Longitudinal hearing outcomes in infant macaques exposed prenatally to Zika virus

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BACKGROUND

- Zika virus (ZIKV), a mosquito-borne virus, is transmitted vertically from mother to fetus.[1]
- Children with congenital Zika syndrome present with structural anomalies, visual and auditory deficits, and other neuropsychomotor and language delays.[2]
- 5.8% of human infants with confirmed congenital Zika syndrome have sensorineural hearing loss in conjunction with microcephaly.[3]
- Another congenital viral infection, CMV, is the most common cause of non-genetic sensorineural hearing loss that can present in early childhood.
- It is unknown if congenital Zika virus infection causes late onset hearing loss, as the pathogenesis of hearing loss in ZIKV infected neonates without severe physical malformations is unknown.[4]
- Model: Rhesus macaques have comparable auditory nervous system development to humans among other developmental milestones that are useful for modeling congenital ZIKV exposure[3]
- Auditory evoked potentials have been studied in Rhesus macaques and remain a reliable method for evaluating the auditory nervous system development.[5]

METHODS

- Translational macaque model of vertical transmission of Zika virus
- Multiple maternal ZIKV infection conditions were utilized:

Treatment group	Inoculation timepoint (Gestational days)	Sample size	Purpose
ZIKV-PR (Puerto Rican isolate)	30gd	n=7	Examine if early in the first trimester inoculation modulates worse hearing outcomes because of the timing and development of the auditory nervous system.
ZIKV-PR	45gd	n=7	Examine if later in first trimester inoculation modulates less severe hearing outcomes than early inoculation.
ZIKV-DAK (Dakar African isolate)	45gd	n=4	Examine if African isolate has worse hearing outcomes compared to Puerto Rican isolate. African isolates of Zika virus are reported to have higher transmissibility and pathogenicity than the contemporary PR isolate.[6]
DENV/ZIKV (Dengue/Puerto Rican isolate)	45gd	n=7	Examine if history of maternal Dengue infection followed by ZIKV-PR infection impacts the severity of disease outcomes. Based on the principles of antibody-dependent enhancement, we hypothesize that previous Dengue immunity has the potential to increase the severity of ZIKV pathogenesis.
Control (Saline)	45gd	n=8	Control animals were inoculated with saline and provide a model for normal development of auditory pathways in macaques.

- Auditory brainstem response (ABR) testing:
  - Examines the function of the inner ear and auditory pathway from the auditory nerve to the brainstem.
  - Electrical activity from the auditory pathway are detected by needle electrodes at the brow ridge (positive input) and behind the right and left pinna (negative inputs).
  - Sound stimuli were presented to the infant via earphones.
  - Auditory pathway responsiveness to the stimuli were quantified by measuring wave IV latency.
- Procedure
  - Evaluation were conducted at 1, 3, 6, and 12 months of age.
  - All infants had normal head circumferences.
  - Auditory brainstem response tests were conducted at three different stimuli procedures and at varying volumes:
    - Click ABR delivered at 70, 50, 30, and 20 dB nHL.
    - Tone burst ABR (1000 Hz) delivered at 80, 60, and 40 dB nHL.
    - Tone burst ABR (500 Hz) delivered at 80, 60, and 40 dB nHL.

We hypothesized that infants would have worse hearing if exposed to ZIKV early in the first trimester, had exposure to the African isolate (ZIKV-DAK), or were born to dams with a history of Dengue infection.

Late 1st trimester (45gd) Zika-exposed infant macaques ***displayed statistically significantly hearing outcomes*** over the first year of life compared to control animals. ***Normal hearing outcomes*** were observed in all groups we hypothesized would face hearing deficits. Although major hearing deficits were not detected in our study, our model supports that ***prenatal Zika virus exposure may result in worse hearing outcomes in early infancy; however further investigation of hearing outcomes is necessary after 1 year of life.***

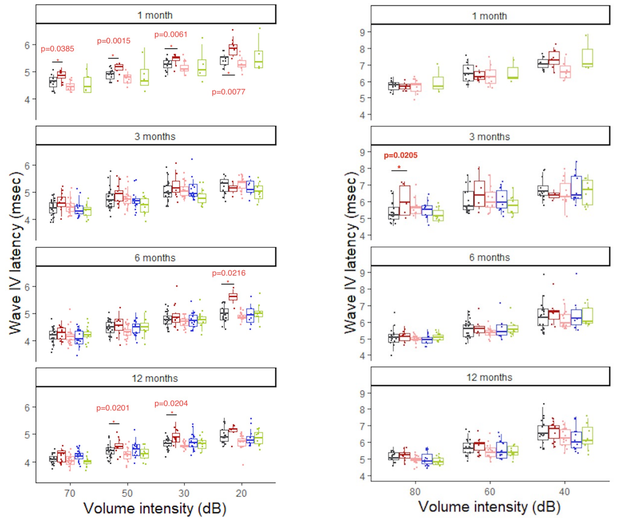
RESULTS

Click stimulus:

- **Significant differences in hearing (wave IV latency) at 1, 6, and 12 months between ZIKV-PR 45gd and control**

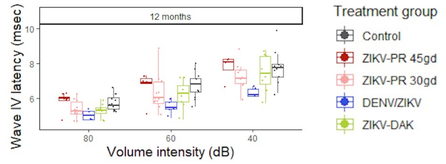
1000 Hz stimulus:

- **Significant difference in hearing (wave IV latency) at 3 months between ZIKV-PR 45gd and control**



500 Hz stimulus:

- **No significant differences in hearing (wave IV latency)**



- Our macaque model displays statistically significant differences in hearing outcomes (ABR wave IV latencies) between control animals and the late infection timepoint ZIKV-PR (45gd) for click and 1000Hz stimulus over year of life.
- Prolonged wave IV latencies were observed in the ZIKV-PR late infection group compared to control animals.
- Among treatment groups that had no significant hearing loss, the following normal developmental trends were observed:
  - Wave IV latency increased as stimulus intensity (dB) decreased over a single testing period.
  - Wave IV latency decreased over the first year of life.

CONCLUSIONS

- Utilizing a macaque model allows us to study the onset and pathogenesis of sensorineural hearing loss and to understand the spectrum of neurodevelopmental consequences associated with exposure.
- Prenatal ZIKV infection may result in hearing deficits occurring during the first year of life, which is similar to three years of life in human children.
- We will measure hearing at three years of life, which is similar to nine years in human development, to determine if delayed onset hearing loss can continue into early childhood.
- Future findings are important for early diagnosis and to create intervention strategies to mitigate these consequences associated with deafness.

ADDITIONAL KEY INFORMATION

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