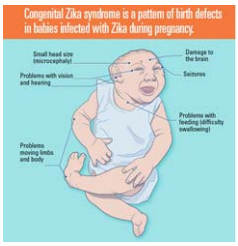
Centers for Disease Control and Prevention

Proving Causation: How Do We Prove that an Infectious Agent is a Teratogen

Sonja A. Rasmussen, MD, MS
Centers for Disease Control and Prevention

National Birth Defects Prevention Network
Virtual Meeting



What is a Teratogen?

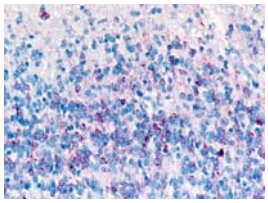
- An exposure in pregnancy that has a harmful effect on the embryo or fetus
- Types of teratogens:
  - Medications
  - Heavy metals
  - Radiation
  - Maternal conditions
  - Infections
  - Procedures
  - Other

History of Zika Virus and Microcephaly

1947	Zika virus identified in macaque in Uganda (Zika Forest)
1953	Zika virus recognized as cause of human illness in Nigeria
2007	Large outbreak of Zika virus illness in the State of Yap, Federated States of Micronesia
2013-2014	Large outbreak of Zika virus illness in French Polynesia
March 2015	Zika virus first identified in the Americas in Brazil
Sept 2015	Increased number of infants born with microcephaly noted in Brazil
Early 2016	Increase in microcephaly retrospectively noted in French Polynesia following the 2013-2014 outbreak
Jan 2016	CDC issues interim travel guidance for pregnant women for areas with ongoing Zika virus transmission

CDC Lab Confirms Zika In Fetal Tissues

- Zika virus shown to be present in fetal tissue
- Evidence of Zika virus has been detected in
  - Amniotic fluid
  - Placenta
  - Fetal brain tissue
  - Products of conception



Immunohistochemical staining of Zika virus antigen (red stain) in fetal brain tissue. This staining is present in the same areas where neuronal cell death/necrosis was identified by microscopic review of tissue morphology.

Reference/attribution for image: Ritter JM, Martines RB, Zaki SR. Arch Pathol Lab Med 2017; 141(1):49-59

Challenges to Determining Teratogenicity of Zika Virus (early 2016)

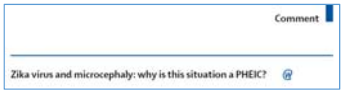
- Large proportion of people infected with Zika infection asymptomatic
- Laboratory testing initially not widely available (most early cases not laboratory-confirmed) and IgM testing challenging (cross-reactivity with other flaviviruses, length of IgM persistence unknown, etc.)
- Consistent and standardized case definitions of microcephaly not being used and baseline rate of microcephaly not well defined
- Mosquito-borne viruses not previously recognized as teratogenic in humans
- Rumors circulating about other possible causes (e.g., insecticides, genetically modified mosquitoes, vaccines)

Does Zika Virus Cause Adverse Pregnancy Outcomes?

- Koch's Postulates
- Bradford Hill Criteria
- Shepard's Criteria for Teratogenicity

Koch's Postulates

- [Micro]Organisms must be consistently detected in the locally affected tissues [...]
- The organisms [that seem plausible candidates for pathogens] must be isolated and grown in pure culture
- It must be possible to reproduce the disease using those pure cultures


Comment

Zika virus and microcephaly: why is this situation a PHEIC?

Heymann et al., Lancet 2016; 387:719-21

Bradford Hill Criteria

- Temporal relationship
- Strength
- Dose-Response Relationship
- Consistency
- Plausibility
- Consideration of Alternate Explanations
- Experiment
- Specificity
- Coherence


Opinion

EMBO Molecular Medicine

Causal or not: applying the Bradford Hill aspects of evidence to the association between Zika virus and microcephaly

Christina Frank, Mirko Faber & Klaus Stark

Frank et al., EMBO Mol Med 2016; 8(4):305-7

How Have Previous Teratogens Been Identified? Two Approaches

- "Astute clinician" approach – rare exposure-rare defect (most teratogens)
- Use of epidemiologic data to confirm an association

Shepard's Criteria for Teratogenicity

TERATOLOGY 50:97-98 (1994)

Letters

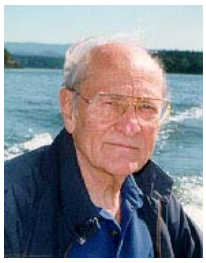
"Proof" of Human Teratogenicity

To the Editor:

Teratology Society Public Affairs Committee Position Paper

Causation in Teratology-Related Litigation

The Public Affairs Committee of the Teratology Society



Shepard, Teratology 1994; 50:97-98

Shepard's Criteria for Teratogenicity (continued)

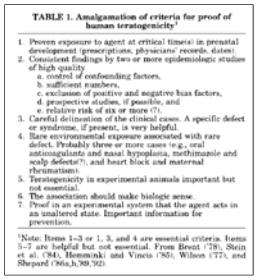


TABLE 1. Amalgamation of criteria for proof of human teratogenicity[1]

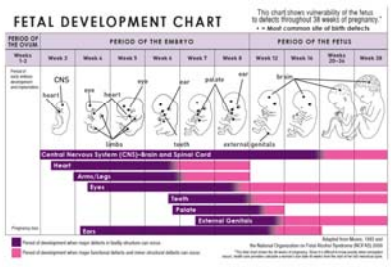
1. Proven exposure to agent at critical time(s) in prenatal development (prescriptions, physicians' records, dates).
2. Consistent findings by two or more epidemiologic studies of high quality
   a. control of confounding factors,
   b. sufficient numbers,
   c. exclusion of positive and negative bias factors,
   d. prospective studies, if possible, and
   e. relative risk of six or more (?).
3. Careful delineation of the clinical cases. A specific defect or syndrome, if present, is very helpful.
4. Rare environmental exposure associated with rare defect. Probably three or more cases (e.g., oral anticoagulants and nasal hypoplasia, methimazole and scalp defects(?), and heart block and maternal rheumatism).
5. Teratogenicity in experimental animals important but not essential.
6. The association should make biologic sense.
7. Proof in an experimental system that the agent acts in an unaltered state. Important information for prevention.

[1]Note: Items 1–3 or 1, 3, and 4 are essential criteria. Items 5–7 are helpful but not essential. From Brent ('78), Stein et al. ('84), Hemminki and Vineis ('85), Wilson ('77), and Shepard ('86a,b,'89,'92).

Shepard, Teratology 1994; 50:97-98

Shepard's Criteria for Teratogenicity

- Shepard Criterion #1 – Proven exposure to agent at critical time(s) in prenatal development
  - Yes, timing for severe microcephaly and intracranial calcifications appears to be late 1st/early 2nd trimester, based on information on some confirmed and many presumed cases of Zika virus infection

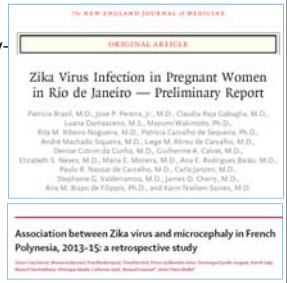


Shepard's Criteria for Teratogenicity

- Shepard Criterion #2 – Consistent findings by two or more epidemiologic studies of high quality
  - Control of confounding factors
  - Sufficient numbers
  - Exclusion of positive and negative bias factors
  - Prospective studies, if possible
  - Relative risk of 6 or more
  - Most data at that time were case series, not epidemiologic studies
  - Two epidemiologic studies that examined this association: Brasil et al. and Cauchemez et al.

Zika Virus and Microcephaly

- Study in Brazil: 42 women with laboratory-confirmed Zika virus infection with prenatal ultrasound
  - 12 (29%) abnormalities detected, including 2 intrauterine fetal deaths
- 2013-14 outbreak in French Polynesia
  - 8 cases of microcephaly identified
  - Modeling estimated infection with Zika during 1st trimester of pregnancy resulted in microcephaly risk of ≈1%


ORIGINAL ARTICLE

Zika Virus Infection in Pregnant Women in Rio de Janeiro — Preliminary Report

Association between Zika virus and microcephaly in French Polynesia, 2013-15: a retrospective study

Brasil et al.; N Engl J Med 2016. Published online March 4, 2016. http://dx.doi.org/10.1056/NEJMoa1602412
Cauchemez et al. Lancet 2016 May 21;387(10033):2125-32

Shepard's Criteria for Teratogenicity

- Shepard Criterion #3 – Careful delineation of clinical cases. A specific defect or syndrome, if present, is very helpful.
  - Phenotype in cases with presumed congenital Zika virus infection – brain abnormalities, including microcephaly and intracranial calcifications, cutis verticis gyrata, eye findings, arthrogryposis, club foot, IUGR, etc. -- some authors have used the term "presumed congenital Zika syndrome"
  - Findings in some cases consistent with fetal brain disruption sequence

Schuler-Faccini et al., MMWR Morb Mortal Wkly Rep 2016; 65(3):59-62
Costa et al., Ann Intern Med 2016; 164(10):689-91
Miranda-Filho et al., Am J Public Health 2016; 106(4):598-600

Shepard's Criteria for Teratogenicity

- Shepard Criterion #4 – Rare environmental exposure associated with rare defect
  - Rare defect
    - Congenital microcephaly – birth prevalence in US - 2-12/10,000
    - Fetal brain disruption sequence – no data on birth prevalence, but rare
  - Zika virus infection - "rare exposure"?
  - Zika virus infection – rare exposure among travelers

Brain Abnormalities in Fetus Born to Traveler and Prolonged Viremia

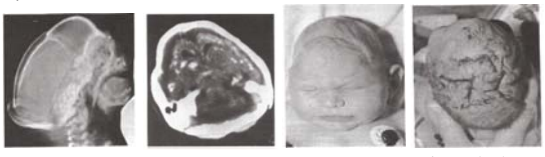
Case report

- Pregnant woman traveled during 11th week of gestation to Mexico, Guatemala, and Belize (Nov 22-29); symptom onset at 12 weeks gestation
- Prenatal ultrasound
  - Decrease in head circumference from 47th percentile at 16 weeks to 24th percentile at 20 weeks
  - Abnormal intracranial anatomy at 19 weeks
  - Fetal MRI at 20 weeks: brain abnormalities, including diffuse cerebral atrophy
- Postmortem evaluation following pregnancy termination at 21 weeks
  - Diffuse cerebral cortical thinning
  - High levels of Zika virus RNA; virus was cultured from brain tissue

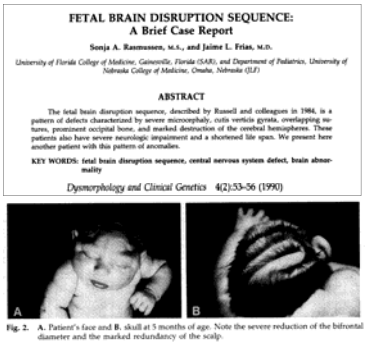
Driggers et al. N Engl J Med 2016; 374(22):2142-51

Zika Virus and Fetal Brain Disruption Sequence

- Findings in some cases were consistent with fetal brain disruption sequence
- First described in 1984 but noted in earlier literature
- Fetal brain disruption sequence includes severe microcephaly, overlapping sutures, prominent occipital bone, scalp rugae, and marked neurological impairment



*Moore, et al. J Pediatr 1990;116:383-386.



FETAL BRAIN DISRUPTION SEQUENCE:
A Brief Case Report

Sonja A. Rasmussen, M.S., and Jaime L. Frias, M.D.

University of Florida College of Medicine, Gainesville, Florida (SAR), and Department of Pediatrics, University of Nebraska College of Medicine, Omaha, Nebraska (JLF)

ABSTRACT

The fetal brain disruption sequence, described by Russell and colleagues in 1984, is a pattern of defects characterized by severe microcephaly, cutis verticis gyrata, overlapping sutures, prominent occipital bone, and marked destruction of the cerebral hemispheres. These patients also have severe neurologic impairment and a shortened life span. We present here another patient with this pattern of anomalies.

KEY WORDS: fetal brain disruption sequence, central nervous system defect, brain abnormality

Dysmorphology and Clinical Genetics 4(2):53-56 (1990)

Fig. 2. A. Patient's face and B. skull at 5 months of age. Note the severe reduction of the bifrontal diameter and the marked redundancy of the scalp.

Shepard's Criteria for Teratogenicity

- Shepard Criterion #5 – Teratogenicity in experimental animals important, but not essential
  - Marked neurotropism in mouse models (Bell et al., Arch Gesamte Virusforsch 35: 183 – 193, 1971; Dick et al., Trans R Soc Trop Med Hyg 46:509–520, 1952)
- No animal model published with Zika virus infection during pregnancy and fetal effects

Shepard's Criteria for Teratogenicity

- Shepard Criterion #6 – The association should make biologic sense
  - Consistent with findings in other viral teratogens
  - Pathologic findings implicate Zika virus is neurotropic and damages brain tissue
  - Other flaviviruses are teratogenic in animals

Shepard's Criteria for Teratogenicity

- Shepard Criterion #7 – Proof in an experimental system that the agent acts in an unaltered state. Important information for prevention
  - Applies to medications, not to infectious causes

Does Zika Virus Cause Adverse Pregnancy and Birth Outcomes?

Criteria for Proof of Human Teratogenicity
Items 1-3 OR 1, 3, 4 are essential criteria,
5-7 are helpful, but not essential

Criterion	Criterion Met?
1. Proven exposure to agent at critical time(s) during prenatal development	Yes
2. Consistent findings by ≥2 high-quality epidemiologic studies	Partially
3. Careful delineation of clinical cases	Yes
4. Rare environmental exposure associated with rare defect	Yes
5. Teratogenicity in experimental animals important but not essential	No
6. Association should make biologic sense	Yes
7. Proof in an experimental system that the agent acts in an unaltered state	NA

Zika Is a Cause of Microcephaly (Released by NEJM on April 13, 2016)

Zika Definitely Causes Birth Defects, U.S. Officials Announce

Zika virus definitely causes birth defects, CDC says

"Never before in history has there been a situation where a bite from a mosquito could result in a devastating malformation."
– Dr. Tom Frieden, former CDC Director, April 13, 2016

"…the last time an infectious pathogen (rubella virus) caused an epidemic of congenital defects was more than 50 years ago…"
– *New England Journal of Medicine*, April 13, 2016

Discussion

- Identification of Zika virus as a human teratogen - too soon or too late?
- Do Shepard's criteria need updating?
- Additional data accumulated since publication of N Engl J Med paper that support teratogenicity
  - Animal models, including mice (Cugola et al., 2016; Li et al., 2016; Miner et al., 2016) and chick (Goodfellow et al., 2016) models
  - Registry data from US and territories (Honein et al., 2017; Reynolds et al., 2017; Shapiro-Mendoza et al., 2017)
  - Epidemiologic data, including case-control study with overall odds ratio of 55.5 (95% CI, 8.6–infinity) (de Araujo et al., 2016)

Thank you!

Sonja Rasmussen, MD, MS
skr9@cdc.gov

For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 www.cdc.gov

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

National Center on Birth Defects and Developmental Disabilities

Congenital Infections

Exploring Their Role in Birth Defects and Other Adverse Pregnancy Outcomes

Cynthia A. Moore, MD, PhD

2017 National Birth Defects Prevention Network Annual Meeting
September 13, 2017

Congenital Infections

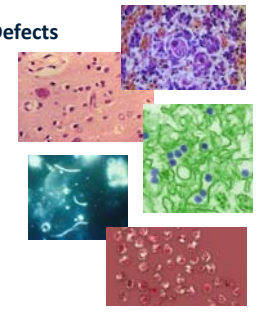
- Infections vertically transmitted from a pregnant woman to her embryo or fetus during pregnancy
- Variable effects depending on type of pathogen, timing during pregnancy, maternal and other factors – and information is limited on many pathogens
- Clinical manifestations also vary (e.g., asymptomatic, mild, non-specific)
- Information greatest on congenital infections encompassed under various iterations of the TORCH acronym
- Emerging infections often lack information on effects during pregnancy
  - New or caused by genetically changed existing organisms
  - Geographic spread of known pathogens to new populations
  - Previously unrecognized infections that emerge because of changes in the ecology
  - Previously controlled infections that have re-emerged (e.g., antimicrobial resistance)

Rasmussen et al., Am J Med Genet, 2007

Congenital Infections and Birth Defects

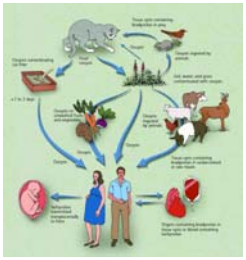
Known Teratogens

- *Toxoplasma gondii*
- *Treponema pallidum*
- Rubella
- Cytomegalovirus
- Herpesvirus 1 & 2
- Varicella-zoster virus
- Lymphocytic choriomeningitis virus
- Parvovirus B19
- Zika virus



Understanding and Responding to Congenital Infections

- Natural history (life cycle) of the pathogen and transmission to human host
- Mechanisms of vertical transmission
- Embryonic/fetal effects (pathogenesis and influencing factors)
- Diagnostic and treatment methods
- Epidemiology
- Prevention and control



The Placental Barrier

- Syncytiotrophoblast barrier occurs by the end of implantation (≈7 days)
- Uteroplacental circulation established and placenta sole barrier (≈12 weeks)
  - ➡ Mechanisms differ early vs late pregnancy
- Mechanisms of vertical transmission
  - Direct or contiguous infection of cell layers (e.g., maternal vascular endothelial cells, extravillous trophoblasts)
  - Trafficking of infected maternal immune cells across the placental barrier
  - Cell-associated transport from maternal blood to fetal capillaries
  - Breach secondary to syncytiotrophoblast layer or villus tree damage
  - Transvaginal ascending infection

Overcoming the Placental Barrier

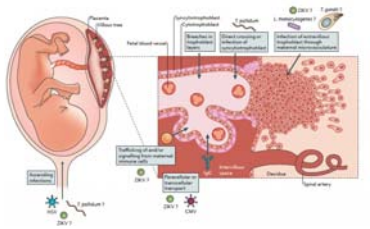
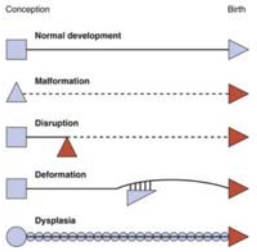


Figure 1. Routes used by TORCH pathogens to overcome the placental barrier.
Reprinted by permission from Macmillan Publishers Ltd: Nature Review Microbiology, 2016 Nov;14(11):707-715.

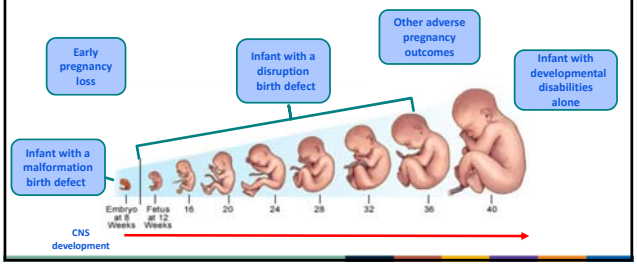
Types of Birth Defects



- Results from an intrinsically abnormal developmental process – neural tube defect
- Results from an extrinsic breakdown of an originally normal developmental process – amniotic band sequence
- Results from extrinsic mechanical forces on an originally normal structure – plagiocephaly
- Results from an abnormal organization of cells into tissues – achondroplasia

Adapted from Goodman & Gorlin, The Malformed Infant and Child, Oxford University Press, 1983

Congenital Infection and Clinical Phenotypes



Congenital Infection and Clinical Phenotypes

Congenital Phenotype	Congenital Infection			
	Rubella	Congenital CMV	Toxoplasmosis	Congenital LCMV
Early pregnancy loss	Fetal death	Possible fetal death	Fetal death	Fetal death
Birth defects – Malformations	Possible Cardiac	None	None	None
Birth defects – Disruptions	Microcephaly Reduced cortical tissue Ventriculomegaly Cardiac (PDA, PPS) Cataract, glaucoma, pigmentary retinopathy, microphthalmia	Microcephaly Reduced cortical tissue Ventriculomegaly Intracranial calcifications Cataract, glaucoma, pigmentary retinopathy, microphthalmia	Microcephaly Obstructive hydrocephaly Intracranial calcifications Cataracts, glaucoma, optic n. atrophy	Microcephaly Hydrocephaly Chorioretinitis
Developmental & sensory disorders	Congenital SNHL Mild to severe ID Motor disabilities Possible autism	Congenital & late-onset SNHL Mild to severe ID Motor disabilities	Possible SNHL Mild to severe ID	Possible SNHL Severe ID Motor disabilities
Other adverse pregnancy outcomes	IUGR Preterm birth Stillbirth	IUGR Preterm birth Stillbirth	Preterm birth Stillbirth	Possible IUGR Hydrops fetalis

Zika Virus (ZIKV) – Newly Identified Teratogen

- Single stranded RNA virus
- Closely related to dengue, yellow fever, Japanese encephalitis, and West Nile viruses
- Primarily transmitted by the *Aedes aegypti* mosquito
- Additional modes of transmission
  - Intrauterine and perinatal transmission
  - Sexual transmission
  - Laboratory exposure
  - Probable blood transfusion, organ transplantation
  - Possible breast milk

History of Zika Virus and Link to Microcephaly

1947	Zika virus identified in macaque in Uganda (Zika Forest)
1953	Zika virus recognized as cause of human illness in Nigeria
2007	Large outbreak of Zika virus illness in the State of Yap, Micronesia
2013 - 2014	Large outbreak of Zika virus infection in French Polynesia
Mar 2015	Zika virus first identified in the Americas in Brazil
Sept 2015	Increased number of infants born with microcephaly noted in Brazil
Early 2016	Increase in microcephaly retrospectively noted in French Polynesia following the 2013 - 2014 outbreak
Jan 2016	CDC issues interim travel guidance for pregnant women for areas with ongoing Zika virus transmission
Feb 2016	WHO declared Public Health Emergency of International Concern (PHEIC)
Nov 2016	WHO declared Zika a significant enduring public health challenge requiring intense action but no longer represents a PHEIC
Mar 2017	Affected infants continue to be born in the Americas and beyond

Zika Virus in Pregnancy

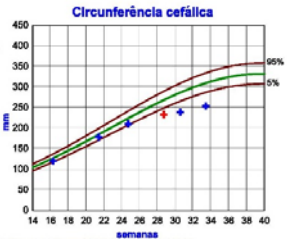
- Incidence of Zika virus infection in pregnant women is not known
- Infection can occur in any trimester
- No evidence of more severe disease compared with non-pregnant women
- No evidence of increased susceptibility during pregnancy
- Pregnant women can be infected
  - Through a mosquito bite
  - Through sex without a condom with an infected partner
- Zika virus can be passed to the fetus during pregnancy or around the time of birth

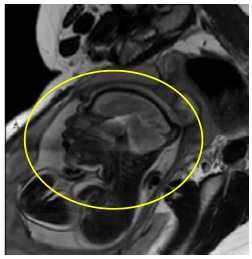
Congenital Zika Syndrome Cranial Morphology

- Severe microcephaly (most more than 3 SD below the mean)
  - Partial collapse of the skull with overlapping sutures
  - Occipital bone prominence
  - Small or absent anterior fontanel
  - Scalp rugae
- Consistent with fetal brain disruption sequence (FBDS)
- Not all with severe microcephaly have FBDS phenotype
- FBDS is rare but not unique to congenital Zika syndrome

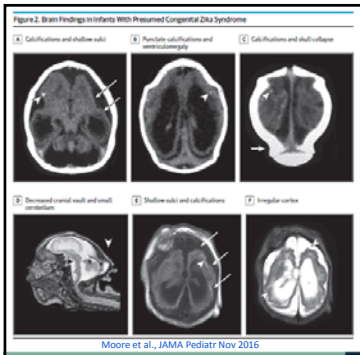
Moore et al., JAMA Pediatr Nov 2016

Congenital Zika Syndrome – *In Utero* Cranial Morphology





Courtesy of NOVA Diagnóstico por Imagem



Congenital Zika Syndrome Brain Anomalies

- Thin cerebral cortex
- Intracranial calcifications primarily subcortical in location*
- Hydrocephalus and hydrocephalus *ex-vacuo*
- Hydranencephaly
- Gyral abnormalities (polymicrogyria by imaging)
- Absent or hypoplastic corpus callosum
- Hypoplasia of the cerebellum or cerebellar vermis

*extremely rare or unique finding

Moore et al., JAMA Pediatr Nov 2016

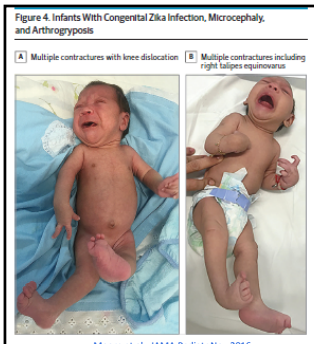
Congenital Zika Syndrome Ocular Findings

- Structural and anterior eye anomalies
  - Microphthalmia, coloboma
  - Cataracts, intraocular calcifications
- Posterior eye anomalies
  - Optic nerve hypoplasia, atrophy
  - Chorioretinal atrophy and scarring*
  - Macular pallor*
  - Gross pigmentary anomalies – generally in the macular area*
- No active chorioretinitis yet reported

*extremely rare or unique finding

Moore et al., JAMA Pediatr Nov 2016

Congenital Zika Syndrome Congenital Contractures



- Isolated and multiple congenital contractures (arthrogryposis) reported
- Clinical picture varies (e.g., large and small joints, upper or lower limbs or both, amyoplasia phenotype)
- Not previously associated with fetal brain disruption sequence phenotype*
- Reported but not well-documented with other congenital infections (e.g., rubella, varicella)
- Report of spinal cord hypoplasia in infants with contractures

***extremely rare or unique finding**

Moore et al., JAMA Pediatr Nov 2016

Congenital Zika Syndrome – Neurologic Sequelae

- Information on long-term medical and developmental outcomes or mortality sparse
- Neurologic sequelae reported to date in addition to contractures include the following:
  - Motor and cognitive disabilities (French Polynesia)
  - Epilepsy
  - Swallowing difficulties
  - Irritability with excessive crying
  - Vision loss and hearing impairment
  - Hypertonia and spasticity with tremors (pyramidal symptoms)
  - Dystonia and dyskinesia (extrapyramidal symptoms)*
  - Paralysis of the diaphragm

*extremely rare or unique finding

Congenital Zika Syndrome – Components of Unique Pattern

- Severe microcephaly with partial skull collapse
- Intracranial calcifications in the subcortical region
- Macular scarring and focal pigmentary retinal mottling
- Congenital contractures
- Neurologic abnormalities both pyramidal and extrapyramidal

Prenatal Zika Virus Infection – Congenital Zika Syndrome

ZIKA VIRUS

Disrupts future developmental processes

and

Destroys existing neural tissue

Prenatal Zika Virus Infection – Congenital Zika Syndrome

ZIKA VIRUS

Disrupts future developmental processes

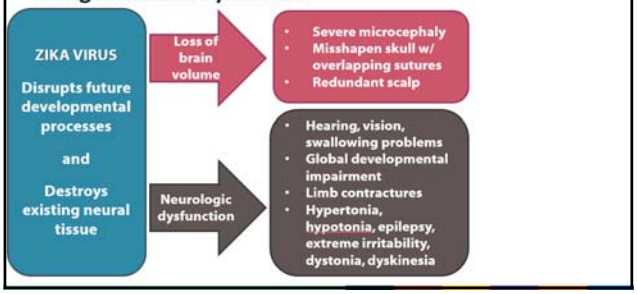
and

Destroys existing neural tissue

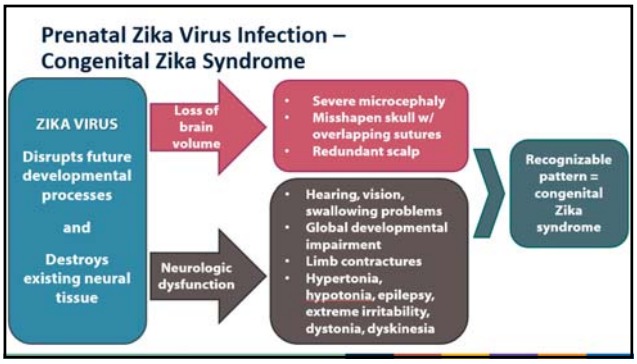
Loss of brain volume

- Severe microcephaly
- Misshapen skull w/ overlapping sutures
- Redundant scalp

Prenatal Zika Virus Infection – Congenital Zika Syndrome



Prenatal Zika Virus Infection – Congenital Zika Syndrome



Congenital Zika Syndrome – Expanding the Phenotype

- Possible expansion to infants with
  - Brain or eye anomalies but no microcephaly
  - Sensory/cranial nerve dysfunction with delayed onset
  - Other CNS anomalies with origin in the embryonic period
  - Non-neurologic congenital anomalies
  - Growth restriction

Congenital Zika Syndrome – Longer Term Medical Sequelae

- Longer term sequelae reported to date include the following:
  - Motor and cognitive disabilities (French Polynesia)
  - Hydrocephaly – some requiring a VP shunt
  - EEG abnormalities in infants without clinical seizures; worsening epilepsy
  - Feeding problems and severe reflux – some requiring a g-tube
  - Respiratory problems – diaphragmatic paralysis
  - Glaucoma
  - Potential cerebral palsy
  - Potential endocrine abnormalities
  - Microcephaly onset after birth

Congenital Zika Virus Infection Many Questions Remain – Some Partially Answered

- What is the full range of potential reproductive health problems that Zika virus infection may cause?
- Are there differences between Asian and African strains in terms of teratogenic potential and if so what underlies the differences?
- How long does the virus persist in various tissues after infection?
- What is the level of risk from a Zika virus infection during pregnancy?
- When during pregnancy does Zika virus infection pose the highest risk to the fetus?
- What are other factors (e.g., preceding or co-occurring infection, nutrition, presence of symptoms) that might affect the risk for birth defects?
- What are the expected patterns of anomalies comprising congenital Zika syndrome?

Studying the Impact of Emerging Infections on Embryo or Fetus – Challenges

- Effects of infection on the fetus are unknown and difficult to predict
  - Vary depending on infectious agent and timing of infection
  - Include spontaneous abortions, preterm birth, intrauterine growth restriction, birth defects and developmental disabilities
  - Later manifestations such as cognitive impairment might occur in infants appearing normal at birth

Rasmussen et al., Birth Defects Research 109:363-371, 2017

Studying the Impact of Emerging Infections on Embryo or Fetus – Challenges

- Infection could be mild or asymptomatic among pregnant women, despite significant effects on the embryo or fetus
- Infections might be missed because appropriate diagnostic tests not done
- Effects on fetus might differ depending on maternal severity and nature of illness, even in the absence of congenital infection

Rasmussen et al., Birth Defects Research 109:363-371, 2017

Studying the Impact of Emerging Infections on Embryo or Fetus – Challenges

- Diagnosis of infection in the fetus or infant can be challenging
  - Application of assays developed for adults to congenital infection can be difficult
  - Detection of specific IgM in infant serum provides strong evidence of infection, but false positives and false negatives occur
  - Sensitivity of diagnostic assays for newly-recognized teratogens might be unknown

Rasmussen et al., Birth Defects Research 109:363-371, 2017

Studying the Impact of Emerging Infections on Embryo or Fetus – Challenges

- Prophylaxis and treatment recommended for the general population might not be appropriate for pregnant women
  - Certain vaccinations or medications are contraindicated during pregnancy because of potential fetal effects
  - Fetal effects of most medications are not known
- Might be difficult to separate effects of infection from effects of treatment

Rasmussen et al., Birth Defects Research 109:363-371, 2017

Challenges in Determining Teratogenicity of Zika Virus (Early 2016)

- Large proportion of people infected with Zika infection asymptomatic
- Laboratory testing initially not widely available (most early cases not laboratory-confirmed) and IgM testing challenging (cross-reactivity with other flaviviruses, length of IgM persistence unknown, etc.)
- Consistent and standardized case definitions of microcephaly not being used and baseline rate of microcephaly not well-defined
- Mosquito-borne viruses not previously recognized as teratogenic in humans
- Rumors circulating about other possible causes (e.g., insecticides, genetically modified mosquitoes, vaccines)

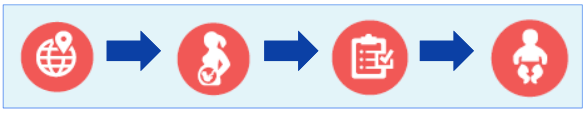
Examples of Studies Used to Examine Fetal Effects of West Nile and Zika Virus – Rasmussen et al., 2017

Type of Study Design	West Nile Virus	Zika Virus
Case Reports/Series	5 case reports of infected born to WNV-infected mothers (1 with congenital anomalies; 4 without)	Case series of infants assessed because of microcephaly demonstrated a consistent phenotype – congenital Zika syndrome
Birth Defects Surveillance Systems	Not used	CDC funded 45 jurisdictions to conduct surveillance for birth defects believed to be associated with congenital ZIKV infection
Pregnancy Registries	WNV Pregnancy Registry showed most infants to infected mothers had no abnormalities at birth or at 1 year Follow up of 11 infants for growth, development, eye outcomes showed no evidence of abnormalities up to age 3 years	United States Zika Pregnancy Registry Zika Active Pregnancy Surveillance System USZPR data used to study prolonged viremia among pregnant women and to estimate the risk of Zika-associated defects in fetuses and infants born to women possible infected with ZIKV during pregnancy

Examples of Studies Used to Examine Fetal Effects of West Nile and Zika Virus – Rasmussen et al., 2017

Type of Study Design	West Nile Virus	Zika Virus
Cohort Studies	Prospective cohort study used to study the effects of WNV during pregnancy including potential effects on developmental outcomes at 24 mos. showed typical development but longer follow-up needed	Prospective cohort of women with rash illness during pregnancy who tested positive and negative for ZIKV showed no differences in fetal deaths but adverse outcomes noted in 46% of Zika-affected pregnancies vs 11% of Zika-unaffected pregnancies
Case-Control Studies	Not used	Preliminary results from case-control study conducted in Brazil demonstrated a substantial association between congenital ZIKV infection and microcephaly with crude OR 55.5, 8.6-∞

Protecting Mothers and Babies from Emerging Threats



Identify emerging threat

Rapidly activate pregnancy and infant registries to monitor effects of threat among pregnant women and infants

Collect information on poor health effects during pregnancy to identify which outcomes to monitor

Adapt rapid birth defects surveillance systems to collect information about babies

Use data to develop recommendations for clinical care, plan for services needed by families affected by emerging threat, and improve prevention efforts nationwide

New Resource for Infections During Pregnancy

March 15, 2017
Volume 109, Number 5

Infections During Pregnancy – Established and Emerging

Guest Editors:
Dana M. Meaney-Delman
Denise J. Jamieson
Sonja A. Rasmussen

Special Thanks to Drs. Sonja Rasmussen, Peggy Honein, and Katie Arnold for their contributions to the presentation.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 www.cdc.gov

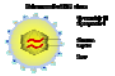
Prenatal Diagnosis and Imaging: CNS Abnormalities & Congenital Infections

CAREY EPPES, MD
BAYLOR COLLEGE OF MEDICINE
DIVISION OF MATERNAL FETAL MEDICINE
CHIEF OF OBSTETRICS BEN TAUB HOSPITAL

MARTINA BADELL, MD
EMORY UNIVERSITY
DIVISION OF MATERNAL FETAL MEDICINE
DIRECTOR, EMORY UNIVERSITY HOSPITAL MIDTOWN PERINATAL CENTER

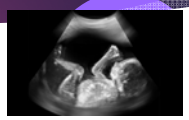
Outline

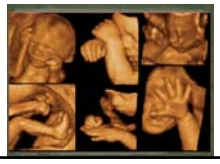
- Prenatal diagnostic modalities for congenital infections
  - Imaging
    - Ultrasound
    - MRI
  - Maternal serology
  - Amniocentesis
- Congenital infections with associated CNS abnormalities
  - Zika
  - CMV
  - Toxoplasmosis



Ultrasonography in Pregnancy: Prenatal diagnosis

- Settings for prenatal ultrasound (U/S)
  - Ob/gyn office
  - Emergency Room
  - Perinatal Center/Maternal-fetal Medicine
- Types of prenatal ultrasound (U/S)
  - 2D
    - First trimester, **anatomy**, growth
  - 3D





MRI in pregnancy: Prenatal Diagnosis

- The principal advantage of MRI is the ability to image deep soft tissue structures
  - Not operator dependent
  - Does not use ionizing radiation
  - No precautions or contraindications specific to the pregnant woman
- Utility
  - Improved visualization of
    - Cerebral biometry, Gyrations/Sulcations, Cerebral parenchyma
  - Demonstrated that fetal MRI yielded additional information compared to US and the ultrasound-based diagnosis and management were modified in 32% and 19% of cases, respectively (*n* = 145)
- Fetal MRI provides superior diagnostic capability compared to US concerning the fetal brain
- Access/Referral

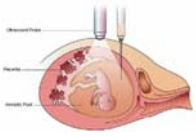
1. Pediatric radiology. , 2004, Vol.34(9), p.694-699
2. Levine D, Barnes PD, Robertson RR, Wong G, Mehta TS.
Fast MR imaging of fetal central nervous system abnormalities.
Radiology 2003;229:51–61.

Maternal serum testing for infection

- Indications for testing:
  - Maternal symptoms
  - Maternal exposure
  - Abnormal fetal ultrasound findings
- Types of testing:
  - IgM/IgG
  - NAT

Fetal Testing: Amniocentesis

- Amniocentesis Procedure
  - Under ultrasound guidance a needle inserted into amniotic sac to obtain small amount of amniotic fluid
  - Pregnancy related loss risk 1/300-1/600
- Indications
  - Genetic diagnosis
  - Infections
  - Other: lung maturity, Rh disease, polyhydramnios



Congenital infections with associated CNS abnormalities

- Zika
- Cytomegalovirus (CMV)
- Toxoplasmosis

Zika Virus

- Zika emerging mosquito borne flavivirus
- One of the rare infections in pregnant women → congenital anomalies
- Zika virus biology not fully defined → challenges managing & counseling about exposures and infection in pregnancy
- Maternal symptoms:
  - Most won't have symptoms or will only have mild symptoms
  - The most common symptoms of Zika are
    - Fever
    - Rash
    - Headache
    - Joint pain
    - Conjunctivitis (red eyes)
    - Muscle pain

Zika: Screening & Diagnosis

- All pregnant women in US should be asked about possible Zika virus exposure before and during the current pregnancy at every prenatal visit
- Pregnant women with possible Zika exposure and symptoms should be tested to diagnose cause of their symptoms
  - NAT & IgM
- Asymptomatic pregnant women *with ongoing* possible Zika exposure should be offered Zika NAT testing 3 times during pregnancy
- Asymptomatic pregnant women with recent possible Zika exposure but *without ongoing* exposure not recommended to have Zika testing
- Pregnant women with possible Zika exposure who have a fetus with US findings concerning for Zika should be tested
  - NAT and IgM

MMWR July 24th, 2017

Zika: Diagnosis

- Serology test results can be difficult to interpret & adverse outcomes caused by Zika during pregnancy are not fully described
  - Pregnant women with lab evidence of recent flavivirus infection are considered to have possible Zika virus infection & should be monitored frequently
    - → More ultrasounds
- Referral to a maternal–fetal medicine (MFM)/perinatologist or infectious disease specialist with expertise in pregnancy management is recommended
  - Maternal infection or abnormal ultrasound

https://www.cdc.gov/zika/healtheffects/birth_defects.html

Congenital Zika Syndrome

- Pattern of birth defects found among fetuses/babies infected with Zika during pregnancy
- **5 features**
  - Severe microcephaly where the skull has partially collapsed
  - Decreased brain tissue with a specific pattern of brain damage
  - Damage to the back of the eye
  - Joints with limited range of motion, such as clubfoot
  - Too much muscle tone restricting body movement soon after birth
- Some infants with congenital Zika virus infection who do not have microcephaly at birth may later experience slowed head growth and develop postnatal microcephaly

https://www.cdc.gov/zika/healtheffects/birth_defects.html

Zika: Fetal Evaluation

- U/S particularly if obtained close to the time of infection, may not preclude later manifestations
  - Some reported only postnatal abnormalities
- Some data suggested most severe adverse outcomes appear to be more common but are not limited to women infected in the **first trimester**
- Data suggest that severe outcomes are not limited to symptomatic pregnant women
- Repeat imaging should be considered if Zika testing suggests infection

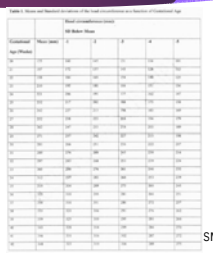
Fetal Evaluation via U/S

- **Prenatal U/S to evaluate for fetal abnormalities consistent with congenital Zika virus syndrome is recommended for all pregnant women tested for Zika, regardless of laboratory findings**
- U/S to assess fetal anatomy (neuroanatomy) and fetal growth
- They should focus on development of findings such as:
  - Microcephaly
  - Intracranial calcifications
  - Ventriculomegaly
  - Arthrogryposis
  - Abnormalities of the corpus callosum
  - Cerebrum
  - Cerebellum
  - Eyes
  - Other brain abnormalities
    - The International Society of Ultrasound in Obstetrics and Gynecology offers a tutorial to help providers

https://www.cdc.gov/zika/healtheffects/birth_defects.html

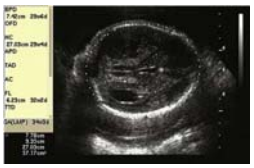
Zika- Microcephaly

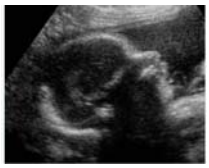
- Head circumference
  - Less than 3 SD below the mean



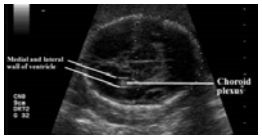
SMFM

Zika: Microcephaly

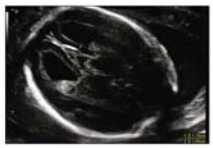




Zika: Ventriculomegaly



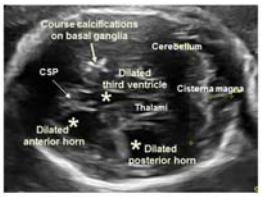
Normal

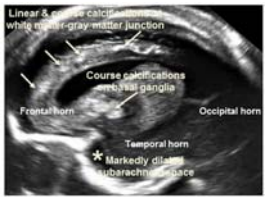


Ventriculomegaly

Mild	>1.0-1.2 cm
Moderate	>1.2-1.5 cm
Severe	>1.5 cm

Zika: Intracranial calcifications





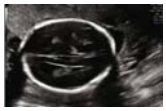
Testing for Zika virus infection in pregnancy: key concepts to deal with an emerging epidemic. Eppes C, et al. Am J Obstet Gynecol. 2017 Mar;216(3):209-225

Zika: -Corpus callosum abnormalities

- Corpus callosum is the largest midline commissure of the brain connecting the neocortex of the cerebral hemispheres
- In complete agenesis of the corpus callosum (ACC) there is a total failure of the commissure to develop

Incidence:
- Among live births ~1.8 /10,000
- Developmental disabilities 2/100
- Existing CNS anomaly ~47 /100

- Associated with a broad range of clinical manifestations ranging from normal to severe psychomotor delay



Zika Fetal Testing Amniocentesis?

- If U/S suspicion for fetal anomaly amniocentesis for Zika virus testing may be considered particularly if being performed for genetic testing
  - **Unknown:**
    - How long after a pregnant woman becomes infected she can transmit the virus to the fetus
    - Duration amniotic fluid will be ZIKV RNA NAT positive
    - Ability of the test is to determine the presence of fetal injury

Zika: Newborn/Infant

- All infants born to mothers who have lab evidence of Zika infection during pregnancy should receive:
  - Comprehensive physical exam
  - Neurologic assessment
  - Head ultrasound
  - Standard newborn hearing assessment
  - Zika virus testing
- Testing is recommended for infants
  - Born to mothers who have laboratory evidence of Zika virus infection
  - Infants who have abnormal clinical findings suggestive of congenital Zika syndrome and a maternal epidemiologic link suggesting possible exposure during pregnancy, regardless of maternal test results.
- A Zika virus RNA NAT test should be performed on both infant serum and urine
- Zika virus immunoglobulin M (IgM) antibody should be performed on infant serum
  - Testing should be performed on specimens collected from infants within 2 days after birth

Interim Guidance for the Evaluation and Management of Infants with Possible Congenital Zika Virus Infection – MMWR, August 19, 2016)

Cytomegalovirus (CMV)

- **Epidemiology**
  - Seroprevalence increases with age
    - Overall in US 59%
  - 1-4% of pregnant women seroconvert
  - Lower SES more likely to have been exposed
  - Day Care, health care workers
- **Primary**
  - Ongoing viral secretion can occur for 6 months
- **Recurrent**
  - Periodic viral shedding

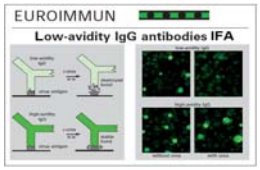
CMV Awareness
www.cmvawareness.org

CMV: Maternal Symptoms

- Asymptomatic, malaise, fever, generalized lymphadenopathy, and hepatosplenomegaly
- Patients who are immunocompromised may develop extremely serious sequelae of infection, including chorioretinitis and pneumonitis

CMV: Screening and Diagnosis

- IgG
- IgM
  - May remain 9 to 12 months after infection
  - May become positive in re-infection, or remain negative
- Avidity
  - New infection: Low avidity
- Reactivation
- Amniocentesis PCR
  - Interval from infection to amnio (7 weeks)
- Neonatal urine and blood PCR



CMV: Transmission

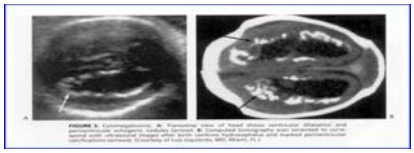
- Rates of transmission correlated with gestational age
  - However severity is the inverse
- Transmission:
  - First trimester- 35%
  - Second trimester- 42%
  - Third trimester- 59%
- Severity: (Impacted by termination rates)
  - First>Second>Third

Feldman et al. AJOG 2011

CMV: Diagnosis

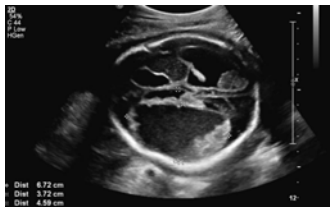
- The most sensitive and specific test for diagnosing congenital CMV infection is the identification of CMV in amniotic fluid by either culture or PCR
- Identification of the virus in amniotic fluid by culture or PCR does not necessarily delineate the severity of fetal injury

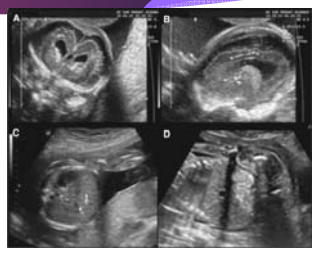
CMV: Ultrasound images



- CNS:
  - Microcephaly
  - Ventriculomegaly
  - Brain Calcifications
- Echogenic bowel
- Growth restriction
- Calcifications: liver, spleen
- Hydrops

CMV: Ultrasound

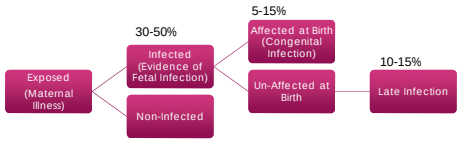




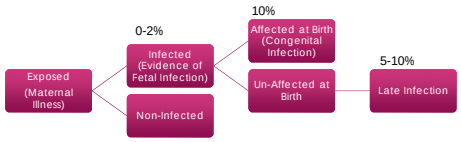
CMV: Neonatal Findings

- Hepatosplenomegaly
- Thrombocytopenia with resultant petechiae
- Intracranial calcifications
- Small for Gestational Age
- Hepatitis and jaundice
- Microcephaly
- Chorioretinitis
- Hearing loss
- Mental retardation
- Seizures

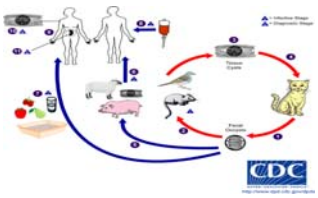
Neonatal Outcomes: Primary CMV Infection



Neonatal Outcomes: Recurrent CMV Infection



Toxoplasmosis



Toxoplasmosis

- Maternal Infection- starts with ingestion of cysts from uncooked/undercooked meat of infected animals or contact with oocysts from infected cats or contaminate soil
- Fetal/neonatal disease:
  - More severe if maternal infection in 1st trimester
  - Incidence of transmission highest in 3rd trimester
- Prevention:
  - Avoid exposure
    - Cat litter
    - Undercooked meats
    - Washing fruits and vegetables

1st 2nd 3rd → Transmission

Severity ← 1st 2nd 3rd

Toxoplasmosis: Symptoms Infected Pregnant Women

- Typically Asymptomatic
- Mononucleosis like symptoms
  - Adenopathy
- Can be severe in immunosuppressed
  - Chorioretinitis
  - Brain abscess

TOXO: Detection

- (#1) IgM
  - Sensitivity 28%
  - Occasionally not detected in early infection
  - Can persist for months to years
  - Often negative in immunosuppressed
- (#2 a) Avidity
  - If IgM positive, this is next step
- (#2 b) IgG
  - Done if avidity not available
  - Repeat titers 3 weeks later
- PCR
  - Amniocentesis or Cordocentesis

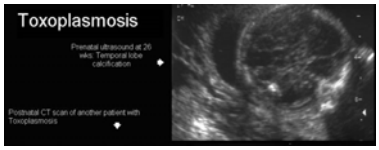
Toxoplasmosis Testing

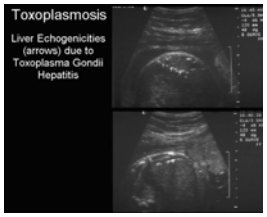
Antibodies	Uninfected	Recent (Acute) Infection	Chronic (latent) infection
IgM	Absent *	Present in almost all cases. Weeks-months	Most often absent, in 5% persist for years**
IgG	Absent	Present. Rise from low (2IU/mL) to high (300-6000 IU/mL) titers Takes 2-6 months to reach peak	Present
IgG Avidity	Absent	Low	High*** Rarely low avidity can persist for a year
IgA	Absent	Present	Absent
IgE	Absent	Present	Absent

Toxoplasmosis: Fetal Ultrasound Findings

- Calcifications
  - Brain
  - liver
- Growth Restriction
- Microcephaly
- Splenomegaly
- Ventriculomegaly
- Hydrops

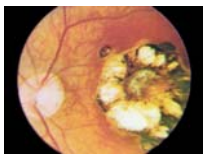
Toxoplasmosis: Ultrasound findings



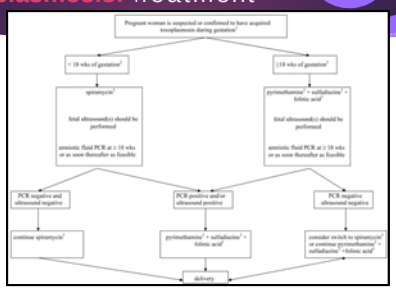


Toxoplasmosis: Neonatal Findings

- Hepatosplenomegaly
- Chorioretinitis
- CNS injury
- Seizures
- Mental Retardation



Toxoplasmosis: Treatment



Goldstein E J C et al. Clin Infect Dis. 2008;47:554-566

Conclusion

- Congenital Infections leading to CNS abnormalities
  - Zika, CMV, toxoplasmosis
    - Exposure or symptoms
    - Ultrasound abnormalities
      - Use of MRI
- Diagnosis
  - Maternal serology
  - Positive Culture
  - Amniocentesis