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Viral Infection of Virus Across the Placental Barrier in Tupaia Belangeri Chinensis

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Abstract: Tupaia (*Tupaia belangeris chinensis*), commonly called the tree shrew is a small non-primate mammal that is classified between rodents and primates. It is reportedly sensitive to various human pathogenic viruses and bacteria which might make it an ideal model for the study ofing human diseases. However, very few articles have reported viral infections inuses on tree shrews that induce many unknown pathogens are not tested. Researchers tested fifty eight progeny tree shrews to assess the presence of 16 test if sixteen pathogens. Fifteen pathogens were tested for using the antibody by Enzyme-Linked Immunosorbent Adsorption Assay (ELISA) approach including Herpes Simplex Virus (HSV), Coxsackie virus, hepatitis A virus, hepatitis B virus, hepatitis D virus, HIV, dengue virus, hemorrhagic fever virus, *Toxoplasma gondii*, adenovirus, human cytomegalovirus, rubella virus, parvovirus, measles virus and poxviruses. Feces were collected to test for rotavirus infection. No viral infections were not detected in this study. Based on these results, no virus was found to infect the offspring of tree shrews. This data may provide us the direction of viral infection on tree shrews and to establish a standardized model for viral infection in these animals.

Key words: Tree shrew, intra-uterine transmission, virus, rubella virus, bacteria

INTRODUCTION

A variety of human viruses cause intrauterine infections such as Cytomegalovirus (CMV), rubella virus, Herpes Simplex Virus (HSV), human papillomavirus, human parvovirus B19, Hepatitis A Virus (HAV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Coxsackie virus, Human Immunodeficiency Virus type-1 (HIV-1), herpes zoster virus, mumps virus and influenza virus (Onakewhor *et al*., 2013; Scott *et al*., 2013; Weisblum *et al*., 2011; Lim *et al*., 2013; Straface *et al*., 2012; Alberico *et al*., 1996; Hsu *et al*., 2007; Prasad and Honegger, 2013; Ouellet *et al*., 2004; Haun *et al*., 2007; Shrim *et al*., 2012; Hanaoka *et al*., 2013; Panda *et al*., 2010). HIV-1 is one of the most prevalent chronic infectious diseases and is associated with significant morbidity and mortality worldwide. Mother-To-Child Transmission (MTCT) of HIV-1 mainly occurs in utero according to research conducted in Europe and North America (Scarlatti, 2004; Magder *et al*., 2005). In China, HBV and HIV-1 are the most prevalent infections. Moreover with regard to HBV and HCV, MTCT remains an important cause of chronic infection in China and both HBV and HCV are associated with chronic persistent infection (Bai *et al*., 2007; Marcellin *et al*., 2002). It is therefore important to study the maternal-fetal transmission of viruses from the viewpoint of controlling the vertical transmission of chronic viral infections.

The chimpanzee is the only validated animal model for testing the authenticity and infectivity of viruses (Hong *et al*., 1999). However, chimpanzees are expensive experimental subjects and also relatively rare. Further, cross-species transmission from infected chimpanzees to other non-human primates has been proven unsuccessful for all species evaluated so far (Abe *et al*., 1993). One of the other models under study is the tree shrew (*Tupaia belangeri chinensis*) which is a non-rodent, small squirrel-like mammal that is the closest relative of the primate (Novacek, 1992). Tree shrews are now widely classified as a separate taxonomic group of mammals that probably diverged from primate species (Maher and DeStefano, 2004; Kumar and Hedges, 1998). They are small non-primate mammals that are indigenous to certain areas of Southeast Asia (Flugge *et al*., 2002). Yunnan Province in China has the highest population of tree shrews. They are evolutionary closely related to humans (Suphamungmee *et al*., 2008; Wong and Kaas, 2009) and have shown susceptibility to infection by several human viruses including HBV, rotavirus, HSV and HCV therefore, the tree shrew has been suggested as an important animal

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Table 1: ELISA results for viral antigens in the tree shrew serum and fecal samples

Virus	Samples	Antibodies tested	Results
Adenovirus	Serum	IgM	-
		IgG	-
Toxoplasma gondii	Serum	IgM	-
		IgG	-
Hepatitis B virus	Serum	HBeAg	-
		HBc	-
		HBsAg	-
		HBb	-
		HBs	-
Coxsackie virus-B	Serum	IgG	-
Cytomegalovirus	Serum	IgG	-
Dengue virus	Serum	IgG	-
Epidemic hemorrhagic fever virus	Serum	IgG	-
Hepatitis A virus	Serum	IgG	-
Hepatitis D virus	Serum	IgG	-
Herpes simplex virus	Serum	IgG	-
Measles virus	Serum	IgG	-
Parvovirus	Serum	IgG	-
Rubella virus	Serum	IgG	-
Varicella-zoster virus	Serum	IgG	-
HIV	Serum	IgG	-
Rotavirus	Feces		-

model for virus research (Yang *et al*., 2013a; Wang *et al*., 2012a; Han *et al*., 2011; Tidona *et al*., 1999; Darai *et al*., 1978; Pang *et al*., 1983). In the 1970s, tree shrews were used as animal models for studying herpes viruses and Borna disease virus infections (Darai *et al*., 1978; Sprankel *et al*., 1978). This model has also been used widely for studying infection with hepatitis virus (A, B, C), HIV, rotavirus, adenovirus (Darai *et al*., 1980), measles virus (Wang *et al*., 2011), influenza virus (Yang *et al*., 2013b) and even HBV and HCV (Yang *et al*., 2013a; Wang *et al*., 2012b).

At present, there is no standard regarding which viruses should be tested to establish SPF normalization in tree shrews. However, intra-uterine viruses remain untested in the tree shrew and there is no reference for viral detection in laboratory tree shrews as most of the published data have been in wild animals. This study aimed to provide such data (Table 1).

MATERIALS AND METHODS

Animals: The domesticated tree shrews used in this study were obtained from the Faculty of Experimental Animals, Kunming Medical University, Yunnan Province, China. Adult tupaias were quarantined for at least 2 months before the experiments were conducted. The progeny were delivered by cesarean section and fed and lived in isolation. Cord blood was conserved for further experiments. Animal experiments were carried out in accordance with the guidelines for the care and use of laboratory animals issued by the Chinese government. Animals were housed at the Laboratory Animal Center of Jinan University.

Sample collection: The adults were anesthetized using ether, cord blood was collected and each sample was centrifuged to separate the serum sample which was then stored in a -20°C freezer. Feces were collected in sterile Eppendorf tubes and mixed with Phosphate-Buffered Saline (PBS) buffer. The solution was centrifuged and supernatant was used for detection of rotavirus.

Diagnostic tests: Serum specimens of the experimental tree shrews were analyzed qualitatively by Enzyme Linked Immunosorbent Assay (ELISA) for several viral markers. The ELISA Method was performed according to each manufacturer's instruction diagnostic kits for detection of viral antigens against the following antibodies were used: IgM antibody against HAV (Lot 201212024; Shanghai Kehua Biotech Co., Ltd. China); IgG antibody against HBV including HBV surface antigen (Lot 201211041; Shanghai Kehua Biotech Co., Ltd.), HBV surface antibody, HBV e antigen, HBV e antibody and HBV core antibody; antibody against HIV (Lot 201211021, Shanghai Kehua Biotech Co., Ltd.); antibody against hepatitis D virus (Lot 20130101; Beier Bioengineering Co., Ltd. China); IgG against dengue fever virus (Lot 20130129), measles virus (Lot 20130402), varicella-zoster virus (Lot 20130402), epidemic hemorrhagic fever virus (Lot 20130105), Coxsackie virus (Lot 20130129; all purchased from Shenzhen Sciarray Biotech Co., Ltd.); Rotavirus Diagnostic kit (Lot LB20121206; Beijing Wantai DRD Co., Ltd.); IgM and IgG antibodies against *Toxoplasma gondii* (Lot 128M/K112 and 128G/K062), IgG antibody against rubella virus (Lot 125G/K023) and IgG antibody against cytomegalovirus (Lot 106G/K033; all purchased from DRG International Inc., Germany); IgM and IgG antibodies against adenovirus (Lot AVM-183 and AVG-181), IgG antibodies against HSV 1/2 (Lot HSVG-058) and IgG antibodies against parvovirus B19 (Lot PARG-101; all purchased from IBL International GMBH, Germany).

Instruments: The study required 37°C incubators (MIR-262, SANYO, Japan), high-speed refrigerated centrifuge (CR22GIII, Hitachi, Japan), a microplate reader (MK3, Thermo Fisher, America) and precision pipette (BSA2002S, Sartorius, Germany).

RESULTS

In this study, researchers conducted serological tests to determine the presence of a variety of intra-uterine viruses in tree shrews at the center.

Detection of hepatitis virus: Chronic hepatitis virus infection is an important global health problem and

chronic HBV infection progresses to cirrhosis and hepatocellular carcinoma in many cases. HBV infection is horizontally and vertically transmitted: intra-uterine infection may breach the placental barrier and infect the fetus. Only very closely related animals such as chimpanzees are susceptible but they are very expensive models. Various hepadnaviruses are known to infect smaller animals but these vary considerably from humans and cannot be used as animal models. There are many reports that strongly support tupaias as a valid model for studies of HBV infection: for example, the primary hepatocytes from tupaias could be infected with HBV and the typical symptoms were exhibited in inoculated animals (Wang *et al*., 2012a, b, 2011). However, whether the tree shrew could be chronically infected with HBV in vivo has been a controversial topic for decades. Wang *et al*. (2012a) showed that outbred neonatal tree shrews could be chronically infected with HBV at a frequency comparable to the infection rate in humans which indicated that this model might be useful for studying chronic HBV infection and viral clearance. However, HBV antibodies were not detected in this study which was consistent with the results of Han *et al*. (2011).

Human Hepatitis D Virus (HDV) is a satellite virus of HBV. Li *et al*. (1995) indicated that adult tupaias could be used as an experimental model of hepatitis D virus (Li *et al*., 1995; Yan *et al*., 2013). However, in the study, none of the hepatitis viruses including hepatitis D virus were detected in any of the samples.

Detection of HIV: HIV-1 is capable of evading the innate and adaptive immune systems. Majority of HIV-infected infants get infected via MTCT. According to the 2011 UNAIDS Progress report about 2.7 million people worldwide was newly infected with HIV-1 in 2010, 14% of whom were infants infected via MTCT. Therefore, this virus should be detected if infecting infant tree shrews. So far, no research has been conducted on the tree shrew as a model of HIV. In this study, all the serum samples were negative for HIV.

Detection of HSV: HSV is an enveloped, double-stranded DNA virus that can be sexually transmitted. According to the Centers for Disease Control and Prevention at least 45 million Americans or one in five adolescents and adults have genital herpes infection. Further, there is a high prevalence of genital herpes in the pregnant population and 85% of perinatal transmission is reported to occur during the intrapartum period. Genital herpes during pregnancy is associated with spontaneous abortion, intrauterine growth retardation and preterm labor and congenital and neonatal herpes infections (Straface *et al*., 2012). It has been shown that juvenile tupaias are more susceptible to HSV than adult animals and display the typical pathological changes in the clinical setting (Darai *et al*., 1978). Wang *et al*. (2011) detected HSV markers in the wild tree shrew and the result was negative which is consistent with the findings of the study.

Detection of CMV: Human CMV infections are congenital and are associated with severe birth defects and intrauterine growth retardation. Systematic screening for maternal CMV infection is not necessary in most countries but 1% of pregnant women are known to contract CMV infection and the rate of vertical transmission is estimated to be 30-50% (Benoist *et al*., 2013). Infants who contract the infection via MTFT will have the disease at birth or develop severe sequelae including convulsive or spastic syndromes, mental retardation and auditory and visual impairment (Benoist *et al*., 2013). The mechanism of human CMV transmission is largely unknown and there are no animal models for this infection. So far, no research has been conducted on tree shrews as a model for CMV infection and researchers did not detect CMV in the serum samples in this study either.

Detection of ADV: ADV is a DNA virus that infects humans, other mammals and birds. It typically causes acute or mild infections of the upper or lower respiratory tract, gastrointestinal tract and conjunctiva. Rare manifestations of ADV infections include hemorrhagic cystitis, hepatitis and colitis (Lynch *et al*., 2011). Chen reported 12 ADV-positive samples from among 90 samples from wild tree shrews. In contrast, Han *et al*. (2011) did not detect any positive samples from among a total of 272 samples. The findings are in agreement with the study of Han *et al*. (2011) as researchers did not detect any ADV-positive serum samples.

Detection of *T. gondii*: *T. gondii* infections occur worldwide but they are especially prevalent in Europe, South America and Africa (Petersen, 2007). Congenital toxoplasmosis is caused by MTFT of *T. gondii* to the fetus. The prevalence of congenital toxoplasmosis ranges from 0.1-0.3 per 1000 live births (Kieffer and Wallon, 2013). Approximately two-thirds of the infants are asymptomatic at birth but they may later develop major clinical manifestations such as chorioretinitis, intracranial calcifications and hydrocephalus (Wang *et al*., 2012a). Isolated *T. gondii* antibodies from *Tupaia glis*, however, researchers did not detect any serum samples positive for *T. gondii*.

Detection of rotavirus: Rotaviruses are non-enveloped viruses consisting of 11 segments of double-stranded RNA enclosed in a triple-layered protein capsid. Rotavirus usually causes severe acute viral diarrhea in infants and children (Dash *et al*., 2012). Wang *et al*. (2011) have detected rotavirus antibodies from wild tree shrew and Pang *et al*. (1983) showed that *Tupaia belangeri* could be infected with rotavirus. In the study, all samples were negative.

Detection of other viruses: Markers for parvovirus B19, rubella virus, Coxsackie virus B, epidemic hemorrhagic fever virus, varicella zoster virus and measles virus were tested in this study, all these viruses have been reported cause intrauterine infection. Some studies have confirmed the susceptibility of the tree shrew to dengue virus, parvovirus B19 and Coxsackie virus B. However, in this study, the serum samples were negative for all of these tested viruses.

DISCUSSION

Intra-uterine viruses are the causative agents of progeny infection which is a global health problem. A series of viruses cause intrauterine infections such as hepatitis virus (such as HAV and HBV), cytomegalovirus, herpes simplex virus and human immunodeficiency virus type-1 amongst others.

In the present study, researchers used conventional methods to assess whether 16 pathogens that cause intrauterine infections had infected fifth-eight infant tree shrews. Some of these viruses such as HBV, HSV, ADV, *T. gondii*, rotavirus, dengue virus, parvovirus B19 and Coxsackie virus B have been previously described as infecting wild tree shrews captured in Yunnan province. In the current study, no virus was detected to infect tree shrew progeny. Differing from other studies, the adult tree shrews in the center were not wild and they were also sourced from a single center. Furthermore, the other studies used diagnostic kits from humans could detected these viruses in this study, none of these have been detected, the animals may not infect these viruses.

Some viruses such as HIV, CMV, EHF, HAV, HDV, measles virus, varicella-zoster virus and rubella virus have never been reported to infect tree shrews and none of these viruses were detected in this study. It may be that these viruses cannot infect tree shrews or that the diagnostic kits used for detection in humans may not be sensitive to tree shrew serum infections. Therefore, as no viral antibody tests for the tree shrew are commercially available, the human antibodies used may have resulted in weak cross-reactivities that were undetectable. Other methods of viral detection in addition to ELISA should be further tested and reference values for virus detection in laboratory tree shrews established. Then, proper measures can be adopted for the management of the progeny tree shrews becomes available.

CONCLUSION

The purpose of this study is to detect intra-uterine viruses which from human, primate and rodents to determine if tree shrews get intrauterine infections. The 16 viruses were screened but all samples were negative. This study may provide data for direction of tree shrew virus detection to establish SPF tree shrews and get closely to a standardized animal model.

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