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Herpesvirus sylvilagus IN COTTONTAIL RABBITS: EVIDENCE OF SHEDDING BUT NOT TRANSPLACENTAL TRANSMISSION[1]

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Abstract: Herpesvirus sylvilagus was inoculated into five cottontail rabbits (*Sylvilagus floridanus*) at various stages of pregnancy; they subsequently had litters in the laboratory. Three other cottontails chronically infected with the virus were bred and bore young in large outdoor pens. Thirty-four living neonates and dead fetuses were weighed, measured and aseptically necropsied. A total of 31 liver, spleen and kidney samples, 16 lymph node, 28 heart and 10 brain samples were collected and processed for inoculation into rabbit kidney cell cultures to attempt virus isolation. Virus was not detected in the 147 tissue samples tested. Pre-conception viremias ranged from 10-21 plaque-forming units per 0.5 ml.

Virus isolation was attempted from 26 oral and lacrymal, 23 genital, nine urine and fecal, and four milk and male ejaculate samples from eight infected rabbits. Virus was recovered from two salivary samples from the same rabbit. Triamcinolone acetonide administered daily for four days to five rabbits did not stimulate excretion of virus.

INTRODUCTION

Previous reports from this laboratory have indicated that *Herpesvirus sylvilagus* or cottontail herpesvirus (CHV) is probably not transmitted among cottontails by fleas and mosquitoes in spite of field evidence implicating the former.[13] Since arthropod-borne transmission appeared unlikely, other mechanisms of virus exit were sought.

A number of herpesviruses are transplacentally transmitted. *Herpes simplex* virus (HSV) has been reported in hospital case history studies to infect the human fetus.[7,12] Experimental inoculation of pregnant laboratory rabbits with high concentrations of HSV also resulted in fetal infection while low concentrations did not cross the placenta.[6] Transplacental transfer has been demonstrated for equine rhinopneumonitis (ERP) in horses, pseudorabies (PR) in swine and infectious bovine rhinotracheitis (IBR) in cows, as cited by Lam and Hsiung.[5] The herpesvirus causing malignant catarrhal fever is transplacentally transmitted among blue wildebeest[8] and cows.[9]

Herpesviruses are spread more commonly via aerosols and body exudates. Transmission has been associated with virus-contaminated saliva for Type 1 HSV, *H. saimiri* and IBR and with respiratory discharge of PR, ERP and infectious laryngotracheitis viruses.[4] *H. saimiri* in carrier squirrel monkeys was detected in nasopharyngeal secretions for a five month period.[2] To determine if CHV could be transmitted by these means, groups of pregnant cottontails were infected with CHV before and during pregnancy and attempts were made to recover virus from living and dead young. To demonstrate virus shedding by chronically viremic rabbits, samples of body exudates were routinely inoculated into cell cultures to recover CHV.

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MATERIALS AND METHODS

Virus and serology

This information has been reported previously.[13]

Experimental animals

Pregnant cottontails used in this study were captured by drive net at the University of Wisconsin-Madison west campus from March to July, 1973. Approximate fetal age was determined by palpation and size estimation of the fetuses *in utero* according to Rongstad.[10]

Transplacental transmission

In the first experiment, two and three pregnant does, estimated to be nine and 18 days post-conception respectively, were inoculated with CHV. Inocula consisted of 0.5 ml doses of field strain virus titering $10^{3.5}$ plaque-forming units (PFU) per ml, injected subcutaneously. These rabbits were housed in 33 x 46 x 53 cm steel cages and provided with water and commercial rabbit pellets *ad libitum*. Blood was sampled for CHV viremia determination from does *post partum* and from young born alive in the cages.

Dead, whole fetuses were individually weighed and measured from rump to crown and at the left hind foot to estimate whether they were to term or aborted a determinable number of days prematurely. Individual samples of liver, spleen, kidneys and, in some cases, lymph nodes, heart and brain were triturated in separate Ten Broeck tissue grinders with sufficient diluent (Medium 199-3 percent fatal calf serum with 1000 units penicillin, 500 μg streptomycin and 50 μg Fungizone® per ml) to produce 20% tissue suspensions. These were placed in individual glass vials and frozen at -70 C or immediately inoculated into rabbit cell culture using previously described methods.[13]

In Experiment 2, three does 17-71 days post infection (DPI) were used. They were bred with CHV antibody-negative bucks in 7.3 x 21.9 m fully enclosed outdoor pens. The mated does were then transferred to individual 3.0 x 6.1 m outdoor pens and allowed to bear their young. A diet of pasture and shrub cover was supplemented with commercial rabbit pellets and water.

Virus shedding

Urine, feces, saliva, milk, male ejaculate and lacrymal and genital secretions were obtained from 15 different CHV-inocculated cottontails. Samples were taken from 18 days to 8 months post-inoculation. Urine, fecal, milk and ejaculate samples were prepared as 20% dilutions in diluent. Dry swabs were taken of the oral cavity, conjunctivae and genital orifice and placed in vials containing 2 ml chilled diluent as before. Collection of urine and ejaculate was by manual stimulation and milk samples were gently expressed from the mammae of lactating females. One-half ml aliquots of the samples were inoculated into individual plaque bottles and processed as above.

In attempt to stimulate virus shedding, the synthetic corticosteroid, triamcinolone acetonide (TA) was tested. A group of six cottontails inoculated approximately 7-11 month previously was tested for CHV viremia and absence of virus in saliva and lacrymal and genital secretions by swabbing as previously described. Five of the rabbits were injected every three days for 12 days with a dose of ten mg per kg of rabbit. Attempts were made to recover CHV from the aforementioned exudates on days 3, 6 and 15 after the initial TA injection.

RESULTS AND DISCUSSION

Transplacental transmission

Virus was not obtained from a total of 31 each of liver, spleen and kidney samples and 16 lymph node, 28 heart and 10 brain samples from eight litters (Table 1). Although 108 of the samples were taken from fetuses dead for as much as 24 hr, 32 samples were harvested from six neonates sacrificed up to five days after birth. Three heart blood samples taken at the day of birth from three live neonates did not produce isolable virus. Ten tissue samples from a live litter of

TABLE 1. Absence of detectable cottontail herpesvirus in tissues from fetuses and neonates from infected cottontail rabbits.

No.	Infected days *pre partum*	No. born alive/total	Tissues[e] (no.) tested L[e]	S	K	LN	H	Be
Experiment 1								
C13	6	4/4[a]	4	4	4	2	4	0
C18	7	3/3[a]	3	3	3	0	0	3
C15	9	4/7[a]	7	7	7	3	7	2
C9	9	1/1[c]	1	1	1	1	1	0
C14	12	2/2[b]	2	2	2	2	2	0
Experiment 2								
C44	45	3/6[a]	6	6	6	3	6	0
C45	50	3/3[a]	3	3	3	3	3	3
C43	99	5/5[d]	3	3	3	2	3	3
C39	control	3/3[d]	2	2	2	0	2	2

[a]found dead
[b]sacrificed at 1, 5 days *post partum* (DPP)
[c]sacrificed at 4 DPP
[d]sacrificed at 5 DPP
[e]20 percent suspension of fetal liver, kidneys, lymph nodes, heart and brain, all CHV negative

TABLE 2. CHV isolation attempts from samples of infected cottontail rabbits.

Sample source	No. samples/no. indivs. tested	Isolation results
Oral swab[2]	26/15	2 recoveries
Urine[1]	9/7	Negative
Feces[1]	9/7	Negative
Lacrymal swab[2]	26/15	Negative
Milk[1]	4/3	Negative
Male ejaculate[1]	4/3	Negative
Genital swab[2]	23/12	Negative
TA Experiment		
Oral swab[2]	15/5	Negative
Lacrymal swab[2]	15/5	Negative
Genital swab[2]	15/5	Negative

[1]20 percent dilution in Medium 199
[2]Swab mixed into 2 ml Medium 199

an uninoculated control doe were negative. *Post partum* viremias of the does in Experiment 1 and pre-conception viremias of those in Experiment 2 ranged from 10-21 and 11-21 PFU per 0.5 ml respectively.

Virus shedding

Virus isolation attempts from nine urine and fecal, 24 oral, 26 lacrymal, four each milk and ejaculate and 17 genital samples were not successful (Table 2). Virus was recovered from two salivary samples from the same rabbit in titers of seven and ten PFU per 0.5 ml. Viremias of the 15 sampled cottontails ranged from 9-24 PFU per 0.5 ml. Due to the chronicity of the disease, viremia was not determined at each sampling.

These experiments failed to demonstrate that *H. sylvilagus* could be transmitted transplacentally among cottontail rabbits. Although most of the fetuses were found dead, 24 of 34 had been born alive. It was not clear from these trials whether CHV infection during pregnancy effected development of the litters. Fetus length and weight measurements at birth did not conform to Rongstad's fetal cottontail growth table[10] so estimation of fetus age at expulsion was not attempted.

Guinea pig herpes-like virus (GPHLV) is similar to CHV in that both are persistent and cell-associated. GPHLV results in frequent transplacental infection as showed by pre-breeding inoculation of the virus into Strain 2 guinea pigs. Transplacental transmission of GPHLV occurred in 52% of the litters.[5]

Although it was suggested that GPHLV may be transplacentally passed via virus-bearing maternal leukocytes, they make up only 0.1% of the total leukocyte population.[5] Regarding CHV, only one in 10^5 leukocytes carries virus.[3] There may be permeability differences between guinea pig and rabbit placentas and it is unknown whether cottontail placental tissue becomes infected. However, the theoretical probability of CHV transfer from maternal circulation is quite small.

In contrast to other herpesviruses, CHV shedding in saliva and other secretions was not induced by administration of corticosteroids to chronically viremic host animals. The present study is in contrast with those concerning herpesvirus shedding in other species. In Holstein bulls having IBR antibody without shedding virus for 4 years, recrudescence was initiated within 3-5 days of treatment with dexamethasone.[11] Similar results were found when 6 calves inoculated 6 months previously with IBR were treated with either dexamethasone or adrenocorticotropic hormone. Virus was recovered from nasal and/or vaginal secretions of these animals 2-5 days later.[1]

The two CHV isolations from saliva of a cottontil suggests a possible route of exit from the infected host in the field as was supported modestly by this study and at length by a series of transmission trials carried out by Hinze (pers. comm., 1974). His donor cottontails were orally rather than parenterally inoculated with the virus which apparently induced a salivary gland infection and consequent shedding of virions. Thus, shedding of CHV in saliva of chronically infected cottontails has been demonstrated although the portal of entry into susceptible rabbits in the field remains unknown. The mechanisms whereby CHV transmission occurs in nature remain to be discovered.

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