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Congenital and Perinatal Infection With Junin Virus in Guinea Pigs

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Junin virus infection in guinea pigs is known to be similar to human Argentine hemorrhagic fever (AHF). The guinea pig was chosen as a model for transplacental transmission of Junin virus, as both guinea pig and man have a similar placental structure. Pregnant guinea pigs were infected with the pathogenic XJ strain of Junin virus intramuscularly route at different stages of pregnancy. The group infected during the last third of pregnancy produced 16 newborn, but mortality reached 100%: 18% were born with typical AHF hemorrhagic signs, 54% without signs, and the remainder were stillborn.

Virus was recovered from organs of newborns, as well as placental tissues. A second group, infected in the second third of pregnancy, died with intrauterine fetuses, all of which showed hemorrhagic signs and virus present. In a last group, infected in the first third of pregnancy, fetuses were free from macroscopic lesions. In order to determine whether lactation may be an alternative infection route in guinea pigs, mother guinea pigs were infected with Junin virus at different times postparturition. The 84% noninfected newborn housed together with their infected mothers died during the suckling period, half with typical AHF signs.

Junin virus transmission from mother to fetus was thus proved, and lactation may be considered as an alternative perinatal infection route.

Key words: junin virus, vertical transmission, guinea pigs

INTRODUCTION

Argentine hemorrhagic fever (AHF) is an endemo-epidemic disease caused by Junin virus, a member of the *Arenaviridae* family. This disease mainly affects rural workers involved in corn and sorghum harvesting.

The arenaviruses characteristically produce persistent infections in their natural rodent hosts and are thereby maintained in nature. Most of the arenaviruses have one main rodent host, and persistent infection has been reported for Junin virus in *Calomys* musculinus and *Calomys* laucha [12]. These wild rodents act as reservoirs, with the virus present in blood and organs, and excreted in urine and saliva [8].

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The greatest AHF incidence is seen in males between 20 and 50 years old. Nevertheless, there is a significant percentage of cases among females. Epidemiological studies have shown both clinical and inapparent infections in women between 20 and 40 years old.

In the broadest sense, viral congenital infection can produce different effects, such as malformations, abnormal function with or without tissue damage, latent infection with subsequent induction of disease, abortion, or stillbirth. Several viruses are able to cross the placental barrier and produce congenital infections: Rubella virus, herpes simplex virus, poliovirus, cytomegalovirus, varicella zoster virus, and Japanese encephalitis virus have been recovered from placental tissue [1–3, 9, 10, 14].

In our laboratory we carried out a preliminary study of the effect of the pathogenic XJ strain of Junin virus on pregnant guinea pigs [13]. Gomez et al studied the effect of an attenuated strain of Junin virus, and observed that the highest mortality for guinea pig moghers occurred when the critical period of disease overlapped with the predelivery period [5].

To date there are no studies reputed concerning on pregnant humans, although Maiztegui et al were able to isolate Junin virus from a sample of human colostrum [7].

Junin virus infection in guinea pigs has been shown to be similar to that observed in humans [6]. Also the guinea pig possesses a placenta with a single trophoblast layer similar to the human structure [4], and therefore we chose this animal as a model for the study of transplacental transmission of Junin virus infection.

The present work was carried out to determine transplacental transmission of Junin virus after inoculation into guinea pigs at different stages of pregnancy. Furthermore, we endeavored to determine whether the infection may be transmitted through colostrum and milk.

MATERIALS AND METHODS

Virus

The XJ pathogenic prototype strain of Junin virus was used with a titer of $10^{7.5}$ LD_{50}/ml in suckling mice and $10^{6.5}$ $TCID_{50}$/ml in Vero cells. The viral stock was prepared as 10% homogenate of infected suckling mouse brain (killed seven days postinfection) in Hank's solution containing 10% calf serum. Virus titers were determined by Cito-pathogenic Effect (CPE) on Vero cells or by intracerebral (IC) inoculation in suckling mice. The $TCID_{50}$ or LD_{50} was calculated by the Reed-Muench method [11].

Tissue Cultures

Vero cells ranging from subcultures 190 to 200 were used throughout the experiments. Cells were cultured in tubes 48–72 hours before inoculation, using Hank's solution containing 0.5% lactalbumin and 10% calf serum, maintained in Eagle medium with 3% serum.

Animals

Outbred guinea pigs at different stages of pregnancy and newborn Rockland mice of either sex were used.

Transplacental Transmission

In order to determine transplacental transmission, 22 pregnant guinea pigs were infected intramuscularly (IM) with 100 $TCID_{50}$ of virus (XJ prototype strain of Junin virus). These animals were split into three groups according to the stages of pregnancy; the average gestation period in the guinea pig is 68–70 days.

Group A. Eleven pregnant guinea pigs infected in the last third of pregnancy.
Group B. Eight pregnant guinea pigs infected in the second third of pregnancy.
Group C. Three guinea pigs infected in the first third of pregnancy.

Transmission by Colostrum/Milk

In order to determine whether among other factors lactation may lead to perinatal infection in newborn guinea pigs, all five mother guinea pigs were infected IM with 100 $TCID_{50}$ of Junin virus (XJ prototype strain). They were inoculated 2,4,6,8, and 12 days postparturition and infected mothers and noninfected young suckling animals then housed together until the mothers died.

Samples and Infectivity Assays

Ten percent homogenates of virus-infected tissues (fetuses and placentas) in HLS were separated and stored at $-70°$ until used for virus titration. Each homogenate was inoculated into Vero cell tubes or intracerebrally to suckling mice.

RESULTS

Transplacental Transmission

All 22 pregnant guinea pigs (groups A, B, and C) died between 13 and 25 days postinfection (PI) with a mean death day of 17. Twenty of them showed typical AHF hemorrhagical signs: Petechiae or larger hemorrhages were seen in the subcutaneous tissues, abdominal lymph nodes, adrenal gland, and intestinal walls. Two guinea pigs presented only widespread congestion. Larger hemorrhages in the uterus and multiple petechias in the yolk sac were particularly noticeable.

Six guinea pigs were used as virus control, and these animals died between 14 and 15 days PI with typical AHF signs.

Table I shows the results discussed below:

Group A. The 11 guinea pigs infected in the last third of pregnancy produced 22 newborn. Of these, 18% died between the first and third week after maternal infection with AHF signs, while 54% died during the same period without obvious signs, mortality occurring throughout from 2 to 10 days of age. The remainder (28%) were stillborn. Therefore, mortality reached 100% in newborn from guinea pigs infected in the last third of pregnancy. Junin virus was recovered from spleen lymph node and lung in all newborn guinea pigs, with a 1–2 log titer.

TABLE I. Junin Virus Infection in Pregnant Guinea Pigs Inoculated During Different Pregnancy Stages

No. of mother guinea pigs	Pregnancy stage	No. of newborn/stillborn or fetuses	Effects of the infection on newborn/ stillborn or fetuses
Group A: 11	Last third	16 newborn 6 stillborn	100% newborn mortality Widespread hemorrhagic lesions Positive viral isolation from lung spleen and lymph node
Group B: 8	Second third	12 fetuses	Widespread hemorrhagic lesions Positive viral isolation from fetal and placental tissues
Group C: 3	First third	2 mothers: 4 fetuses 1 mother: 5 aborted fetuses	No macroscopic lesions Viral isolation was not performed on these fetuses owing to their small size

TABLE II. Viral Isolation and Mortality of Suckling Guinea Pigs in Contact With Their Infected Mothers

Guinea pig No.	No. of newborn	Time of challenge to mother (days postpartuntion	Effect on suckling newborn	Viral isolation from mammary gland
1	2	2	1 died with typical AHF signs 1 survived	Positive
2	1	4	1 died with typical AHF signs	Positive
3	2	6	1 died without signs 1 survived	Positive
4	5	8	2 died without signs 3 died with typical AHF signs	Positive
5	2	12	1 died without signs 1 died with typical AHF signs	Positive
Total: 5	12 newborn		6 died with typical AHF signs 4 died without signs 2 survived	All positive

Group B. The eight pregnant guinea pigs infected in the second third of pregnancy died with a total of 12 intrauterine fetuses. Each mother had a maximum of five fetuses. Junin virus was recovered from all fetuses, with hemorrhagic signs, and also from placental tissue.

Group C. The three pregnant guinea pigs infected at the beginning of their gestation produced nine fetuses free from macroscopic lesion; five, all from the same mother, were aborted before midpregnancy.

Transmission by Colostrum/Milk

All five mothers infected at different times after parturition died between 14 and 16 days PI with typical AHF signs.

Table II shows that out of the 12 noninfected young animals housed together with their infected mothers, 84% (10/12) died during the suckling period between 7 and 38 days after birth. Half of the latter died with typical AHF signs, the other half died without obvious AHF signs. The two survivors were challenged with the pathogenic XJ strain of Junin virus and died with typical signs, three to four weeks later. Virus was recovered from mammary gland of these five mothers, as well as from the 19 animals belonging to groups A and B. Tests were not performed on the three mothers of group C.

DISCUSSION

The present work was carried out in order to demonstrate transplacental transmission of Junin virus after inoculation into guinea pigs at different stages of pregnancy. In addition, lactation was studied as an alternative route for Junin virus infection.

With regard to transplacental transmission the results presented here—which include (1) typical hemorrhagic signs seen in newborn from guinea pigs infected with Junin virus during the last third of pregnancy; (2) fetuses "in utero" showing multiple petechias and widespread hemorrhages; and (3) the isolation of Junin virus from fetal and placental tissues, as well as from organs (spleen, lymph node and lung) in all newborn guinea pigs—prove that Junin virus is able in these cases to cross the placental barrier and

produce congenital infection in guinea pigs. These newborn guinea pigs might also be infected by close contact, for example, during lactation; although they all died within 11 days after birth and the inoculation period of AHF in guinea pigs is usally longer. Thus, considering the high viremia of the infected mothers, transplacental transmission seems the most likely route of infection in these cases.

It was noticeable that out of the 22 infected pregnant guinea pigs, only one aborted during the experiment, this animal having been inoculated in the first third of pregnancy.

Virus isolation from mammary gland and the high mortality (84%) of the uninoculated suckling animals (50% of which showed typical AHF signs) suggests that lactation may be a route of viral transmission in the guinea pig. Junin virus has been detected in human milk [7], and our findings support that lactation may be a route for Junin virus infection.

At any rate, other forms of transmission, especially among suckling guinea pigs, should be kept in mind, because the guinea pigs inoculated with Junin virus exhibited viremia, viruria, and hemorrhages.

This work proves that transplacental transmission of Junin virus may occur in guinea pigs, the animal model most commonly used in AHF studies, and suggests that lactation may be an alternative route of perinatal infection. With regard to humans infected with Junin virus, congenital and perinatal infection must therefore be taken into account.

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