Infection Regularity of Rhesus Cytomegalovirus in Indigenous RhCMV Rhesus Monkey Model

Liang-Qin SHI1,a, Qi-Hui LUO1,2,b, Xiao-Li LI1, Chao HUANG1,2, Yu XIA1, Wen ZENG3, Wen-Tao LIU1,2, Zheng-Li CHEN1,2,c,*

1Laboratory of Animal Disease Model, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu, Sichuan 611130, China
2Key Laboratory of Animal Disease and Human Health of Sichuan Province, College of Veterinary Medicine, Sichuan Agricultural University, Ya’an, Sichuan 611130, China
3Sichuan Primed Biological Technology Co., Ltd/ National Experimental Macaque Reproduce Laboratory, Ya’an, Sichuan 625014, China
ashiliangqin87@163.com, blqhbiology@163.com, cchzhli75@163.com
*Corresponding author

Keywords: RhCMV, Natural infection, Vertical Transmission, Histological distribution, Rhesus monkey.

Abstract. RhCMV, which is a counterpart of HCMV, some specific phenotypes of natural infection are not fully understood. The present research was designed to study the seroprevalence of indigenous infection of RhCMV among different age groups by ELISA, and detected its histological distribution in adult and their own fetal monkeys by the ISH and PCR methods. The ELISA result showed that the average seroprevalence of RhCMV was 76%, the concentration of RhCMV-IgG in each group was between 10-30 pg/ml and there was no significant difference between each group (P>0.05). The ISH and PCR results showed that RhCMV infected a wide range of tissues, including immune system, respiratory tract, gastrointestinal tract and urinary tract, and the positive signal of RhCMV in mother monkeys was stronger than that of in their own fetal monkeys. In addition, we also found that RhCMV positive signal also existed in mother’s reproductive organs, including uterus, ovary and placenta. The above results indicate that RhCMV can infect all age groups monkeys and hide in a wide range of organs, and can transmit vertically to their own fetal. The infection regularity of indigenous RhCMV rhesus monkey model can provide support for further understanding the persistence and pathogenesis mechanism for HCMV. Furthermore, these results can remind the researchers to give more attention to the potential and adverse effect induced by RhCMV when doing reproductive work and some other research work.

Introduction

Human cytomegalovirus (HCMV), widespread only in humans with the seroprevalence rate ranging from 60% to 99% in adults [1-4], is a main cause of non-hereditary congenital birth defects[5]. However, to date, there have been no approved vaccines for HCMV or anti-HCMV drugs that can be administered to gravid women who were infected by HCMV before and during pregnancy. Therefore, effective strategies need to be developed to minimize the infection rate. Animal models can provide a convenient way to study the persistence and pathogenesis of HCMV and test the novel therapeutic strategies [6]. However, these...
artificially established models [7-9] are not enough to meet current research needs as they cannot exactly reproduce almost all the features of HCMV.

Rhesus cytomegalovirus (RhCMV) also belongs to the herpesviridae family of viruses, and share so many phylogenetically conserved features with HCMV. As a counterpart of HCMV, RhCMV usually causes inapparent infection in rhesus monkeys. RhCMV widely spreads both in wild and captive monkeys across the world. Serological surveys show that its infection rate in wild monkeys ranges within 75.00-90% [10], and this infection rate reaches to 87.3% in captive animals [11-13]. Although a series of evidences have already shown that the clinical symptoms, pathogenesis, epidemiology and risk group of RhCMV are similar to HCMV in immunosuppressive or artificial disturbance situations, some specific naturally infected phenotypes of RhCMV, such as the infection rate among different age groups, the differences in histological distribution between adult and fetal monkeys, and the possible transmission route of RhCMV, remain far from clear. In addition, an incredible research found that RhCMV can asymptotically infect persons who work with rhesus macaques or who are hurt by rhesus macaques [14]. Although the frequency was <0.5% (<1/200 primate workers), the result indicates that the infection regularity of RhCMV can provide direct platform for understanding the persistence and pathogenesis mechanism for HCMV.

Therefore, in the present study, we detected the seroprevalence of RhCMV in captive rhesus macaques from different age groups who may naturally infected with RhCMV, as well as it’s histological distribution of RhCMV in adult and their fetal monkeys. The result showed that the seroprevalence was 76%, less than the average infection rate of 87.3%, but there was no significant difference between each group. Meanwhile, the histological evidence of RhCMV distributed in reproductive organs (including uterus, ovary, umbilical cord and placenta) of mother monkeys and almost all the organs of fetal monkeys indicates that RhCMV from indigenous infected monkeys could be transmitted vertically to their own infant. Furthermore, the infection regularity of indigenous RhCMV rhesus monkey model can provide further information for understanding the persistence and pathogenesis mechanism for HCMV.

**Materials and Methods**

A total of 110 female rhesus monkeys (used for ELISA test) with no abnormal clinical phenotype were provided by the National (Sichuan) Experimental Rhesus Monkey Resources Base (Certificate No: SCXK [Chuan]:2013-105). Experimental procedures were in conformity with the guidance of “the National Institutes of Health Guide for the Care and Use of Laboratory Animal” of the United States. Monkeys were housed in stainless steel monkey cages (one animal per cage). The study rooms were under controlled conditions, with a regulated temperature of 19-26°C, a relative humidity of 40-70%, regular ventilation (10 times/ hour), and a 12 (day)/ 12 (night) hours lighting cycle. Tap water (used as drinking water, taken freely by monkeys via the automatic bubbler) and standard diet (approximately 200 g, fed twice daily, containing 18% protein, 69% carbohydrates, 3% fat, and10% water) were supplied to each animal, and vegetables or fruits were provided around 4 P.M.

Before the experiment, all the animals were trained by pulling the arms out of the cage before fed so that the animals cooperated with the experimenters and could be restrained easily during the experiment. Therefore, anesthesia was not used during blood collection.
In addition, three pregnant animals (pregnanted, fetus aged approximately 4-5 months) died of trauma were obtained. Meanwhile, two healthy and seronegative monkeys were selected as negative group.

**Tissue Collection**

In order to know whether RhCMV is likely to be the cause of death, the organs (including the heart, liver, spleen, lung, kidney, thymus, trachea, intestines, stomach, umbilical, placenta, uterus, ovaries and lymph nodes) of mother monkeys and their own infant were obtained in two hours. Lymph nodes were stored in liquid nitrogen for PCR assay, while the other samples were stored in 4% Paraformaldehyde for at least 48h and then were used for histopathology analysis and the ISH assay. The samples from negative group were also obtained in the same way.

**ELISA Assays**

**Qualitative assay of RhCMV IgG.** Blood samples were collected at the cephalic veins in the morning before eating. After centrifugated at 5000 rpm for 8 min at 4°C, the obtained serum were stored at -70°C until use.

The qualitative assay of the RhCMV IgG was analyzed using a standardized commercial ELISA kit (HuShang Biological Technology Co., Ltd, ShangHai, China), and all procedures were performed according to the manufacturer’s instructions. Briefly, the procedures were as follows: Serum samples (5 fold diluted) were separately added to each well (coated with purified monkey CMV IgG antigen to make solid-phase antigen, performed by manufacturer), and incubated for 30 min at 37°C. Afterwards, 50 μl HRP-labeled CMV IgG antigen conjugate reagent was added to each well to become antigen-antibody-enzyme-antigen complex, except the blank well, then the plate was incubated again for 30 min at 37°C. Subsequently, 100μl freshly made TMB substrate solution was added into each experimental well, and evade the light preservation for 15 min at 37°C. After adding stop solution, the absorbance (OD values) was read in a microplate reader (Thermo, USA) at 450nm within 15min. Between each step above, each test well was washed five times with washing buffer. Each 96-well plate was set two negative controls, two positive controls and one blank control.

When the average value of positive controls was greater than or equal to 1.00 and the average value of negative controls was less than or equal to 0.10, the tests were thought to be valid. A sample was considered positive if the OD value was greater than calculate critical (CUT OFF= the average of Negative control well + 0.15), and considered negative if the OD value was less than or equal to CUT OFF value.

The 110 monkeys were divided into four groups according to their age (Table. 1), and the OD values of all samples were obtained at a wavelength of 450nm.

**Quantitative assay of RhCMV IgG.** In order to obtain an accurate result, we subsequently carried out the quantitative assay. The standard curve between different concentrations of standard substance (RhCMV IgG: 60, 40, 20, 10 and 5 pg/ml) was established using linear regression analysis. Each concentration of standard substance, all serum samples (5 fold diluted) and blank control were set twice repeat wells. The other steps were similar to the qualitative assay. The standard curve of the quantitative analysis was performed with the
Richards model, and the equation was 
\[ y = \frac{a}{(1 + e^{b-x})^{1/d}} \]  
(a=1.84396856536E+000, b=3.42143299833E+000, c=1.05456809687E-001, d=1.48411986544E+000, r= 0.99961663).

**PCR Assay and Molecular Identification of RhCMV**

DNA was isolated from the lymph node samples using a Genomic DNA extraction kit (Code No.9765, TaKaRa Biotechnology Co., Ltd. Dalian, China) and stored at -20°C until use. PCR was performed using the Bio-rad system in 20-μl reactions containing 1μl of DNA, 10μl of 2×Taq PCR MasterMix, 0.5μl of each primer (20 pmol) and ddH2O. The PCR procedure was carried out using the following conditions: 5-minute enzyme activation at 95°C, followed by 35 cycles of 95°C for 30 seconds, 68°C for 30 seconds, and 72°C for 30 seconds, 72°C for 10 minutes in the end. Primer sequences (designed based on the envelope glycoprotein B (gB) gene, GenBank Accession No: AF033184.1) are represented as follows: (F)5'-TTG TCA AGA ACA CCG TGC GCA AC-3', (R)5'-TGA GAG CTG CGG CCG TGA CAC C-3'. The total length of the expected fragment was 414 bp. PCR formation was determined by agarose gel electrophoresis and visualized by gold view staining and recycled using a gel extraction kit (Lot No. P5215, TIANGEN biotech (Beijing) Co., Ltd China) to sequencing (performed by Shanghai SANGON Biological Technology Co., Ltd., China). After obtaining the sequencing result, we compared our sequences with the sequences (belonging to the 68-1 strain of RhCMV) availabled from the gene bank.

**In Situ Hybrization Assay**

The specific sequence of the oligonucleotide probes marked by digoxigenin (performed by Takara Biotechnology Co. Ltd, Dalian, China) was designed to detect DNA of RhCMV and its sqeunce (designed based on the gB gene) was as follows: 5'-TTG TCA AGA ACA CCG TGC GCA ACT CTG T-3', and it's located at 2590-2617 nucleotides. Briefly, these procedures were performed according to the manufacturer’s instructions (Code No.MK1032, BOSTER, Wuhan, China) and as follows[21]:

The sections were washed in 3% H2O2 to destroy the activity of endogenous enzymes, and subsequently digested with pepsin diluted with 3% citric acid. Then the sections were incubated in prehybridization solution for 2.5 hours to prevent non-specific staining. After adding the hybridization probe, the sections were incubated at 41°C for one night. Next, the sections were washed with SSC, blocked, incubated in biotinylated labeled digoxin antibody for two hours, exposed to strept avidin-biotin complex (SABC) for 20 minutes, incubated in biotin peroxidase for 60minutes, stained with DAB for no more than 30 minutes, then washed, dehydrated, and mounted.

Prehybridization solution with no hybridization probe was used in the negative sections, the other steps were the same as above.

**Image Analysis and Processing**

For analysis of ISH data, the sections were observed by a light microscope and photographed by a digital microscope (Nikon, Japan). The positive areas of RhCMV and the average OD values were quantified by Jiangsu Jieda 801 series morphologic analysis.
system[22]. Five pieces of each section in different positions were chosen at randomly, and the detection was performed in triplicate.

Statistical Analysis

Statistical analysis was carried out using SPSS 17.0. ELISA data was analyzed by Chi-Square test, and the other data were expressed as mean±SD and subjected to one-way analysis of variance (ANOVA). P<0.05 was considered statistically significant.

Results

Results of Qualitative and Quantitative Assays for RhCMV-IgG

Similar with previous research, the results in the present study show that the average seropositivity rate was 76%, the infection rate in young monkeys- the major breeding group was higher than that in other age groups, but there was no significant difference between each group (P>0.05). Meanwhile, the results of quantitative analysis showed that the concentration of RhCMV-IgG in each group was between 10- 30 pg/ml, and there was no significant difference between groups (P>0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Qualitative assay</th>
<th>Quantitative assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive rate of each group</td>
<td>Total positive rate</td>
</tr>
<tr>
<td>Nonage (3-4 years)</td>
<td>20</td>
<td>70.00%(14/20)</td>
<td>76%</td>
</tr>
<tr>
<td>Youth (5-8 years)</td>
<td>28</td>
<td>85.71%(24/28)</td>
<td>76%</td>
</tr>
<tr>
<td>Middle-aged (9-13 years)</td>
<td>37</td>
<td>78.38%(29/37)</td>
<td>76%</td>
</tr>
<tr>
<td>Elderly (≥14 years)</td>
<td>25</td>
<td>64.00%(16/25)</td>
<td>76%</td>
</tr>
</tbody>
</table>

PCR Results for RhCMV

After the amplification process, the agarose gel was visualised using the Image Lab 4.1 System, and the specific sequence amplicons were located between 400 bp and 500 bp (Fig. 1). All the lymph node samples from three mother monkeys and their babies were positive for RhCMV. After recycling the purpose gene fragments and sequencing test, the results showed that the sequence shared the highest identity (99.9%) with the 68-1 strain, which is the prototypic strain of RhCMV that has been used for pathogenesis and vaccine development.
In Situ Hybrization Assay

In order to know whether the other organs were infected with RhCMV, the ISH method was used in the present paper. In mother monkeys, we found that the RhCMV virus mainly distributed in immune organs (including the spleen and thymus), and then distributed in the respiratory passages organs (including lung and trachea), the gastrointestinal tract organs (including the stomach and intestine) and the urinary tract organ (kidney) (Fig. 2A). Meanwhile, we found RhCMV virus also distributed in hypothalamus (Fig. 3A), but the statistical analysis result showed no significant difference among several hypothalamus nucleus (P>0.05) (Fig. 3B). Subsequently, we detected the virus in their own fetus organs. Similar with their mother monkeys, almost all the organs in fetal monkeys can be detected with RhCMV (Fig. 2B), but the positive signals almost in all organs were significant lower than that of in their mother monkeys (P<0.01) (Fig. 2D). Ultimately, we detected the virus in the reproductive organs of mother monkeys. Surprisingly, the positive signals were detected in the ovary, uterus and placenta (Fig. 4).

Positive signals in negative control group and negative sections were not seen (Fig. 2C).
Comparison of Infection Regularity between RhCMV and HCMV

In order to provide more convincing evidence that the naturally infected monkeys can be used for pathogenesis and efficacy evaluation for new drugs of HCMV, we compared the infection regularity between RhCMV and HCMV (data came from the present paper and previous studies). Both naturally and artificially infected RhCMV models share many features of HCMV (Table 2 and 3). Currently, the artificial models were often used for HCMV study, but it’s need more effort and cost to keep the model going. Regardless of the transmission route, pathological characteristics or histological distribution, the indigenous infection models were more suitable for studying the pathogenesis and efficacy evaluation of new drugs for HCMV.

Figure 2. ISH results. (A) The distribution of RhCMV in the internal organs and brains of adult monkeys. (B) The positive signals of RhCMV in the internal organs and brain of fetal monkeys. (C) Negative picture. (D) Contrastive analysis of the positive areas of RhCMV between adult (n=3) and fetal monkeys (n=3). (E) OD values of the internal organs and brain of adult and fetal monkeys. **P<0.01 versus “fetal organs”.

Figure 3. ISH results in the hypothalamus of adult macaque. (A) The positive areas of RhCMV in nucleus of hypothalamus. (B) Average optical density of RhCMV in the nucleus of the hypothalamus.
Figure 4. The ISH result of RhCMV in reproductive organs. (A) The distribution of RhCMV in the uterus, ovary and placenta. (B) Positive areas of RhCMV in reproductive organs. (C) Average OD values of RhCMV in reproductive organs. **P<0.01 versus “fetal organs”.

Table 2. Comparison of the epidemiology and histological distribution between natural RhCMV infection and HCMV.

<table>
<thead>
<tr>
<th>Item</th>
<th>RhCMV infection</th>
<th>HCMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host</td>
<td>Rhesus, human[20]</td>
<td>Human[23]</td>
</tr>
<tr>
<td>Susceptible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seropositivity rate</td>
<td>75-90%[1]</td>
<td>60-99%[3, 4, 24]</td>
</tr>
<tr>
<td>Infection stage</td>
<td>During prenatal and postnatal life</td>
<td>During prenatal and postnatal life[6]</td>
</tr>
<tr>
<td>Fetal monkeys, infants</td>
<td>and immunocompromised individuals[10, 25]</td>
<td>Fetuses, infants, immunocompromised individuals, and transplant recipients</td>
</tr>
<tr>
<td>Vulnerable age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Characteristic</td>
<td>Inapparent infection</td>
<td>Yes[6]</td>
</tr>
<tr>
<td>Lifelong persistence</td>
<td>Yes[26, 27]</td>
<td>Yes[6]</td>
</tr>
<tr>
<td>Congenital infection</td>
<td>Yes[10]</td>
<td>Yes[24]</td>
</tr>
<tr>
<td>Mononucleosis</td>
<td>Yes[24]</td>
<td></td>
</tr>
<tr>
<td>Transmission rout</td>
<td>Urine</td>
<td>Yes[26, 28]</td>
</tr>
<tr>
<td></td>
<td>Saliva</td>
<td>Yes[28, 29]</td>
</tr>
<tr>
<td></td>
<td>Breast milk</td>
<td>Yes[10, 29]</td>
</tr>
<tr>
<td></td>
<td>Tears</td>
<td>Not clearly</td>
</tr>
<tr>
<td></td>
<td>Feces</td>
<td><strong>Probably</strong></td>
</tr>
<tr>
<td></td>
<td>Semen</td>
<td>Yes[29]</td>
</tr>
<tr>
<td></td>
<td>Genital secretions</td>
<td>Yes[33]</td>
</tr>
<tr>
<td></td>
<td>Intrauterine transmission</td>
<td>Yes[33]</td>
</tr>
<tr>
<td></td>
<td>Vertical transmission</td>
<td><strong>Probably</strong></td>
</tr>
</tbody>
</table>

Note: The bold “Yes” or “**Probably**” represent the results obtained from or suggested by the present study.
### Table 3. Comparison of histological distribution between RhCMV and HCMV.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Naturally infected</th>
<th>Fetal inoculation</th>
<th>HCMV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult monkey</td>
<td>Fetal monkey</td>
<td>Fetal monkey</td>
</tr>
<tr>
<td>Spleen</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Thymus</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes [37]</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Yes</td>
<td>Yes</td>
<td>Not clearly</td>
</tr>
<tr>
<td>Brain</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes [10]</td>
</tr>
<tr>
<td>Nasal mucosal Membrane</td>
<td>Yes [41]</td>
<td>Not clearly</td>
<td>Not clearly</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>Not clearly</td>
<td>Not clearly</td>
<td>Not clearly</td>
</tr>
<tr>
<td>Lung</td>
<td>Yes [41]</td>
<td>Probably</td>
<td>Yes</td>
</tr>
<tr>
<td>Kidney</td>
<td>Yes [30, 42]</td>
<td>Yes</td>
<td>Yes [10]</td>
</tr>
<tr>
<td>Adrenal</td>
<td>Yes</td>
<td>Probably</td>
<td>Yes [10]</td>
</tr>
<tr>
<td>Heart</td>
<td>Yes</td>
<td>Yes</td>
<td>Not clearly</td>
</tr>
<tr>
<td>Live</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes [44]</td>
</tr>
<tr>
<td>Bile ducts</td>
<td>Yes [41]</td>
<td>Probably</td>
<td>Not clearly</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Probably</td>
<td>Probably</td>
<td>Yes</td>
</tr>
<tr>
<td>Stomach</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Intestines</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes [10]</td>
</tr>
<tr>
<td>Cochlea</td>
<td>Not clearly</td>
<td>Probably</td>
<td>Yes</td>
</tr>
<tr>
<td>Muscle</td>
<td>Not clearly</td>
<td>Probably</td>
<td>Yes</td>
</tr>
<tr>
<td>Skin</td>
<td>Not clearly</td>
<td>Probably</td>
<td>Yes [10]</td>
</tr>
<tr>
<td>Vessel</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Uterus</td>
<td>Yes</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ovaries</td>
<td>Yes</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Testis</td>
<td>Yes</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Placenta</td>
<td>Yes</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Umbilical cord</td>
<td>Yes</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Note: The bold “Yes” represents results obtained from the present study.

### Discussion

HCMV, a member of the herpesviridae family, has the ability to maintain a lifelong persistent infection, since primary and recurrent infections occur within an immunocompetent host. Although it is usually asymptomatic in hosts, it can cause severe and even fatal diseases in transplant recipients and fetuses/newborns [5, 55-60]. One of the obstructions in the development of vaccines and researches on HCMV is that HCMV could not be directly studied in any animal model. Genetic evidence indicates that all CMVs originated from a common progenitor [61]. RhCMV as a counterpart of HCMV, shares many features of HCMV. In particular, the genome of RhCMV strain 68-1 and 180.92, which were deeply studied, are highly closely related to HCMV and at least 90% of proteins are orthologs at the level of protein families [62-64]. Accordingly, RHCMV infection holds promise as a highly relevant surrogate for human HCMV infection, so the exploration of rhesus macaque model is vital to understand the persistence and pathogenesis mechanism for HCMV, and to prioritize both potential vaccine strategies and antiviral therapies for humans [30]. Currently, the artificial models are often used for HCMV study, but more effort and cost are needed to keep the model going. From the transmission route, pathological characteristics and histological
distribution to consider, the indigenous models were more suitable for studying the pathogenesis and efficacy evaluation of new drugs for HCMV.

RhCMV infection is ubiquitous in wild and captive rhesus monkeys[14], and its seroprevalence reach 100% around one year of age[65] well before the age of sexual maturity group (3-4 years)[15, 66]. In the present study, the supplementary result we found is that seroprevalence in young monkeys was higher than the other groups. The PCR and ISH results showed that RhCMV virus harbored mainly in immune organs, including in thymus, spleen and lymph nodes, the result combined with the asymptomatic phenotype indicates that the immune system is stronger to resist the virus and control the host in subclinical conditions [67-70]. However, although the defense system in rhesus monkeys is strong, some viruses always have the ability to slip through their immune system. In humans, HCMV can incessantly transmit and reproduce through saliva, tears, urine, feces, semen, milk and intrauterine exposure. Similar routes appear to exist in rhesus monkeys. In the present study, we found that RhCMV virus harbored in the epidermis of respiratory tract, gastrointestinal tract and urinary tract organ, the result indicate that RhCMV probably can be effluenced from the host through saliva, feces, urine to the surrounding environment, thus, infecting more animals. 

But currently, neither vertical transmission from mother to fetus nor histological evidence of RhCMV disease in fetuses or neonates has been documented. In the present study, we provide the histological evidence that RhCMV is not only distribute in mother’s reproductive organs (including uterus, ovary, placenta and umbilical cord), but also in its infant’s organs (including thymus, spleen, trachea, intestine, stomach and kidney). However, due to the protective effect of the placental barrier and the arrest and antitoxic effects of the thymus, which is matured earlier than the spleen in four or five mouth fetal monkey[71, 72], and some other immune organs, infection intensity in infant was milder than their own mother. The result indicate that RhCMV probably could be transmitted vertically to their own infant.

Conclusions
Taken together, the infection regularity in indigenous RhCMV rhesus monkey model can provide support for understanding the persistence and pathogenesis mechanism for HCMV. Furthermore, these results can remind the researchers to give more attention to the potentially and badly influence induced by RhCMV when doing reproductive work and some other research work.

Acknowledgments
This research was financially supported by the National Science Foundation.

References


