

In the format provided by the authors and unedited.

Miscarriage and stillbirth following maternal Zika virus infection in nonhuman primates

Dawn M. Dudley¹, Koen K. Van Rompay^{2,3*}, Lark L. Coffey³, Amir Ardeshir², Rebekah I. Keesler², Eliza Bliss-Moreau², Peta L. Grigsby⁴, Rosemary J. Steinbach⁴, Alec J. Hirsch^{5,6}, Rhonda P. MacAllister⁷, Heidi L. Pecoraro⁸, Lois M. Colgin⁸, Travis Hodge⁷, Daniel N. Streblow^{5,6*}, Suzette Tardif^{9*}, Jean L. Patterson¹⁰, Manasi Tamhankar¹⁰, Maxim Seferovic¹¹, Kjersti M. Aagaard^{11,12}, Claudia Sánchez-San Martín¹³, Charles Y. Chiu^{13,14}, Antonito T. Panganiban^{15,16*}, Ronald S. Veazey¹⁷, Xiaolei Wang¹⁷, Nicholas J. Maness¹⁵, Margaret H. Gilbert¹⁸, Rudolf P. Bohm¹⁸, Kristina M. Adams Waldorf^{19*}, Michael Gale Jr^{20,21}, Lakshmi Rajagopal^{22,23}, Charlotte E. Hotchkiss²⁴, Emma L. Mohr²⁵, Saverio V. Capuano III²⁶, Heather A. Simmons²⁶, Andres Mejia²⁶, Thomas C. Friedrich^{26,27}, Thaddeus G. Golos^{26,28} and David H. O'Connor^{1,26*}

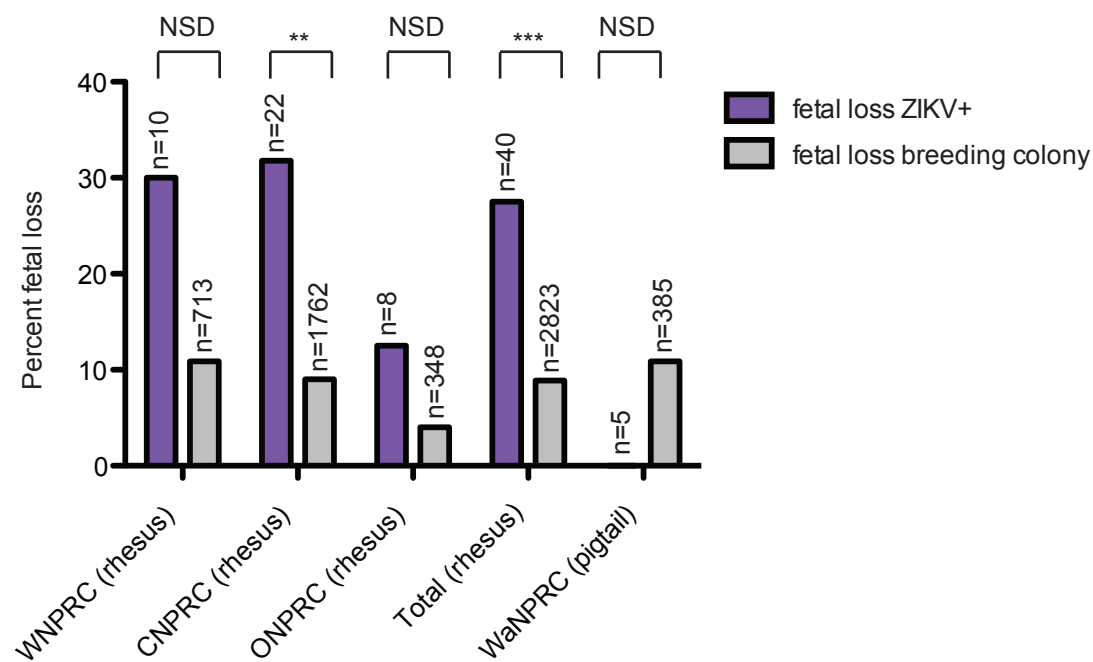
¹Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI, USA. ²California National Primate Research Center, University of California-Davis, Davis, CA, USA. ³Department of Pathology, Microbiology, and Immunology, University of California-Davis, Davis, CA, USA.

⁴Department of Reproductive and Developmental Sciences, Oregon National Primate Research Center, Portland, OR, USA. ⁵Vaccine and Gene Therapy Institute, Oregon Health and Science University, Portland, OR, USA. ⁶Division of Pathobiology and Immunology, Oregon National Primate Research Center, Portland, OR, USA. ⁷Division of Comparative Medicine, Oregon National Primate Research Center, Portland, OR, USA. ⁸Pathology Services Unit, Division of Comparative Medicine, Oregon National Primate Research Center, Portland, OR, USA. ⁹Southwest National Primate Research Center, Southwest Foundation for Biomedical Research, San Antonio, TX, USA. ¹⁰Department of Virology and Immunology, Texas Biomedical Research Institute, San Antonio, TX, USA. ¹¹Departments of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, USA. ¹²Department of Molecular and Human Genetics at Baylor College of Medicine, Houston, TX, USA. ¹³Department of Laboratory Medicine, University of California, San Francisco, San Francisco, CA, USA.

¹⁴Department of Medicine/Infectious Diseases, University of California, San Francisco, San Francisco, CA, USA. ¹⁵Division of Microbiology, Tulane National Primate Research Center, New Orleans, LA, USA. ¹⁶Department of Microbiology and Immunology, Tulane University Health Sciences Center, New Orleans, LA, USA. ¹⁷Division of Comparative Pathology, Tulane National Primate Research Center, New Orleans, LA, USA. ¹⁸Division of Veterinary Medicine, Tulane National Primate Research Center, New Orleans, LA, USA. ¹⁹Department of Obstetrics and Gynecology, University of Washington, Seattle, WA, USA. ²⁰Department of Immunology, University of Washington, Seattle, WA, USA. ²¹Center for Innate Immunity and Immune Disease, University of Washington, Seattle, WA, USA. ²²Department of Pediatrics, University of Washington, Seattle, WA, USA. ²³Center for Global Infectious Disease Research, Seattle Children's Research Institute, Seattle, WA, USA. ²⁴Washington National Primate Research Center, University of Washington, Seattle, WA, USA.

²⁵Department of Pediatrics, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI, USA. ²⁶Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI, USA. ²⁷Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA. ²⁸Department of Comparative Biosciences and Obstetrics and Gynecology, University of Wisconsin-Madison, Madison, WI, USA.

*e-mail: kkvanrompay@ucdavis.edu; streblow@ohsu.edu; stardif@txbiomed.org; apanagani@tulane.edu; adamsk@u.washington.edu; dhoconno@wisc.edu



Supplementary Figure 1: Percent fetal loss in Zika inoculated pregnant animals or animals in breeding colonies from each macaque cohort. The raw data of fetal losses was compared to the raw data of live births in either Zika virus -exposed or Zika virus naive animals at each center using a Fisher's exact test. The raw numbers from each center in each group of rhesus macaques were then added together and compared in the Total column using a Chi-Square test. p-values are reported from each test respectively. NSD-not statistically different. **p=0.003. ***p<0.0001.

Supplementary Table 1: Average rates of fetal demise among nonhuman primate cohorts infected with ZIKV during pregnancy.

	ZIKV isolate	Dose (PFU)	Route of inoculation ^a	Ave. inoculation gestational age (GA)	inoculation GA that ended in demise	Fetal Demise n (%)
Rhesus macaque (n=6)	Brazil SPH_2015 + PRVABC59	4 x10 ³	IA+IV	48	45, 45, 51, 44	4 (66.7)
Rhesus macaque (n=4)	Brazil SPH_2015	2 x10 ⁵	IA+IV	61	41	1 (25)
Rhesus macaque (n=2)	FP/2013	1x10 ⁴	SC	33	N/A	0
Rhesus macaque (n=2)	FP/2013	1x10 ⁴	SC	112	N/A	0
Rhesus macaque (n=12)	PRVABC59	1x10 ³	SC	30	30, 30	2 (16.7)
Rhesus macaque (n=8)	PRVABC59	1x10 ⁴	SC	45	46, 46	2 (25)
Rhesus macaque (n=6)	PRVABC59	1x10 ⁵	SC	49	54	1 (16.7)
Rhesus macaque (n=2)	PRVABC59	1x10 ⁵	SC	115	N/A	0
Rhesus macaque (n=1)	PRVABC59	5x10 ⁷	SC	50	50	1 (100)
Pigtail macaque (n=2)	FSS 13025_Cambodia	5x10 ⁷	SC	101	N/A	0
Pigtail macaque (n=3)	Brazil 2015 Fortaleza	5x10 ⁷	SC	61	N/A	0
Common marmoset (n=2)	Brazil SPH_2015	2.5x10 ⁵	IM	76	79/83, 68/72	2 (100)

^aIA=intra-amniotic; IV=intravenous; SC=subcutaneous; IM=intramuscular. N/A=not applicable

Supplementary Table 2: Rates of spontaneous abortion or stillbirth in healthy breeding colony rhesus (CNPRC, ONPRC, WNPRC) and pigtail (WaNPRC) macaques.

Institution	Breeding season	Spontaneous loss	Total pregnancies	Percent losses (%)
WNPRC	2012	20	114	17.5
	2013	11	113	9.7
	2014	10	142	7.0
	2015	10	103	9.7
	2016	12	124	9.7
	2017 (partial)	15	117	12.8
			Weighted average	10.9
CNPRC	2008-2009	19	235	8.1
	2009-2010	23	242	9.5
	2010-2011	15	210	7.14
	2011-2012	23	230	10.0
	2012-2013	24	173	13.9
	2013-2014	15	165	9.1
	2014-2015	18	128	14.1
	2015-2016	10	187	5.4
	2016-2017 (partial)	11	192	5.7
			Weighted average	9.0
ONPRC ^a	2007	6	47	12.8
	2008	3	36	8.3
	2009	0	37	0.0
	2010	1	18	5.6
	2011	1	11	9.1
	2012	0	9	0.0
	2013	0	6	0.0
	2014	0	20	0.0
	2015	1	39	2.6
	2016	1	75	1.3
	2017 (partial)	1	50	2.0
			Weighted average	4.0
WaNPRC	2014	13	114	11.4
	2015	7	91	7.7
	2016	15	122	12.3
	2017 (partial)	7	58	12.1
			Weighted average	10.9

^aPregnancies were confirmed at greater than gestational day (GD) 30 and delivered by cesarean section between GD 135 and GD 155, and therefore the risk of very early or late-term abortion in healthy pregnant animals is not captured in these statistics from ONPRC. Other primate centers include data from animals with natural or C-section delivery between GD 155 and GD 165.

Supplementary table 4: Detection of Zika virus RNA in maternal reproductive/fetal extraembryonic and fetal tissues from fetal demise and neonatal cases.

Institution	Animal ID	Pregnancy outcome	Maternal reproductive/fetal extraembryonic tissues with positive ZIKV RNA*	Fetal tissues with positive ZIKV RNA*	Limit of detection of viral load assay	Publication including data
CNPRC	5303	Fetal death GD48	Placenta, chorioamnion, umbilical cord, amniotic fluid	Brain, liver, heart, lung, intestine, kidney	100 RNA copies/ml fluid or 1000 copies per gm tissue	Coffey et al (NCOMMS-17-31047B, in press)
CNPRC	5508	Fetal death GD60	Placenta, amniotic membranes, umbilical cord, amniotic fluid	Eye, lung, stomach, intestine, heart, kidney/adrenal, skin, urinary bladder	100 RNA copies/ml fluid or 1000 copies per gm tissue	
CNPRC	5374	Fetal death GD35	Placenta, amniotic membranes	(No tissue available)	100 RNA copies/ml fluid or 1000 copies per gm tissue	
CNPRC	5463	PPROM with imminent abortion GD115	Placenta	Brain area 46, brain occipital lobe, kidney, lung, ileum	100 RNA copies/ml fluid or 1000 copies per gm tissue	
CNPRC	5592	Fetal death GD59	Placenta, amniotic membranes, umbilical cord	Brain, eye, lung, liver, stomach, intestine, heart, kidney/adrenal, skin, urinary bladder	100 RNA copies/ml fluid or 1000 copies per gm tissue	
CNPRC	5266	Stillbirth GD166	Fetal placenta, maternal placenta, umbilical cord	None	100 RNA copies/ml fluid or 1000 copies per gm tissue	
CNPRC	5804	Fetal death GD86	Placenta, amniotic membranes	Brain (parietal lobe), kidney, lung, ileum	100 RNA copies/ml fluid or 1000 copies per gm tissue	
WNPRC	527453 (dam) 291635 (fetus)	Fetal death GD95	Amniotic/chorionic membrane, decidua, placental disk 1, umbilical cord, and placental bed.	Adipose-omentum, aorta-thoracic, cerebrum, colon, cornea, dura mater, heart, jejunum, liver, lung, muscle-quadriceps, pericarium, retina, kidney, sclera, prostate, spleen, stomach, testis, thymus, urinary bladder, axillary LN.	100 copies/ml plasma or 192 copies/reaction for tissues	Mohr et al. PLoS One. Jan 30;13(1): e0190617. doi:10.1371/journal.pone.0190617.eCollection 2018. PMID:29381706.
WNPRC	125500 (dam) 411377 (fetus)	Stillbirth GD155	Amniotic/chorionic membrane, decidua, placental disc 1, umbilical cord, and placental bed.	Adipose-omentum, adrenal gland, cerebrum (5 sections), cervical spinal cord, cornea, dura mater, epidermis/dermis abdomen, esophagus, optic nerve, femur, heart, jejunum, liver, lumbar spinal cord, lung, mesenteric LN, ovary, pancreas, muscle-quadriceps, retina, kidney, sclera, spleen, stomach, thymus, thyroid, tongue, tonsil, urinary bladder, uterus.	100 copies/ml plasma or 192 copies/reaction for tissues	
WNPRC	795784 (dam) 499874 (neonate)	Live birth GD155; clinically indicated neonatal euthanasia GD162	Decidua (some still pending)	None	100 copies/ml plasma or 192 copies/reaction for tissues	
WNPRC	664184 (dam) 416597 (fetus)	Fetal death GD133	None (some still pending)	None (some still pending)	100 copies/ml plasma or 192 copies/reaction for tissues	
SNPRC	34103	Fetal deaths GD95	Placenta (ISH)	Fetus abdomen/thorax (viral load) Ocular muscle (ISH)	15 RNA copies/ml	http://dx.doi.org/10.1101/259317
SNPRC	33722	Fetal death GD86	Placenta (ISH)	Fetus abdomen/thorax (viral load) Ocular muscle, ophthalmic nerve, neuroprogenitor cells of the developing cortical plate (ISH)	15 RNA copies/ml	http://dx.doi.org/10.1101/259317
ONPRC	24005 (dam) 36633 (fetus)	PPROM GD118 and emergency cesarean; alive at delivery but non-viable due to gestational age	None	Submandibular gland, tonsil, parotid SG, thyroid gland, ovary, lumbar SC, trigeminal ganglion, bladder, triceps (qPCR)	400 copies/ug RNA	
ONPRC	24002 (dam) 36634 (neonate)	Preterm cervical dilation GD126 and emergency cesarean; neonatal death GD128	MFI tissues unavailable for analysis	Esophagus, tonsil, trigeminal ganglion, uterus, bladder, finger, basal ganglion, bicep, ovary, eye, dorsal root ganglion, knee, sciatic nerve, adrenal gland, brachial radialis, thoracic SC, cervical SC, wrist stomach, liver, heart, ileum, parietal lobe (brain), kidney, cervical LN, inguinal LN, thymus, frontal lobe (brain), gall bladder, quadriceps (qPCR)	400 copies/ug RNA	
ONPRC	25689 (dam) 36635 (neonate)	Live birth GD145; clinically indicated neonatal euthanasia GD162	Pending analysis	Elbow, lumbar SC, retroperitoneal LN, thoracic SC, tonsil, cervix, pituitary, brachial plexus, toe (qPCR)	400 copies/ug RNA	

*Refer to <https://bit.ly/2GYd57g> for viral load quantifications.

Supplementary table 5: Histopathology and ultrasound analysis of fetal demise and neonatal death cases following Zika virus infection.

Institution	Animal ID	Pregnancy outcome	Major maternal reproductive/fetal extraembryonic histopathology**	Major Fetal histopathology**	Ultrasound abnormalities*	Citation/corresponding author for more information	doi
CNPRC	5303	Fetal death GD48	Mild fibrinosuppurative placentitis with necrosis of trophoblasts (fetal); placenta had positive ISH	Fetal tissues had moderate autolysis; several tissues had positive ISH	None prior to fetal death	Coffey/Van Rompay	Coffey et al (NCOMMS-17-31047B; in press)
CNPRC	5508	Fetal death GD60	Possible placental separation, deciduitis	Moderate to severe autolysis of fetal tissues	None prior to fetal death	K. Van Rompay	
CNPRC	5374	Fetal death GD35	Possible placental separation, deciduitis	Moderate autolysis of fetal tissues	None prior to fetal death	K. Van Rompay	
CNPRC	5463	PPROM with imminent abortion GD115	Neutrophilic amnionitis and placentitis with hemorrhage and trophoblastic necrosis (fetal)	Minimal hemorrhage in retina; neutrophils and erythrocytes within lungs and stomach and overlying the skin	None prior to abortion	K. Van Rompay	
CNPRC	5592	Fetal death GD59	Mild fibrinosuppurative placentitis with necrosis of trophoblasts (fetal)	Moderate to severe autolysis of fetal tissues	None prior to fetal death	K. Van Rompay	
CNPRC	5266	Stillbirth GD166	Large areas of trophoblastic necrosis and degeneration (fetal); basal decidual necrosis; mild neutrophilic funisitis	Large infant (664 grams); few pulmonary neutrophils; brain: mild ventricular hemorrhage, mild loss of ependyma in lateral ventricle; congestion in other organs (likely dystocia)	None prior to fetal death	K. Van Rompay	
CNPRC	5804	Fetal death GD86	Placental necrosis with decidual hemorrhage and edema; trophoblastic proliferation and attenuation; hemorrhage within amniotic membranes	Severe autolysis of fetus	None prior to fetal death	K. Van Rompay	
WNPRC	527453 (dam) 291635 (fetus)	Fetal death GD95	Severe diffuse fibrinopurulent and necrotizing chorioamnionitis with multifocal mineralization of the decidua and amniotic and chorionic membranes. Diffuse suppurative and lymphoplasmacytic endometritis with multifocal necrosis and perivascular lymphoplasmacytic myometritis. Myometrial radial arteries show leukoclastic vasculitis. Suppurative placentitis with multifocal villous infarction, syncytial knots, marked multifocal mineralization, suppurative vasculitis, and multiple remote infarctions.	Choroidal coloboma, anterior segment dysgenesis, and dysplastic retina. Lung with numerous alveoli filled with fibrin, cellular debris and squamous cells admixed with neutrophils; alveolar walls are lined by hyperplastic type II pneumocytes with segmental thickening of the alveolar septa with infiltrated neutrophils.	Placental abruption and subchorionic hemorrhage which resolved. There was subsequent fetal echogenic and dilated bowel. Scant placental calcifications.	Mohr et al. PLoS One. Jan 30;13 (1):e0190617. PMID:29381706.	10.1371/journal.pone.0190617. eCollection 2018
WNPRC	125500 (dam) 411377 (fetus)	Stillbirth GD155	Severe segmental subacute chorioamnionitis and placental coagulative necrosis and hemorrhage infarction. Scattered areas of villous infarcts. Marked multifocal chorionic and subacute villi mineralization. Possible acute non-occlusive umbilical cord thrombus at site of insertion into the chorionic plate.	Multifocal lymphoplasmacytic and neutrophilic pericarditis. Amniotic fluid and squamous cell aspiration in lung tissue.	Mild placental calcifications. Overall unremarkable fetal anatomy.	David O'Connor	
WNPRC	795784 (dam) 499874 (neonate)	Live birth GD155; clinically indicated neonatal euthanasia GD162	Uterus: minimal lymphocytic endometritis. Decidua: mild multifocal lymphoplasmacytic and minimal multifocal suppurative deciduitis with multifocal failure of physiologic transformation and remodeling of spiral arteries (persistently muscularized arteries). Placenta: multiple chronic (remote) infarctions with mild multifocal mineralization, suppurative placentitis and intervillitis.	Lungs with neutrophilic interstitial and alveolar inflammation. Suppurative otitis media. Minimal perivascular lymphoplasmacytic and neutrophilic pericarditis.	Echogenic bowel and pericardial effusion were noted and the echogenic bowel resolved within a few weeks. Mild placental calcifications. A possible small pericardial effusion persisted until delivery.	David O'Connor	
WNPRC	664184 (dam) 416597 (fetus)	Fetal death GD133	Uterine diverticulum with transmural suppurative metritis, suppurative endometritis, multifocal myometrial vascular infarction, multifocal acute placental ischemia, and clinical dystocia.	Moderate to severe autolysis with post mortem cerebellar herniation through the foramen magnum due to dystocia. Lungs with moderate numbers of squamous cells (aspirated amniotic fluid) within alveoli. Minimal suppurative otitis media with luminal keratinized squamous cells.	Fetal heart rate downtrended during the last 6 weeks of pregnancy. Scant placental calcifications.	David O'Connor	
SNPRC	34103	Fetal deaths GD95	MFI not available	Regions of disorganization of the cortical neurons	none	Jean Patterson	http://dx.doi.org/10.1101/259317
SNPRC	33722	Fetal death GD86	None detected by hematoxylin and eosin staining	None	none	Jean Patterson	http://dx.doi.org/10.1101/259317
ONPRC	24005 (dam) 36633 (fetus)	PPROM GD118 and emergency cesarean; alive at delivery but non-viable due to gestational age	Prominent foci of mineralization; increased perivillous and villous accumulation of fibrin; inflammatory infiltrates adjacent to remodeled vessels	None detected by hematoxylin and eosin staining	Moderate preterm placental calcification; BPD and HC trending below mean	Dan Streblow	
ONPRC	24002 (dam) 36634 (neonate)	Preterm cervical dilation GD126 and emergency cesarean; neonatal death GD128	None detected by hematoxylin and eosin staining	Minor autolysis of neonatal tissues; multifocal suppurative cerebral encephalitis; diffuse cerebellar external granular layer apoptosis and necrosis	Mild preterm placental calcification	Dan Streblow	
ONPRC	25689 (dam) 366354 (neonate)	Live birth GD145; clinically indicated neonatal euthanasia GD162	None detected by hematoxylin and eosin staining	Left ventricular hypertrophic cardiomyopathy	Mild preterm placental calcification	Dan Streblow	

*Additional information about the results of each US test and some images can be found at <https://bit.ly/2GYd57g>

**Note: necrosis may be the result of fetal death in utero prior to tissue collection and may not be part of the etiology caused directly by ZIKV infection.

Images representing the findings listed here can be found at <https://bit.ly/2GYd57g>