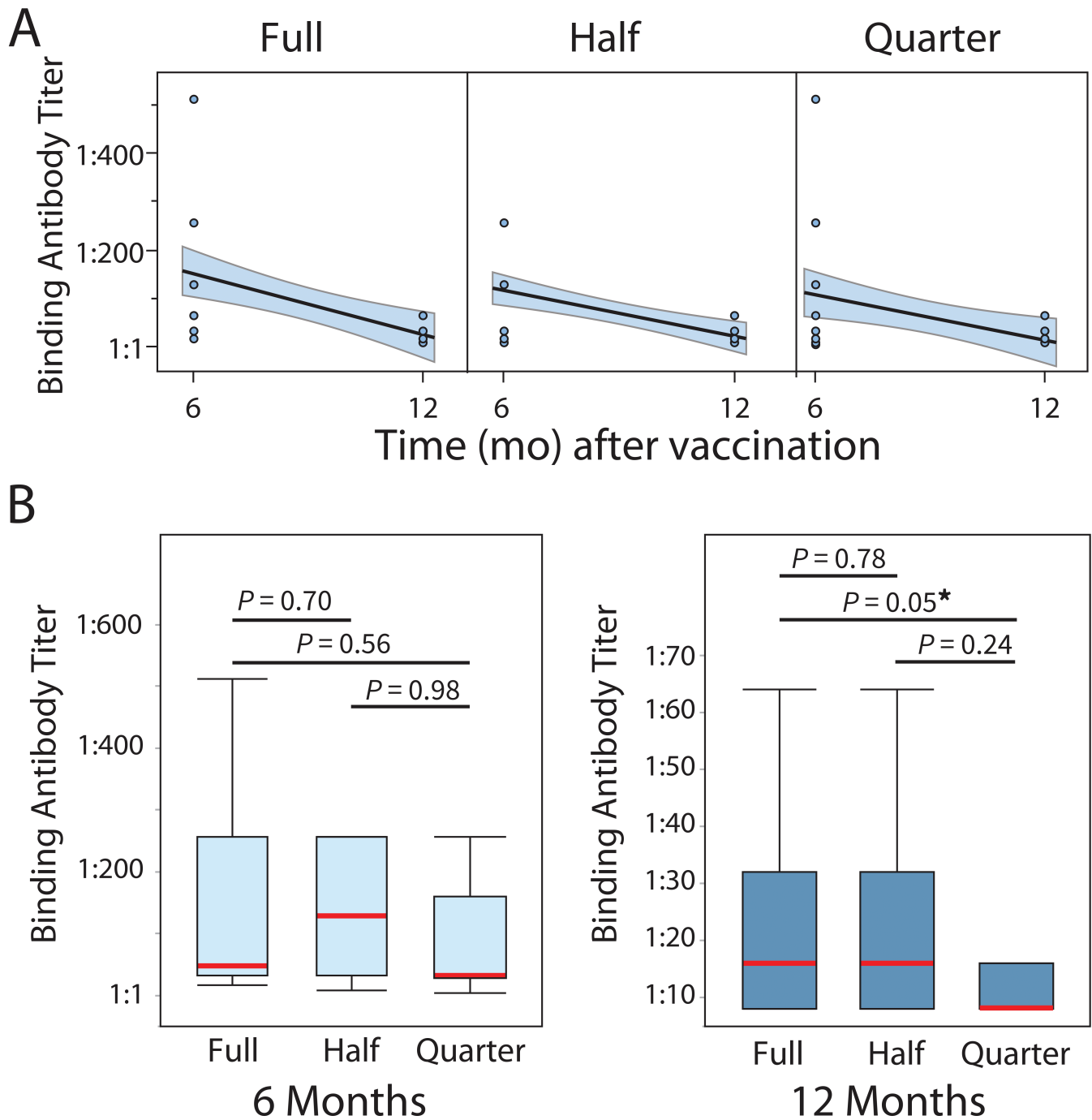
Figure 1. (A) Line of fit for measles binding antibody titers, reported as the highest dilution of test serum showing antibodies against measles, with 95% CI (shaded region). A longitudinal downward trend is observed for all doses. (B) Box plots of post-vaccination antibody dilution measurements (6 and 12 mo after vaccination) suggest no difference between groups in the binding antibody titers at 6 mo after vaccination. However, animals in the quarter-dose group showed slightly lower binding antibody titer (marginally significant, $P = 0.05$) at 12 mo after vaccination. Red lines illustrate the medians, whiskers depict the minimal and maximal values, and the bands are the 1st and 2nd quartiles. *, $P = 0.05$.

addition, there are potential zoonotic concerns regarding NHP-to-human transmission. This underscores the importance of measles vaccination programs in biomedical research facilities housing NHP, as previously recommended.[18,85]

Vaccination is the most effective strategy against morbilliviruses.[24] The latest attenuated measles vaccines are among the safest in use worldwide today.[31] Local reactions at the site of injection are negligible. The main reported reaction is a mild, measles-like syndrome, which occurs in 2% to 30% of humans at approximately 1 wk after vaccination.[31]

In addition, morbilliviruses have recently been shown to induce cross-protection within genera.[11] Cross-protection against canine distemper virus from MV vaccination has been demonstrated previously in dogs, macaques, and mice.[7,9,25,77] However, some concerns exist regarding potential attenuated replication of canine distemper virus due to CDMV in NHP, especially given that NHP have been shown to be susceptible to infection by canine distemper virus through both experimental vaccination as well as natural occurrence.[17,21,24,25,56,64,70,73,78,86] Although no evidence supporting this

Table 3. Neutralizing antibody titers, reported as the highest dilution of test serum showing antibodies against measles, for all 3 dose groups at 6 mo after vaccination

	Median titer	Mode titer	Titer range	% negative (no antibody)
Full dose	40	40	<20–202	9.0%
Half dose	80	40	20–202	0%
Quarter dose	40	40	<20–160	18.2%

Data regarding titers are given as reciprocal values.

Table 4. Neutralizing antibody titers, reported as the highest dilution of test serum showing antibodies against measles, for all 3 dose groups at 12 mo after vaccination

	Median titer	Mode titer	Titer range	% negative (no antibody)
Full dose	30	40	<20–120	22.7%
Half dose	20	20	<20–120	23.8%
Quarter dose	20	20	<20–70	27.2%

Data regarding titers are given as reciprocal values.

concern exists in the literature, the principal danger with an attenuated vaccine is that the organism, because it is still alive, can sometimes recover its virulence and cause disease in vaccinees.[62] However, MV vaccination has been shown to induce partial protection against canine distemper virus in macaques,[25] which should further reduce this concern. In addition, MV vaccination may provide general protection against all infectious diseases and protect polymicrobial herd immunity for as long as 3 y,[1,53] which may be particularly helpful in high-density, outdoor NHP colonies, where diseases can spread readily. Moreover, due to their pleomorphic nature and long-lasting stimulation of the immune system, morbilliviruses are now being considered as vaccine vectors for protection against other infectious disease agents, including oncolytic viruses.[11]

The most commonly used, commercially available measles vaccines investigated for use in NHP include Attenuvax (Merck), M-Vac Vaccine (Serum Institute of India, Pune, India) and CDMV.[18,85] In addition, a human measles–mumps–rubella vaccine (Merck) is available and includes an Attenuvax component, but its polyvalent formulation results in increased costs and vaccination against additional agents, making it less desirable for use in large research NHP colonies; consequently this vaccine was not investigated in previous studies. Since the completion of the CDMV safety and efficacy study in 1996, the facility has adopted the use of CDMV with the only hiatus being from 2007 to 2013 resulting from unavailability of the vaccine. Thus far, more than 9100 rhesus macaques, 450 long-tailed macaques (*Macaca fasicularis*), 60 titi monkeys (*Callicebus moloch*), and 6 squirrel monkeys (*Saimiri* spp.) have been vaccinated at our center. As in the original study, animals continue to be observed closely both clinically and pathologically by board-certified laboratory animal veterinarians and pathologists, respectively, for any adverse effects that could possibly be linked to morbillivirus infection, and none have been noted. Local injection site reactions have been nonexistent. In addition, we have received anecdotal reports that many other NHP facilities have been and still are using CDMV in macaques and other NHP species without any reported adverse effects.

In the current study, the mean, median, and mode titers for all 3 doses were within 1 dilution of each other. A single-dilution difference is within the typical normal range of testing variation for these and other immunoassays; thus, at least a 4-fold (2-dilution) difference is typically required to report seroconversion or a change in titer.[33,85] As previously shown, the production of neutralizing antibodies against MV is considered protective in rhesus macaques,[18] such that it formed the basis of comparison. Although there were no statistically significant differences between the neutralizing titers for all 3 groups at 6 mo after vaccination, there was a marginally significant difference between the full and quarter doses (Figures 1 B and 2 B), and there was a higher percentage of animals with negative antibody tests in the quarter-dose group. The full- and half-dose groups had similar titers at 12 mo, but the half-dose group had fewer subjects with no detectable antibody at 6 mo. In addition, we noted an increased range of titers within the quarter-dose group at 6 mo, perhaps suggesting that interindividual variation in immune response becomes a factor with the quarter dose of CDMV, making it potentially less reliable.

In humans, especially in developing countries, the optimal MV vaccine strategy is a 2-dose approach, with the first dose as early as 6 mo and the second ranging from 12 mo to school entry, depending on the national immunization program standards, because booster vaccination has been shown to provide increased immunity in subjects with low to no detectable antibody responses.[8,68,69,76] In light of these considerations, we selected as subjects previously unvaccinated, juvenile (age, 11 to 17 mo) rhesus macaques of both sexes housed outdoors in half-acre breeding corrals; it was beyond the scope of the current study to evaluate the optimal timing of booster measles vaccination. At our facility, we elect not to vaccinate juveniles until they are at least 6 mo of age, to sufficiently reduce documented interference and inhibition of the immune response by maternal antibody.[1,51]

The implications of our current results are substantial. The potential cost associated with the implementation and maintenance of a measles vaccination program can be a deterrent to vaccination. As compared with the manufacturer's recommended 1.0 mL dose, using a half dose results in a 50% cost reduction yet provides comparable immunity. With rising management costs, NHP facilities must strive to decrease costs without diminishing animal welfare. This 50% cost reduction may provide sufficient incentive to implement and maintain a new NHP MV vaccination program or to modify an existing one, for example, by ensuring a 2-dose approach as is standard in humans. Alternately, the monetary savings from half-dose vaccination with CDMV could be used to improve another aspect of animal welfare. Moreover, the 50% dose volume reduction has the additional advantage of reducing potential local muscle damage when administered to young and small NHP species, given that the degree of muscle trauma and subsequent pain and inevitable rise in creatinine protein kinase are related to the volume of injectate.[10,37] Lastly, live-attenuated virus vaccines have been shown to undergo a dose-sparing effect due to the subsequent replication of the vaccine vector in vivo, which is illustrated by our results and offers a powerful practical advantage when considering a measles vaccination protocol in large NHP colonies.[66,81] Therefore, the results of the current study indicate that using a reduced dose of measles vaccine is safe, efficacious, and more affordable than previously thought, allowing facilities to support the protection of these valuable animal model resources via a measles vaccination protocol.

In conclusion, vaccination remains the most effective intervention strategy to combat morbillivirus infections, and we
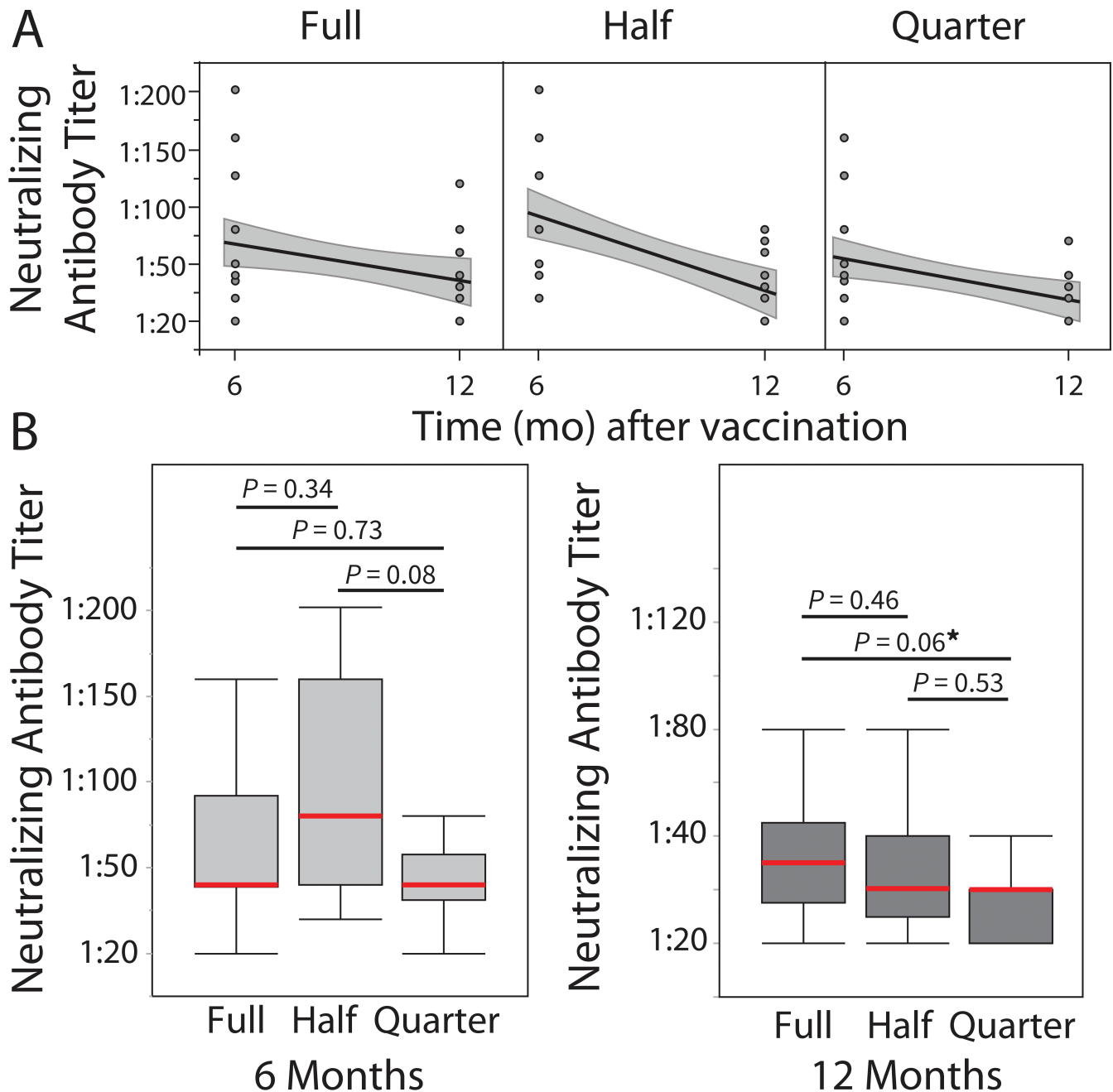
Figure 2. (A) Line of fit for neutralizing titers, reported as the highest dilution of test serum showing antibodies against measles, with 95% CI (shaded region). Similar to results from binding antibody measurement, a longitudinal downward trend is observed for all doses. (B) Box plots of post-vaccination neutralizing dilution titers (6 and 12 mo after vaccination) suggests no difference between groups in the neutralizing antibody titers at 6 mo after vaccination. However, animals in the quarter-dose group showed slightly lower albeit nonsignificant ($P = 0.06$) neutralizing titers at 12 mo after vaccination. Red lines illustrate the medians, whiskers depict the minimal and maximal values, and the bands are the 1st and 2nd quartiles. *, $P = 0.05$.

can now provide adequate protection against measles by using half of the manufacturer-recommended dose of CDMV yet still achieve full-dose immunity to MV, because the production of neutralizing antibodies against MV in rhesus has previously been shown to be protective.[18] In addition, using this decreased dose may lead to a 50% vaccine cost reduction, and savings might be an economic incentive to implement or augment a measles booster program to prevent disease outbreak or, with the financial savings, to enhance animal welfare in other ways.

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