**PLASMODIUM COATNEYI IN THE RHESUS MONKEY (MACACA MULATTA) AS A MODEL OF MALARIA IN PREGNANCY**

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Abstract. Pregnant women with *Plasmodium falciparum* infection are at increased risk for complications such as anemia and cerebral malaria. In addition, the infants of these women suffer intratuberine growth retardation (IUGR), low birth weight (LBW), congenital infection, and high infant mortality. Although much has been learned from studies of malaria during human pregnancy, progress has been limited by the lack of a suitable animal model. Nonhuman primates are of particular interest because, other than the armadillo, they are the only animals with a discoidal, villous, hemochorial placenta like that of humans. We have established a model of malaria during human pregnancy by inoculating pregnant rhesus monkeys (*Macaca mulatta*) with *Plasmodium coatneyi* (a sequestering parasite) during the first trimester. In our initial experiment, four monkeys were inoculated with a fresh inoculum containing 10⁶ viable parasites from an infected donor monkey. All four monkeys became parasitemic seven days postinoculation (PI) and three monkeys aborted 7–10 days PI coincident with high peak parasitemias (41,088–374,325 parasites/mm³). Although abortion is one of the outcomes observed in *Plasmodium*-infected women, the intent of this study was to examine the effects of *Plasmodium* infection throughout gestation. Since the rapid onset of high parasitemia may have been responsible for the abortions, a decision was made to reduce the size of the effective inoculum. Six additional pregnant monkeys were inoculated with a frozen isolate taken from the same donor containing 10⁶ parasites. These six animals became parasitemic seven days postinoculation (PI) and, along with monkey E412, carried their infants to term. These seven infants weighed significantly less at term than the infants of uninfected mothers (*P* = 0.0355). Symmetrical IUGR was detected by ultrasound in one fetus with an LBW of 334 g. Another LBW infant (300 g) had asymmetrical growth retardation, which has been associated with uteroplacental insufficiency and was consistent with the lower placental weights found in infected dams compared with controls (*P* = 0.0455). The infant with symmetric IUGR died at five days of age, while the other is alive but congenitally infected. The IUGR, LBW, congenital infection, postnatal infant mortality, and early abortions observed in these animals suggest that *P. coatneyi* in pregnant rhesus monkeys is a valid model of malaria in human pregnancy. This model should provide the opportunity to study questions about malaria in pregnancy that have been difficult to study in humans.

Pregnant women are more likely to develop symptomatic malaria than their nonpregnant counterparts.¹–⁶ Malaria during pregnancy leads to many complications in women and their infants including anemia, high fever, hypoglycemia, pulmonary edema, renal failure, cerebral malaria, abortion, preterm delivery, intratuberine growth retardation (IUGR), low birth weight (LBW), congenital infection, and increased fetal and maternal mortality.⁷–⁹ The risks of symptomatic maternal malaria, maternal complications during pregnancy, and fetal compromise are influenced by the level of malaria endemicity (greater in nonendemic areas), by parity (primigravidas and secundigravidas are more susceptible than multigravidas),⁴ and gestational age (prevalence of infection and parasite density are highest in the first half of the pregnancy).¹⁰,¹¹ Although a number of investigators have studied malaria during human pregnancy, the results are often difficult to interpret because of the confounding variables inherent in these studies¹² such as the mother’s health, imprecise estimates of gestational age, tissue availability, patient compliance, socioeconomic conditions, and moral, ethical, and financial constraints. As a result, we are left with many questions that are difficult or impossible to address in human studies.

The mechanisms responsible for increased susceptibility to malaria during pregnancy have not been elucidated.¹³ The reasons for the increased susceptibility of primigravidas and secundigravidas remain unknown. Neither the pathophysiological mechanisms responsible for LBW infants nor the role of placental malaria in LBW and other adverse fetal outcomes has been defined. The incidence of congenital infection and the mechanism of transplacental transmission have not been established. In addition, the effects of maternal anemia are poorly understood.

Current animal models of malaria in pregnancy are based on pregnant mice and rats with *Plasmodium berghei*.¹⁴–¹⁹ Although these rodent models are well-established, their relevance to malaria in human pregnancy is questionable because of the many differences between rodent and human pregnancies. Rats cannot be used as models of the human maternal-fetoplacental unit because progesterone is produced by the corpus luteum of the ovary in rats, rather than the placenta as in humans and because the rodent placenta is labyrinthine hemochorial, rather than villous hemochorial, like the human placenta.²⁰,²¹ The usefulness of rodents in the study of congenital malaria is also questionable.²²

An effective disease model should closely mimic the pathogenesis of malaria in pregnant women. Nonhuman primates are ideal candidates because they are susceptible to many species of *Plasmodium* and have humoral and cellular responses similar to those of humans.⁵ In addition to macaques, great apes and baboons have discord villous hemochorial placentas similar to those of humans.²³ However, neither great apes nor baboons are practical candidates for a model of malaria in pregnancy. Great apes cannot be used because they are an endangered species. Although baboons
are used in reproductive studies, they are expensive to house because of their size. In addition, baboon malaria models are less well-developed than macaque malaria models. Thus, of the three primates with discord villous hemochorial placentas, only macaques are readily available and have been used frequently in malaria research. Macaques used in malaria research include pig-tailed monkeys (Macaca nemestrina), cynomolgus or long-tailed monkeys (M. fascicularis), and rhesus monkeys (M. mulatta). Of these, the rhesus monkey is the best characterized, most widely used, and most available. In addition, the rhesus placenta has been studied extensively. Thus, the rhesus monkey was a logical choice for these studies.

The macaque placenta is anatomically similar to that of humans and produces progesterone as does the human placenta. Organogenesis in macaques is also comparable with humans. The immune responses of pregnant rhesus monkeys infected with simian immunodeficiency virus (SIV) are similar to those of pregnant women infected with human immunodeficiency virus. To our knowledge, there are only two previous reports on malaria during pregnancy in nonhuman primates. In 1934, Das Gupta inoculated one pregnant rhesus monkey with Plasmodium cynomolgi and was killed 17 days postinfection (PI). Although the placenta was heavily parasitized, the fetus did not become congenitally infected. Saxena and others infected 15 pregnant rhesus monkeys at gestational day (GD) 56–70 with P. cynomolgi in 1988. Five animals aborted and one died with the infant in utero. Results of scanning electron microscopic examinations of placental biopsies obtained at the time of peak parasitemia were reported. No results were reported concerning the remainder of pregnancy or fetal outcome.

Plasmodium coatneyi was discovered by Eyles in Anopheles hackeri. One year later in 1962, P. coatneyi was isolated by Eyles from M. fascicularis, one of its natural vertebrate hosts, in which it produces a mild persistent infection. However, the best experimental vertebrate host has been the rhesus monkey (M. mulatta), in which blood-induced infections may be explosive with mortalities of 33% in intact monkeys and up to 100% in splenectomized monkeys. A rhesus monkey model of cerebral malaria has been described recently with P. coatneyi, and P. coatneyi-infected rhesus monkeys have been proposed as models for the development of malaria vaccines. Plasmodium coatneyi was the parasite of choice for these studies because it sequesters in the cerebral capillaries of rhesus monkeys via endothelial cell receptors.

The goal of this study was to develop a model of malaria during pregnancy that would closely approximate the human condition. This was accomplished by inoculating rhesus monkeys with P. coatneyi during the first trimester of pregnancy.

MATERIALS AND METHODS

General study design. Ten pregnant, malaria-naive, Indian-origin, rhesus monkeys (M. mulatta) were inoculated intravenously (IV) with P. coatneyi during the first trimester between GDs 30 and 54. These 10 females had been born at the Tulane Regional Primate Research Center (TRPRC) and therefore had no previous exposure to malaria parasites. Three dams were primigravidas (M181, L226, and L422), one was a secundigravida (L414), and six were multigravidas (D673, E412, J653, L412, L434, and M488).

In the initial experiment, fresh blood from a P. coatneyi-infected rhesus monkey with a parasitemia of 7% was inoculated into four monkeys (M181, M488, L412, and E412). Three of four monkeys aborted at 7–10 days PI coincident with high peak parasitemias (41,088–374,325 parasites/mm³). Although dam E412 also had an early high peak parasitemia (255,000 parasites/mm³), she carried her infant to term. The size of our effective inoculum was reduced by using frozen blood from the same animal and six additional monkeys were inoculated (D673, J653, L226, L414, L422, and L434). All six became parasitemic by 14 days PI, and carried their infants to term.

The 10 pregnancies resulted from natural mating in outdoor breeding colonies. Rhesus monkey gestation averages 165 days (±10 days). Thus, the first trimester in the rhesus monkey is from GD 0 to 55, equivalent to GD 0–90 in the human; the second trimester is from GD 56 to 110, equivalent to GD 91–180 in the human; and the third trimester is from GD 111 to 165, equivalent to GD 181–270 in the human. Infants were delivered by cesarean section near term (GD 155) to ensure collection of the placenta, which is otherwise consumed by the dam. Infants were weighed, measured, and returned to their dams within 24 hr of delivery; infants rejected by their mothers were nursery-reared.

Animal housing and clinical evaluations. Following ultrasonic examination to determine the gestational age of the conceptus, 10 pregnant monkeys were removed from the breeding colony at the TRPRC. They were housed individually in accordance with the Guide for the Care and Use of Laboratory Animals, published by the U.S. Department of Health and Human Services, National Institutes of Health. Prior to examination each week, the dams were anesthetized with 10 mg/kg of ketamine hydrochloride given intramuscularly. Parameters monitored during these weekly examinations included weight, spleen size, and general physical condition. Diagnostic tests included complete blood counts (CBCs), thick and thin blood smears for parasite counts, and ultrasound examinations. Appetite, stool consistency, and behavior were monitored daily. Previous ultrasonographic evaluations using ketamine hydrochloride weekly during pregnancy (50 control pregnancies) resulted in no adverse effects on the fetus. All fetal growth parameters were within the normal ranges throughout gestation (Davison B, unpublished data). Other studies evaluating the effects of SIV on pregnancy outcome had normal fetal growth patterns with the weekly use of ketamine hydrochloride.

Parasite inoculations. After anesthesia with ketamine hydrochloride (10 mg/kg), baseline blood samples were drawn from the femoral vein for a CBC and thick and thin blood smears. Four monkeys (M181, M488, L412, and E412) received 1 ml of fresh blood IV in the saphenous vein containing 10⁸ viable parasites from an infected donor monkey. Three of the four animals aborted their infants 7–14 days after inoculation (M181, M488, and L412). Because we were interested in studying the long-term effects of malaria during pregnancy (not short-term abortions), we inoculated six additional monkeys (D673, J653, L226, L414, L422, and
stained blood smears from venous blood were used to mon-
venipuncture of the femoral vein. Thick and thin Giemsa-
their infants to term. These seven dams and their infants
E412 from the initial experiment, these six monkeys carried
became parasitemic by day 12 PI. In addition to monkey
6.0/7.5 MHz scanhead, linked to a computerized database
collection that is known to be ac-
Parasite enumeration. Blood was collected weekly by
thick and thin Giemsa-
parasites were enumerated by counting
number of parasites in 10 microscopic fields (1,000×
magnification) and recorded as a percent of red blood cells
(RBCs) parasitized. The RBC count on the same day was
used to calculate the number of parasites/mm³ of blood.
Ultrasonographic examinations. Ultrasonographic ex-
aminations were performed using a real-time sector scanner
(model Sonoline SI-200; Siemens, Schaumburg, IL) with a
6.0/7.5 MHz scanhead, linked to a computerized database
containing established control parameters for the rhesus
monkey during gestation.⁴⁰ Gestational age of the fetus and
the delivery dates were calculated during early gestation
based on ultrasound measurements that are known to be ac-
currate within ± five days of the delivery date.⁴¹ The growth
parameters assessed during the early stages of gestation in-
cluded the greatest length of the embryo, which is the dis-
tance from the crown of the head to the base of the tail, and
the mean dimension of the developing gestational sac.⁴¹ Measurements collected later in gestation included the bi-
parietal diameter (BPD), which is the diameter of the head
at the level of the thalami, and femur length (FL). General
anatomic configuration of the developing fetus, head circum-
fERENCE, abdominal circumference, fetal heart rate, and pla-
cental location were also assessed.
Definition of IUGR. There are two basic types of IUGR.⁴²
The most common is asymmetrical growth retardation or
head spared growth retardation; the second is symmetrical
growth retardation. Asymmetrical growth retardation may
result from uteroplacental insufficiency as often reported in
association with malaria in human studies although the ab-
solute mechanisms remain unknown. Uteroplacental insuf-
ciency has been described as one factor that may compro-
mise the nutritional status of the fetus. Asymmetrical growth
retardation may also result from a variety of maternal dis-
orders during pregnancy such as hypertension, diabetes mel-
itus, and the post-mature syndrome. In these growth-retard-
ed babies, the fetal liver is small and subcutaneous fat is
markedly reduced. In contrast, babies with symmetrical
growth retardation have a high incidence of congenital de-
fects. Their growth derangements are thought to result from
these congenital defects, to occur early in pregnancy, and to
cause reductions in body weight and organ size. Placentas
from infants with IUGR are typically small with extensive
thrombosis and infarction.
In this study IUGR was evaluated using the parameters
listed previously. The BPDs and FLs for each fetus were
compared with the standard curves established previously at
the California Regional Primate Research Center (CRPRC).

Symmetrical growth retardation was defined by BPD and FL
measurements falling below the lower end of the control
range.⁴⁰ In addition to the ultrasound measurements, the birth
weight, subcutaneous body fat, and overall clinical impres-
sion of the fetus, neonate, and placenta were used in making
a diagnosis of asymmetrical IUGR.

Cesarean section. Because rhesus monkey gestation av-
erages 165 days (± 10 days), elective cesarean sections were
performed near term (GD 155) to ensure the collection of
placental tissues and cord blood samples, and to avoid the
potential complicating effects of labor. Just before surgery,
blood samples were taken from the dam for a CBC and
parasite smears. Animals were pre-anesthetized with glyco-
pyrrolate followed by ketamine hydrochloride and isoflurane
gas. To avoid contamination with maternal blood, the uterus
was exteriorized and an incision was made in an avascular
area on the ventral surface near the cervix. The initial inci-
sion was then extended until the amniotic membrane bulged
from the incision. At this point an amniotic fluid sample was
obtained with a needle and syringe. After the infant was
removed, a blood sample was taken from the umbilical cord
before extraction of the placenta to avoid contamination with
maternal blood. The placenta was then extracted, weighed,
and placed in a sterile tray to obtain samples for freezing in
liquid nitrogen and for thick and thin placental blood smears.
Post-surgical recovery of the dams was uneventful.

Pathologic evaluations of dams and infants. Complete
necropsies and histopathologic examination of tissues were
performed on all dams and infants that died or were killed
during the project. Representative sections of all major tis-
ues were fixed in 10% neutral-buffered formalin, sectioned
at 5–6 μm, and stained with hematoxylin and eosin.

Controls. The placentas from 10 dams with infants that
were cesarean delivered near term (GD 155) in a previous
study at the TRPRC served as one set of controls for pla-
cental weight comparisons. Another set of controls, used to
compare weight changes in the dams during pregnancy, con-
sisted of eight individually caged age-matched pregnant fe-
nales that underwent weekly ultrasound examinations in an-
other study.

Three groups of infants that were cesarean delivered near
term (GD 155) and one group of vaginally delivered infants
were used as controls for birth weight and growth parame-
ters: 1) eight infants born to dams previously inoculated with
SIV during pregnancy, 2) eight infants that underwent man-
ipulation in utero for a gene therapy study, 3) 25 infants
delivered on GD 155 at the CRPRC, and 4) 57 vaginally
delivered infants from the TRPRC outdoor breeding colony.
The dams in groups 1 and 2 were manipulated in exactly the
same manner as the *Plasmodium*-infected dams: cage-
housed, anesthetized weekly with ketamine hydrochloride,
IV inoculated, weekly ultrasound examinations, weekly
blood collection, and cesarean delivery near term (GD 155).
The SIV group was included as another model of maternal
infection during pregnancy for comparison of fetal outcome.

Statistical analysis. The entire group of 10 *P. coatneyi-
infected monkeys was used in calculating the abortion rate.
For all other tests, statistical analysis was performed on the
seven dam/infant pairs that carried their offspring to term.
Although one monkey (E412) was inoculated with fresh in-
oculum and the other six were inoculated with frozen ino-
Figure 1. Highest parasitemia in a rhesus monkey model inoculated with Plasmodium coatneyi during the first trimester. Of the seven monkeys that carried their infants to term, dam E412 (multigravida) had the highest initial peak parasitemia (255,000 parasites/mm$^3$) late in the first trimester. However, parasite levels decreased early and there was no resurgent parasitemia during the third trimester. This monkey’s healthy infant (V443) is now 11 months old with normal growth parameters (see Table 3).

RESULTS

Timing, levels, and patterns of parasitemia and anemia. All 10 rhesus monkeys became parasitemic within 7–14 days PI. Three of the four monkeys inoculated with fresh material (M181, M488, and L412) aborted 7–10 days PI at the time of high peak parasitemias (41,088–374,325/mm$^3$). The other seven monkeys carried their pregnancies to term, including one inoculated with fresh material and six inoculated with frozen material. The rapid abortions resulting from our initial study prompted our decision to reduce the size of the effective inoculum. Although abortion is an important outcome that represents one of the many sequelae of Plasmodium infection during pregnancy, the goal of this study was to study the effects of malaria over time during pregnancy. By using a frozen inoculum we were able to achieve this goal.

Two patterns of peak parasitemia were observed in the seven monkeys that carried their pregnancies to term. High peak parasitemias of 20,000–255,000/mm$^3$ were observed in three monkeys (Figures 1 and 2), whereas low peak parasitemias of 7,800–10,000/mm$^3$ were observed in four monkeys (Figure 3). When comparing Figures 1, 2, and 3, the variation in the coordinates of the Y axis must be taken into account. Six of the seven monkeys that carried their infants to term had their peak parasitemias late in the first trimester or early in the second trimester (GDs 51–73); only one monkey peaked late in the second trimester (GD 92).

Contrary to expectation, high peak parasitemia occurred with approximately the same frequency in primigravida (2 of 3), secundigravida (1 of 1), and multigravidas (3 of 6). The significance of this finding is problematic due to the small group size used in this pilot study. An increased sample size may be required to demonstrate differences between groups.

Following the initial peak parasitemia that occurred after inoculation, an additional resurgence of between 7,800 and 10,000/mm$^3$ occurred during the third trimester in four of the seven animals that carried their infants to term. Two of these four animals had initial low peak parasitemias (8,820 and 9,800/mm$^3$) and two had initial high peak parasitemias (70,712 and 115,260/mm$^3$). The two dams with initial high peak parasitemias and third trimester resurgence produced infants with IUGR and the lowest birth weights. One dam was a primigravida whose infant died at five days of age; the other was a secundigravida whose infant is alive, but congenitally infected. The two dams with low initial peak parasitemias and third trimester resurgence produced one infant that died at four days of age (from a primigravida) and another who is alive and healthy (from a multigravida).
FIGURE 2. High parasitemia in two rhesus monkeys inoculated with *Plasmodium coatneyi* during the first trimester. Of the seven monkeys that carried their infants to term, two monkeys had high initial peak parasitemias of 70,000 and 115,000 parasites/mm³ during the second trimester with resurgent parasitemia (> 7,800 parasites/mm³) during the third trimester. The infant of monkey L226 (secundigravida) had intrauterine growth retardation and LBW (334 g) and died at five days of age. The infant of monkey L414 (primigravida) had the lowest birth weight of the seven infants (300 g) and is alive but congenitally infected with *P. coatneyi*.

FIGURE 3. Low parasitemia in four rhesus monkeys inoculated with *Plasmodium coatneyi* during the first trimester. Of the seven monkeys that carried their infants to term, four monkeys had low initial peak parasitemias (7,800–10,000 parasites/mm³) late in the first or early during the second trimester. One primigravida (L422) had a resurgence of parasites to > 7,800/mm³ during the third trimester and her infant died at four days of age. One multigravida (D673) also had a third trimester resurgence (> 7,800 parasites/mm³) and her healthy 11-month-old infant (V513) has normal growth parameters (see Table 3).
FIGURE 4. Red blood cell (RBC) counts and hemoglobin levels in rhesus monkeys inoculated with *Plasmodium coatneyi* during the first trimester. **Top**, a very similar pattern of anemia occurred in all seven *Plasmodium*-infected pregnant monkeys. The RBC counts decreased to their lowest levels of $3.0 - 3.38 \times 10^6$/mm$^3$ late during the second trimester (mean gestational day [GD] = 90). At delivery, monkeys D673, E412, and L414 had the most complete recovery from anemia (GD = 140–150) and their infants are still alive. The remaining infants, born to the four persistently anemic dams, died within one month of delivery. **Bottom**, hemoglobin levels decreased coincident with anemia and lowest levels ranged from 6.5 to 7.5 g/dL.

Thus, the mothers with high initial peak parasitemias and third trimester resurgent parasitemias $\geq 7,800$/mm$^3$ had infants with IUGR and the lowest birth weights.

Following their initial peak parasitemias, three of seven dams did not have resurgent parasitemias ($\geq 7,800$/mm$^3$) during the third trimester, although they remained persistently parasitemic throughout pregnancy. One of these three had the highest initial peak parasitemia of the seven (255,000 parasites/mm$^3$) shortly after infection. However, the parasitemia decreased rapidly and only low parasitemias ($< 3,000$/mm$^3$) occurred during the third trimester. This dam’s infant is alive and well. The other two dams had low initial peak parasitemias and infants that died within the first month after delivery, although only low parasite levels were detected during the third trimester. Although neither the initial peak parasitemia nor third trimester resurgence correlated...
with infant mortality, mortality was related to persistent anemia during the third trimester.

Anemia (RBC count \(< 4.5 \times 10^6 /\text{mm}^3\) occurred during the second trimester in all seven monkeys between GDs 66 and 101. The most severe anemias followed the peak parasitemias with RBC counts ranging from 3.0 to 3.38 \(\times 10^6 /\text{mm}^3\) (Figure 4). Hemoglobin levels decreased in parallel with the RBC counts and ranged from 6.5 to 7.5 g/dL (mean = 7.2 g/dL, normal = 11.2–13.7 g/dL) (Figure 4). In contrast, the lowest RBC count observed in a previous study of 30 normal rhesus pregnancies was 4.7 \(\times 10^6 /\text{mm}^3\) (Davison B, unpublished data).

Based on the individual variations observed after the lowest RBC count, the seven monkeys could be divided into two distinct groups based on their patterns of recovery from anemia. These two patterns are illustrated in Figure 4 and the clearest distinction is observed between GD 140 and 150 when the RBC counts of monkeys D673, E412, and L414 are separated from monkeys J653, L226, L422, and L434 by approximately one million RBCs. Monkeys D673, E412, and L414 recovered from anemia with steady increases in their RBC counts to \(\geq 4 \times 10^6 /\text{RBC/mm}^3\) by the day of delivery. The three infants from these dams are alive at 11 months of age. In contrast, the infants born to the four monkeys who did not recover from their anemia before delivery died at 4, 5, 18, and 33 days of age. These observations suggest that failure to recover from anemia after malaria during pregnancy may be an important risk factor for IUGR, LBW, failure to thrive, and early infant death.

Other clinical findings in the dams. Anorexia and weight loss occurred sporadically in the seven \(P.\) coatneyi-infected monkeys that carried their infants to term. In contrast, as shown in Table 1, the age-matched controls consistently gained weight during pregnancy (\(t = 5.93, \text{df} = 13, P = 0.00005\)). Both groups underwent weekly ultrasonographic examinations.

TABLE 1
Comparison of weight changes during pregnancy between \(P.\) plasmodium-infected dams and controls

<table>
<thead>
<tr>
<th>Plasmodium-infected</th>
<th>Weight gain/loss</th>
<th>Controls</th>
<th>Weight gain/loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>D673 13 (+) 400</td>
<td>G696 9 (+) 1,750</td>
<td>E412 7 (+) 1,650</td>
<td>J653 7 (+) 1,650</td>
</tr>
<tr>
<td>E412 12 (+) 400</td>
<td>H510 8 (+) 2,300</td>
<td>L226 7 (+) 300</td>
<td>L414 7 (+) 700</td>
</tr>
<tr>
<td>J653 13 (+) 400</td>
<td>I950 7 (+) 2,000</td>
<td>L422 7 (+) 100</td>
<td>L434 7 (+) 1,950</td>
</tr>
<tr>
<td>L226 7 (+) 300</td>
<td>I343 7 (+) 2,300</td>
<td>L414 7 (+) 1,750</td>
<td>L434 7 (+) 2,000</td>
</tr>
<tr>
<td>L414 7 (+) 400</td>
<td>J743 7 (+) 1,750</td>
<td>L426 7 (+) 100</td>
<td>L434 7 (+) 2,100</td>
</tr>
<tr>
<td>L422 9 (+) 100</td>
<td>J381 6 (+) 1,950</td>
<td>J381 6 (+) 1,950</td>
<td>J381 6 (+) 1,950</td>
</tr>
<tr>
<td>L434 8 (+) 100</td>
<td>N697 12 (+) 4,300</td>
<td>N697 12 (+) 4,300</td>
<td>N697 12 (+) 4,300</td>
</tr>
</tbody>
</table>

Mean = (+) 28.57 Mean = (+) 1,706.25
SD = (+/-) 423.14 SD = (+/-) 630.44

* Age is in years.
† Weight is in grams.
‡ The age-matched pregnant controls gained significantly more weight than the \(P.\) plasmodium-infected pregnant dams throughout gestation (\(P = 0.00005\)). Both groups underwent weekly ultrasonographic examinations.

Figure 5. Absolute lymphocyte counts (ct.) in seven rhesus monkeys inoculated with \(P.\) coatneyi during the first trimester. Following inoculation with \(P.\) coatneyi, absolute lymphocyte counts increased steadily throughout gestation and were 2–3 times higher than those observed in 30 normal control pregnancies.
FIGURE 6. Comparison of birth weights for infants born to Plasmodium-infected mothers and control infants born at the California Regional Primate Research Center. The malaria group has a lower average birth weight than the control group. However, the difference was not statistically significant and this may be attributed to genetic variation between the colonies at these two primate centers. Bars show the mean ± SD.

Fetal/infant outcome. Three of 10 pregnant dams inoculated during the first trimester aborted (GD 37 at 7 days PI; GD 43 at 8 days PI; GD 56 at 14 days PI). In contrast, no abortions were observed in a study of 30 control pregnancies in rhesus monkeys that were also monitored by ultrasound. The difference in abortion rates was significant (P = 0.01215). The normal (background) spontaneous abortion rate in the TRPRC colony is 4% (Falkenstei, KR, TRPRC, unpublished data).

The mean birth weights of infants born to P. coatneyi-infected dams, of infants born to normal (uninfected) mothers, of infants born to SIV-infected mothers, and of infants born within the TRPRC breeding colony are presented in Table 2. The first three groups of infants described above were delivered by cesarean section near term (GD 155). One infant in the P. coatneyi-infected group was vaginally delivered and was not included in the analysis of infant weight data. There was a significant difference between the three groups of infants delivered near term (GD 155) (F = 3.9994, P = 0.0355, by ANOVA). The infants of infected mothers weighed less (t = 3.9994, P = 0.0019). Three of these seven infants have survived to date. Figure 7 shows the mean weights of both groups after 19 weeks of observation. By the age of 11 weeks, the weights of the three surviving infants from P. coatneyi-infected mothers were similar to those of infants of control unoinculated mothers.

Four of the seven infants born to P. coatneyi-infected dams died at 4, 5, 18, and 33 days of age, respectively. Although their mortality was greater than the normal controls (4 of 7 versus 2 of 8 with one as a result of maternal trauma), the difference was not significant (P = 0.15618). Similarly, although the relative risk of death was six times greater (95% confidence interval = 0.58–61.84) for infants of infected mothers during the first 30 days of life, this was also not statistically significant.

One of the three surviving infants developed congenital malaria, despite a negative cord blood smear for malaria parasites at birth. Although this infant’s hematocrit decreased at 42 days of age (from 38.9% to 30.3%), weekly thick and thin peripheral blood smears remained negative until 80 days of age, when a parasitemia of 0.15% was first detected. This infant has remained persistently parasitemic with parasite counts ranging from 2,200 to 120,000/mm³ and hematocrits from 23% to 42%. Absolute lymphocyte counts ranged from 2,618 to 32,912/mm³ and have remained high since parasitemia was first detected (Figure 8). As in the pregnant dams, decreases in lymphocyte numbers were followed by increases in parasitemia. This persistently parasitemic infant is smaller than the two other live noninfected infants from malaria-infected dams and remains 500 g below his expected weight for age at 11 months.

Intrauterine growth retardation. The infant born to dam L226 suffered from symmetrical IUGR. This infant’s BPD and FL were below the standard curve at GD 100 (Figure 9), and remained lower than the standard curve until term. The infant’s birth weight was 334 g, while the average birth

Table 2. The first three groups of infants described above were delivered near term (GD 155) (F = 3.9994, P = 0.0355, by ANOVA). The infants of infected mothers weighed less than all other groups (P < 0.05). TRPRC colony versus all groups (F = 4.1021, P = 0.0094, significant difference). Duncan post hoc malaria group weighs less than all other groups (P < 0.05).

<table>
<thead>
<tr>
<th>Group† (dams uninfected)</th>
<th>N</th>
<th>Mean weight (grams)</th>
<th>Standard deviation</th>
<th>Range (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal† (dams uninfected)</td>
<td>8</td>
<td>456</td>
<td>48.1</td>
<td>370–530</td>
</tr>
<tr>
<td>Malaria†</td>
<td>6</td>
<td>376</td>
<td>48.11</td>
<td>300–424</td>
</tr>
<tr>
<td>SIV†</td>
<td>8</td>
<td>466</td>
<td>84.8</td>
<td>300–550</td>
</tr>
<tr>
<td>Breeding colony§ TRPRC</td>
<td>57</td>
<td>475</td>
<td>66.2</td>
<td>360–620</td>
</tr>
</tbody>
</table>

† Electro-abortions, determined from the infected dams, resulted in either spontaneous abortion or stillbirth.

§ The dams in the first three groups were given ketamine HCl weekly throughout gestation. First three groups (F = 3.9994, P = 0.0355, by analysis of variance [ANOVA]). Malaria weights versus uninfected weights (P < 0.05). Malaria weights versus simian immunodeficiency virus (SIV) (P < 0.05). Uninfected weights versus SIV weights = no statistical differences (P > 0.05). TRPRC colony versus all groups (F = 4.1021, P = 0.0094, significant difference). Duncan post hoc malaria group weighs less than all other groups (P < 0.05).

† Dams experimentally infected with SIV; infants are uninfected.

§ Vaginal deliveries, assumed to be term. TRPRC = Tulane Regional Primate Research Center.
weight for the TRPRC colony was 475 g. This is consistent with the mean birth weights reported by others.\textsuperscript{43, 44} We have defined LBW for TRPRC rhesus monkey infants to be greater than or equal to one standard deviation below the TRPRC mean, establishing LBW at < 409 g. Low birth weight, obvious emaciation at birth (Figure 10), and atrophy of internal organs were noted in this infant at necropsy. The placental weight was low at 83 g (mean bidiscoidal placental weight [GD 155] = 123.9 g). This infant died at five days of age, but did not have detectable \textit{Plasmodium} in blood or tissues. Another infant, born to L414, weighed only 300 g at birth but was much stronger than the infant born to L226. The infant’s placenta weighed 111 g. Although ultrasound had not revealed symmetrical growth retardation, this emaciated infant was found to be asymmetrically growth retarded and growth parameters were below the normal growth curve until three months of age. Although this infected infant is still alive at 11 months of age, he is congenitally infected with

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**FIGURE 7.** Mean weight trend of infants born to \textit{Plasmodium}-infected mothers compared with control infants for 19 weeks. The weight trends became similar by 11 weeks of age. This trend continued in the uninfected infants but changed in the congenitally infected infant when \textit{Plasmodium} infection became apparent. DOB = date of birth.

**FIGURE 8.** Parasitemia and absolute lymphocyte counts in a congenitally infected infant rhesus monkey. Lymphocyte counts increased following parasitemia in this congenitally infected infant. There was a reciprocal increase in lymphocyte numbers with decreases in parasite levels in a fluctuating pattern. RBC = red blood cell.
FIGURE 9. Intrauterine growth retardation as it occurred in the fetus of monkey L226. The biparietal diameter of the head and femur length were recorded weekly during ultrasonographic examination. These measurements were compared with a standard curve and began to decrease below the curve at 100 days gestation following a peak parasitemia on gestational day 90. Measurements remained below normal until birth and this infant’s birth weight was 334 g. Bars show the mean ± SD.

*P. coatneyi.* A decrease in weight gain began at three months of age when parasitemia was first noted and has continued in this persistently parasitemic infant throughout the first year. A weight comparison between this infant and the other two surviving infants is shown in Table 3.

**Placental findings.** Six placentas were obtained from the six cesarean sections. One monkey delivered vaginally at term and the placenta was not recovered because it was eaten by the mother. Placental weights in infected dams were lower than those of the 10 uninfected normal controls (*t* = 2.195, *df* = 14, *P* = 0.0455). The weights of placentas from infected dams ranged from 83 to 115 g with a mean of 106.83 g versus a mean of 123.9 g in the controls. The placenta that weighed 83 g belonged to the infant with the most severe IUGR and an LBW of 334 g.

**DISCUSSION**

These studies suggest that the pregnant rhesus monkey with *Plasmodium coatneyi* infection is an excellent model of malaria during human pregnancy. Malaria during pregnancy leads to complications in these monkeys and their infants, which are similar to those observed in humans. These include anemia, abortion, IUGR, LBW, congenital infection, and perinatal and maternal mortality. The monkey model also demonstrated the worst outcomes in primigravidas and secundigravidas, as have been reported in human studies. One critically important difference between this model and human studies is that one can follow the natural course of infection in the monkey without treatment. Thus, for the first time, it should be possible to observe the natural history of this process in primates with placentas similar to humans.

One of the other important observations in these studies was that the pattern and timing of recovery from anemia, not the level or pattern of parasitemia, correlated with infant survival after birth. This was exemplified by the three infants that are still surviving, whose mothers had the most complete recoveries from anemia during the last half of gestation. The RBC counts increased steadily in these three monkeys, despite high initial peak parasitemias or resurgent high parasitemias during the third trimester. The lowest RBC counts observed were similar in all seven animals following the peak parasitemia, and occurred late in the first trimester or early in the second trimester in six of seven monkeys (late during the second trimester in one monkey). The peak magnitude of the parasitemia has also been reported to occur at this same time in humans. All four infants whose dams did not recover from anemia prior to delivery (RBC count < 4 × 10^6/mm^3) died within one month of delivery. These observations suggest that persistent anemia during the last trimester can have a devastating impact on the infant, presumably because this is a time when fetal oxygen demands are high. One human study reported that average birth weights decreased progressively with the degree of anemia and that anemic primiparous and multiparous women had babies with lower birth weights than women with normal or near-normal hematocrits. Other studies have also associated anemia with LBW and have cited maternal anemia as a risk factor for perinatal mortality. Nosten and others have related infant mortality to maternal anemia at term, independent of birth weight. These results suggest that an emphasis on the treatment of anemia may improve the fetal outcome in women with malaria during pregnancy.

Low parity and high peak parasitemia with resurgence of parasitemia (> 7,800/mm^3) during the third trimester were related to poor infant outcome in the model, which manifested itself as abortion, early infant death, IUGR, LBW, and congenital malaria. Similar relationships have been noted in human studies. One primigravid monkey aborted at the time of a very high parasitemia. Similarly, in one human study in Zimbabwe, a high proportion of women with malaria aborted during the first and second trimesters. One other primigravid and one secundigravid monkey had resurgence of parasitemia (> 7,800/mm^3) during the third trimester, which were associated with IUGR and the two lowest birth weight infants in this study. One of these infants is alive but congenitally infected; the other died at five days of...
age. At delivery, these mothers had the two highest peripheral blood and placental parasitemias. The third primiparous dam had a resurgence of parasitemia (> 7,800/mm$^3$) during the third trimester and an infant that died at four days of age. In one recent human study, two factors associated with IUGR-LBW and fetal malaria were parasitemia at enrollment and placental malaria. Other studies have cited peripheral parasitemia$^b$ and placental parasitemia$^b$ as risk factors for IUGR-LBW and infant mortality.$^c$

As observed in human studies, infants born to dams with malaria had lower birth weights than controls.$^d$ In this study, LBW was also related to IUGR, as defined by weekly ultrasound measurements of fetal growth. This association is consistent with the consensus in the human literature that malaria, not prematurity, leads to IUGR.$^e$ By performing weekly ultrasound studies in the monkey, we were able to detect not only IUGR, but also the time when IUGR began. Relating fetal measurements to maternal, placental, and fetal outcome in larger groups of monkeys should help to define the mechanisms responsible for IUGR.

Latent congenital infection was seen in one rhesus monkey infant that did not become parasitemic until 80 days of age. This delayed parasitemia could have resulted from many factors, including transplacental transfer of maternal antibody,$^f$ immunologic tolerance that developed in utero,$^g$ fetal hemoglobin, or the $p$-aminobenzoic-deficient

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**Table 3**

Comparison of the monthly weights (in kilograms) among the three surviving infants born to *Plasmodium*-infected dams$^*$

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>V443 C-sec</td>
<td>0.488</td>
<td>0.658</td>
<td>0.794</td>
<td>0.969</td>
<td>1.186</td>
<td>1.3333</td>
<td>1.576</td>
<td>1.685</td>
<td>1.821</td>
<td>2.031</td>
<td>2.096</td>
</tr>
<tr>
<td>V513 Vaginal</td>
<td>0.516</td>
<td>0.748</td>
<td>0.912</td>
<td>1.09</td>
<td>1.28</td>
<td>1.41</td>
<td>1.501</td>
<td>1.464</td>
<td>1.712</td>
<td>1.891</td>
<td>1.932</td>
</tr>
<tr>
<td>V435 C-sec</td>
<td>0.422</td>
<td>0.574</td>
<td>0.704</td>
<td>0.802</td>
<td>0.955</td>
<td>1.086</td>
<td>1.182</td>
<td>1.256</td>
<td>1.396</td>
<td>1.512</td>
<td>1.53</td>
</tr>
<tr>
<td>Breeding colony$^\dagger$</td>
<td>Mean</td>
<td>0.54892</td>
<td>0.7779</td>
<td>0.98011</td>
<td>1.10377</td>
<td>1.26484</td>
<td>1.39995</td>
<td>1.53227</td>
<td>1.62892</td>
<td>1.77059</td>
<td>1.82235</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.11602</td>
<td>0.15081</td>
<td>0.36872</td>
<td>0.56529</td>
<td>0.62664</td>
<td>0.23813</td>
<td>0.2887</td>
<td>0.41016</td>
<td>0.25549</td>
<td>0.32618</td>
</tr>
</tbody>
</table>

$^*$ Parasites were detected in the peripheral blood of infant V435 at 80 days of age. This was followed by a gradual decrease in this infant’s weight gain when compared with the uninfected infants. C-sec = cesarean section.

$^\dagger$ Congenitally infected infant. At 11 months, this infant weighed 484 g, 25% less than the mean of the other two infant’s weights. This infant’s weight was $> 1$ standard deviation below the Tulane Regional Primate Research Center breeding colony mean at 11 months.

$^\ddagger$ Breeding colony animals are term vaginal deliveries born outdoors.
nature of breast milk.\textsuperscript{57} Under field conditions in an endemic area, one would assume that an infant with infection 80 days after birth had been mosquito-infected. However, there have been reports of delayed onset in human infants with congenital malaria.\textsuperscript{58} Among 49 cases of congenital malaria in the United States, the mean age of onset was 5.5 weeks (with a range of 0–60 weeks).\textsuperscript{59}

Absolute lymphocyte counts were 2–3-fold higher in the \textit{P. coatneyi}-infected pregnant monkeys than in the 30 pregnant controls. Absolute lymphocyte counts were also elevated in the congenitally infected monkey after his parasitemia was first diagnosed at 80 days of age. As in the pregnant dams, high parasitemias were followed one week later by an increase in the absolute lymphocyte count and a subsequent decrease in the parasitemia. The lymphocyte counts in the congenitally infected infant (20,000–35,000/mm\textsuperscript{3}) were much higher than in the pregnant monkeys (6,084–9,620/mm\textsuperscript{3}), and have persisted at those levels for the past 11 months. Although lymphocytosis has been reported in human infants with congenital malaria,\textsuperscript{59–61} those infants were treated with antimalarials soon after diagnosis, and it is therefore not clear whether the lymphocytosis would have persisted if they had not been treated.

The results presented here establish that the \textit{P. coatneyi} rhesus monkey model of malaria in pregnancy has many of the features associated with \textit{P. falciparum} infection in human pregnancy: anemia, abortion, IUGR, LBW, congenital malaria.\textsuperscript{58} Although some of the results from this study demonstrate patterns that have not been described in humans, these patterns may not have become apparent because of an inability to conduct a controlled experiment. In the monkey model we can time the inoculation, withhold therapeutic intervention, and there are no socioeconomic factors or other disease processes to influence outcome. Extensive data collection is possible in the form of weekly ultrasound examinations, complete blood counts, parasite determinations, and systematic evaluation of placentas. This would be difficult, if not impossible, to achieve in studies of humans and is therefore a major advantage of the monkey model. This model will also be useful in testing the safety and efficacy of new antimalarial drugs and vaccines for use in pregnancy. In addition, it will be a valuable model for studies of pregnancy immunosuppression, the fundamental causes of IUGR, the effects of anemia on fetal and placental growth and development, the role of the placenta in disease transmission, and other studies involving maternal-fetal-placental relationships.

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