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*A Murine Model for Studying Transplacental Transmission of *Borrelia burgdorferi**



Sousan Sayahtaheri Altaie, Ph.D.
Assistant Professor of Pediatrics,
SUNY at Buffalo and
Microbiology Review Officer,
U. S. Food and Drug Administration



Background (1)

Gardner, T. 1995. Lyme Disease. In J. S. Remington and J. O. Klein (eds.) *Infectious diseases of the fetus & newborn infant*, 4th ed.

- ◆ A review of the congenital and gestational Lyme borreliosis literature yielded 161 cases for which the outcome of the episode of gestational Lyme borreliosis was noted.
- ◆ The 46 cases of adverse outcomes of these 161 cases were found to include miscarriage, stillbirth, perinatal death, congenital anomalies, systemic illness, early-onset fulminating or mild sepsis, and late-onset chronic progressive infection.

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Background (2)

Gardner, T. 1995. Lyme Disease. In J. S. Remington and J. O. Klein (eds.) *Infectious diseases of the fetus & newborn infant*, 4th ed.

Fetal/ Neonatal abnormality	No. with finding/Total (%)
Cardiac	11/46 (24)
Neurologic	9/46 (20)
Orthopedic	6/46 (13)
Ophthalmic	3/46 (7)
Misce. Anomalies	8/46 (17)
Fetal/Neonatal demise	22/46 (48)
Rash	5/46 (11)



Background (3)

Gardner, T. 1995. Lyme Disease. In J. S. Remington and J. O. Klein (eds.) *Infectious diseases of the fetus & newborn infant*, 4th ed.

Trimester	Antibio.	No./Total (%)	
		Adv. Out.	Norm. Out.
First	Yes	8/17 (47)	9/17 (53)
	No	8/10 (80)	2/10 (20)
Second	Yes	5/22 (23)	17/22 (77)
	No	4/5 (80)	1/5 (20)
Third	Yes	0/8 (0)	8/8 (100)
	No	1/2 (50)	1/2 (50)

(3)



Borrelia burgdorferi Strains

- ◆ Strain 297, a Northeastern isolate
 - ◆ isolated from human CSF
- ◆ Strain W18, a Midwestern isolate
 - ◆ isolated from *I. scapularis* nymph off a Veery bird along St. Croix River in Minnesota
- ◆ Strain Son-1 from the Pacific region
 - ◆ isolated from *I. pacificus* nymph in Sonoma County, California



Mice

- ◆ Five- to six-week-old inbred C3H/HeJ mice were obtained from Jackson Laboratories.
 - ◆ Animals had access to food and water ad libitum and were exposed to a 12-h light/12-h dark cycle.
 - ◆ Three to five days prior to infection and mating, the animals were anesthetized by inhalation of Methoxyflurane and were surgically splenectomized.

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B. burgdorferi propagation

- ◆ The isolates were maintained in our laboratory by animal inoculation and recovery at 32°-34°C in modified Barbour-Stoener-Kelly medium (BSK II) with the following antibiotics: Phosphomycin, 400 µg/mL; 5-Fluorouracil, 100 µg/mL; Trimethoprim, 10 µg/mL; Sulfamethoxazole, 50 µg/mL.



Animal Inoculation

- ◆ Splenectomized test animals were inoculated subcutaneously on the right lower quadrant of the ventral surface, with 10^6 - 10^7 *B. burgdorferi* in 0.25 mL BSK II medium.
- ◆ Splenectomized control animals were inoculated with 0.25 mL sterile BSK II medium.

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Culture Method

- ◆ Using aseptic techniques various tissues were harvested and rinsed in sterile saline. After rinsing, a small section of the tissue was placed in 4 mL of BSK II and incubated at 32°-34°C for nine weeks. The cultures were examined by darkfield microscopy every three weeks for presence of motile spirochetes.

Experimental Design: In-utero

- ◆ Early-gestation
 - ◆ Infected 6-7 days after observation of coital plugging and sacrificed 6 days after infection
- ◆ Middle-gestation
 - ◆ Infected 9-10 days after observation of coital plugging and sacrificed 6 days after infection
- ◆ Late-gestation
 - ◆ Infected 12-13 days after observation of coital plugging and sacrificed 6 days after infection

(b)



Experimental Design: In-utero

- ◆ Sacrificed adults by exsanguination and soaked them in ethanol until necropsy
 - ◆ Cultured adult brain, ear, heart, bladder, liver, kidney, and spleen after rinsing in sterile saline
- ◆ Harvested fetuses and placentas aseptically
 - ◆ After rinsing in sterile saline, cultured full minced fetuses in early gestation and cultured 1/2 fetuses in mid- and late- gestation.
 - ◆ After rinsing in sterile saline, cultured 1/2 placentas



Experimental Design: Postnatal

- ◆ Infected and mated adults the same day
- ◆ If coital plugging did not occur within a week of mating, animals were discarded.
- ◆ Allowed pregnancies to go to term and sacrificed adults and pups at 1, 7, 14, and 21 days post delivery

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◆ *Experimental Design: Postnatal*

B = Brain
 E = Ear
 H = Heart
 BL = Bladder
 L = Liver
 K = Kidney
 S = Spleen

- ◆ Sacrificed adults by exsanguination and soaked in ethanol until necropsy (10-20 min.)
 - ◆ Cultured adult B, E, H, BL, L, K, and S after rinsing in sterile saline
- ◆ Sacrificed pups by exsanguination and soaked in ethanol until necropsy (20-30 min.)
 - ◆ Cultured, Ear and skin from scruff in one tube, B, H, BL, K, and S in another tube, and milk content of the stomach in a third tube
 - ◆ Milk was cultured on all 1-day-old and some of the 7- and 14- day-old pups.

◆ *Results: In-utero (1)*

- ◆ 100% of experimentally infected animals were culture positive.
- ◆ None of the uninfected mating pairs were culture positive; i.e., no sexual transmission.
- ◆ All the fetuses and placentas were culture negative.

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Results: In-utero (2)

- ◆ However, by PCR, from 34 experimentally infected females in groups A and C, 7 (21%) transmitted *B. burgdorferi* to their fetuses and/or placentas.
 - ◆ 4/11 (36%) during early-gestation
 - ◆ 3/12 (25%) during middle-gestation
 - ◆ No transmission during late-gestation
- ◆ No PCR-positive samples in group B



Results: In-utero (3)

- ◆ Of the 7 female mice that transmitted *B. burgdorferi*,
 - ◆ one was infected with strain 297
 - ◆ three were infected with strain W18
 - ◆ three were infected with strain Son-1
- ◆ Low numbers but no difference among strains, thus data from strains were pooled

(a)



Results: In-utero (4)

PCR positive samples/Total No.

<u>Gestation</u>	<u>F & P</u>	<u>P only</u>	<u>F only</u>	<u>Total</u>
Early	1/30 (3%)	3/30 (10%)	1/30 (3%)	5/30 (17%)
Middle	2/57 (3%)	1/57 (2%)	2/57 (3%)	5/57 (9%)
Late	0/47 (0%)	0/47 (0%)	0/47 (0%)	0/47 (0%)
Total	3/134 (2%)	4/134 (3%)	3/134 (2%)	10/134 (7%)



Results: Postnatal (1)

- ◆ 100% of experimentally infected animals were culture positive.
- ◆ None of the uninfected mating pairs were culture positive; i.e., no sexual transmission.



H.H.S. proved milk transmission
in 1997 - Work done by FDA

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Results: Postnatal (2)

- From the 25 experimentally infected females in which the milk was cultured in groups A and C, 2 (8%) transmitted *B. burgdorferi* to their pups on day one via their milk.
 - 2 out of 4 pups, group C, strain 297
 - 1 out of 8 pups, group A, strain W18
- None of the milk samples collected after day 1 were culture positive.
- Transmission rate via milk from infected dams to offspring was 2.3% (3/131).



Results: Postnatal (3)

- Milk samples collected on days 1 and 7 were positive for anti-*B. burgdorferi* antibodies by ELISA.
- No milk samples collected for ELISA from pups older than 7 days
- Milk collected from control animals up to day 14 were negative by ELISA for anti-*B. burgdorferi* antibodies.

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Results: Postnatal (4)

- ◆ Among 49 experimentally infected dams from groups A and C, 5 (10.2%) transmitted *B. burgdorferi* to their pups in-utero
 - ◆ 3/5 dams were infected with strain 297
 - ◆ first litter: 2/6 pups were culture positive
 - ◆ second litter: 1/2 pups was culture positive
 - ◆ third litter: 1/7 pups was culture positive



Results: Postnatal (5)

- ◆ 2/5 dams were infected with strain Son-1
 - ◆ first litter: 1/7 pups was culture positive
 - ◆ second litter: 2/6 pups were culture positive
- ◆ None of the dams infected with strain W18 had culture positive pups.
- ◆ All control pups were culture negative.
- ◆ Thus, the in-utero transmission rate from infected dams to offspring was 3% (7/224) in this study.

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Results: Postnatal (6)

- ◆ Close contact transmission
 - ◆ Four of 24 litters from mating pairs in group B had culture positive pups (rate, 17%)
 - ◆ (9/132 pups; rate, 7%)
 - ◆ one male infected with strain 297
 - ◆ one male infected with strain W18
 - ◆ two males infected with strain Son-1
 - ◆ All four females were culture-, ELISA-, and PCR- negative.



Results: Postnatal (7)

◆ Close contact transmission

- ◆ Litter 1, 7-days-old : 3/5 culture positive, strain 297
 - ◆ 2 only E/S positive; 1 E/S and other tissues positive
 - ◆ no milk or serum was collected
- ◆ Litter 2, 7-days-old: 1/8 culture positive, strain W18
 - ◆ E/S positive; Milk and serum ELISA-negative
- ◆ Litter 3, 7-days-old: 3/6 culture positive, strain Son-1
 - ◆ E/S positive; Milk and serum ELISA- negative
- ◆ Litter 4, 21-days-old: 2/4 culture positive, strain Son-1
 - ◆ E/S positive; no milk collected, serum ELISA-negative

E/S = Ear and
Skin from
the scruff

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Summary (1)

- ◆ Experimental infection rate is 100% in this model.
- ◆ The three tested strains seem to behave similarly in this model.
- ◆ If groups A and C are combined from both phases
 - ◆ from 83 experimentally infected females 12 (14%) transmitted *B. burgdorferi* to their offspring in utero
 - ◆ the in-utero transmission rate from infected females to offspring is 5% (17/358) in this model.



Summary (2)

- ◆ In-utero transmission seems to occur during the early- and middle-gestation periods.
- ◆ From the 25 experimentally infected females in groups A and C, 2 (8%) transmitted *B. burgdorferi* to their pups via colostrum.
- ◆ Transmission rate via colostrum from infected dams to offspring is 2% (3/131).

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Summary (3)

- ◆ Close contact transmission does not occur among mice >3-week-old in this model.
- ◆ From 24 experimentally infected males in group B, 4 (17%) transmitted *B. burgdorferi* to their pups.
- ◆ The close contact transmission rate from infected males to offspring is 9/132 (7%).



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