Table S1. Primers used to complete the sequencing of the crocodile-derived WNV.

CROCKUNV3F	GATGCGGAAATCACAGGTTCC
CROCKUNV3R	CAGCCAGCCCATCTCATTGG
CROCKUNV4F	CTGAAATCATTCAAAGACTTCGC
CROCKUNV4R	GCCTCACGCTCTTCATCCACC
CROCKUNV5F	GGAACGAGAGCAGTGGGAAGAC
CROCKUNV5R	TGCGGCACGGGGTCTCCACTAACC
CROCKUNVseq1F	CCTGATTGCTGGTGTGG
CROCKUNVseq1R	GCTGTCACCTTCAAGGACC
CROCKUNVseq2R	GGAATGGCCATAGAGTCC
CROCKUNVseq3F	GGAACAGTTTGGAGGTGG
CROCKUNVseq3R	CCAAGAACACGACCAGAAGG
CROCKUNVseq4F	GCTGATGTCTCCTCATAGG
CROCKUNVseq4R	CCCAGTCATCGTTCTTGC
CROCKUNVseq5F	CGTACCACACCCATCATGC
CROCKUNVseq5R	GGTGTCTGAATTGAGTAGAGG
CROCKUNVseq6R	CCAGAGTATGGAACATCGC
CROCKUNVseqEF	GGAGTTTGAAGAACCACATGC
CROCKUNVseqER	CCCTGAGTTATCCAAGACATG
WNV NS1 F	CATGCTGACACTGGATGTGCCATAG
WNV NS2B R	CTCCTCTTTGTGTATTGGAGAGTTATC

Standard	Log 10 (standard)	CT score
10 ^{5.92} TCID ₅₀ /ml (neat)	5.92	12.788361
1:10	4.92	18.738192
1:100	3.92	22.422401
1:1000	2.92	25.790836
1:10000	1.92	28.877739
1:100000	0.92	32.220005
1:1000000	-0.08	36.105015
1:10000000	-1.08	39.028511

Table S2. Standard for estimation of $TCID_{50}$ -equivalents of WNV_{KUN} in pen-water as determined by qRT-PCR.

Table S3. Summary of histopathological changes

Tissue/	Tongue	Conjunctiva	Kidney	Stomach	Intestine	Liver	Lung	Brain	Pancreas	Adrenal
Organ										gland
Group										
Control		Transepithel	Rare minute				Lymphoid			
		ial lymphoid	interstitial				aggregates			
		aggregate in	lymphoid				in lung			
		the	infiltrates				interstitia			
		lachrymal								
		glands								
10 ⁵ IU	Locally	Lymphoplas	Interstitial	Activated		Multifocal	Congestion	Gliosis		
	extensive	macytic	lymphoplas	lymphoplas		lymphoplas	with a			
	lymphoplas	perivascular,	macytic &	macytic		macytic	mononuclea			
	macytic	non-	histiocytic	infiltrates in		infiltrate in	r cell			
	infiltrate	glandular	infiltrate in	the mucosa		the portal	infiltrate			
	with high	consistent	the	and		area				
	endothelial	with focal	interstitium	submucosa						
	postcapillary	reaction to	consistent			Blood clot in				
	venules	insult	with			the portal				
			interstitial			area with no				
	Lymphoplas		nephritis			signs of				
	macytic					inflammatio				
	infiltrates					n suggesting				
	with		Focal			an				
	activated		lymphoplas			iatrogenic				
	dendritic		macytic in			incident.				
	cells &		the renal			There is an				
	transendoth		medulla			infiltrate of				
	elial					dead and				
	migrating					dying cells in				
	cells					the affected				
						areas				

10 ⁵ IU	Locally	Lymphoplas	Focal	Multifocal	Mucosal	Lymphoplas	Interstitial	Lymphoplas		Lymphoplas
in-	extensive	macytic	lymphoplas	lymphoplas	lymphoplas	macytic	lymphoplas	macytic &		macytic
contact	lymphoplas	aggregate	macytic in	macytic &	macytic	heterophilic	macytic	heterophilic		infiltrate
control	macytic	with	, the renal	histiocytic	infiltrate	infiltrate	infiltrate	infiltrate		
	infiltrate	transepitheli	medulla	infiltrates				with		
	with high	al migration		with				activated		
	endothelial	and damage		dominance				dendritic		
	postcapillary	on the		of				cells		
	venules	epithelium		lymphocyte						
				s (lymphoid						
				aggregates)						
				with a few						
				monocytes						
				in the						
				mucosa of						
				the stomach						
10 ⁴ IU	Lymphoplas	Subepithelial		Subepithelia		Multifocal	Vascular	Lymphoplas	Lymphohisti	
	macytic	lymphoplas		l multifocal		lymphoplas	centric and	macytic	ocytic	
	histiocytic	macytic		lymphoplas		macytic	interstitial	histiocytic	infiltrate	
	infiltrate	histiocytic		macytic		infiltrate in	lymphoplas	infiltrate	with	
	with high	infiltrate		histiocytic		the portal	macytic		isolated	
	endothelial	with		infiltrate		area	infiltrate		dying cells	
	post	activated		compressing						
	capillary	high		the crypts						
	venules with	endothelial		with						
	a mixed	post		activated						
	population	capillary		high						
	(migrating)	venules		endothelial						
	cells			post						
		Lymphoplas		capillary						
	Vascular	macytic		venules						
	centric	infiltrate								
	lymphoplas	compressing								
	macytic	the								
	infiltrate	epithelium								

	Histiocytic infiltrate destroying the lachrymal gland	in the lachrymal gland						
10 ⁴ IU	Focal		Lymphoplas		Locally		Activated	
in-	iymphopias		macytic		extensive		iymphopias	
contact	macytic		infiltrate		lymphohistio		macytic	
control	infiltrate in		along with		cytic		infiltrated	
	the mucosa-		exudative		infiltrate		compressing	
	muscularis		accumulatio				the	
	plagued		n in the				pancreatic	
	endothelial		vascular				acini	
	cells,		lumen					
	transepitheli							
	al and							
	surrounding							
	vessels							

r		DI	D2	D3	04	D5	06	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21
Challenge	Animal ID	TCIDSO/mi	TCI050/ml	TCIDS0/ml	70050/ml	TCIOSO/ml	TC/050/ml	TCID50/ml	TCID50/ml	TCIDS0/ml	TCID50/ml	TC/D50/ml	TCID50/ml	TCIDSG/ml	TCID50/ml	TCID50/ml	TC:050/ml	TCi050/ml	TCID50/mi	TCIDS0/ml	TCIDS0/ml	TCIDEQ/ml
10^4	11	641 65933			27101.0581			33116.6209			65.4971252			52.4808255		- accountation						
10^4	39	108.224225			253.031085						749,306707											
10^4	55	24,3462745									133.08567						627.599163					
10^4	8	-	5304 1635			19963 1579			76 4851891													
10^4	9		690.641429			22.9495105			325,255764		_	374.253039										
10^4	14		1175 73695			\$9248.0892	1		3451 73657		-	45.6100265						-				
10^4	6		1	134726.175			5626.98813	-			-					9.81576236						
10^4	22			83.572997				-		80,5432308			89.9787254			42.6765968						
10^4	23			6668.77212			4590.30183			812.719848						-					1	
10^4	57							-														
10*4 ctr	45									1						1 C - 1 C - 2					-	
10*4 ctr	67																					
10*4 ctr	33											201.148268	-		133734.85			2916.20899			36.5033918	
10*4 ctr	37													-								
10^4 ctr	27																					
10*4 ctr	54																					47.3257227
10^5	12	245 565592			48.7446323			153.134001			299.877413									-		
10^5	30	115 66207									65.9826304											
10^5	32	7673 37126			598885-714			2810.48787														
10^5	13		999.419167			14697.2533			2937.82573			78.7783515										
10^5	17	1													162,454115			67 4608459				
10^5	35		219350.918			137744.459			1478.3202			37.0897167						Sector Sector	-			
10^5	62	-	1900.16097			42,6709968			402.938899													
10^5	15	-					154.269125			330.005629			574.372613									
1045	29			545,433768			208,824658			238.514502			89.9787214									
10^5	38			1575837.82			15477.0392			38.2017326												
10^5 ctr	3																					
10^5 ctr	60			1					_									1.1.1.1	1	1.15		
10^5 ctr	20														1149.97396			5503.69844			48.029938	
10^5 ctr	36												2									
10^5 ctr	1																					
10^5 ctr	40			1.1						_			727.495106	5			-					
control	24																					
control	45																		1.			
control	19																					
control	53			1.00																		
control	41																					
-control	47												1									1.1.2
NI	51																				1.1.1	1.1

Table S4. Plasma viremia as detected by qRT-PCR and converted to TCID_{50} equivalents.

Figure S1.



Figure S1. Standard curve to determine WNV_{KUN} infectious units equivalents from plasma qRT-PCR CT scores. Ten-fold dilutions of WNV_{KUN} (10⁻¹ to 10⁻⁷) were simultaneously assessed for infectious titre by TCID₅₀ assay and levels of viral RNA by Taqman qRT-PCR. An exponential trend line was generated from the derived CT scores and calculated infectious units of the standard dilution series using the Excel Growth Function. Infectious unit equivalents were then predicted for each plasma sample from their derived CT scores. R² value indicates line of best fit (closest to 1).



Figure S2.

Figure S2. Overview of relationship between first detection of virus-neutralizing antibodies (VN Ab) and viremia as detected by RT-PCR, viral RNA (vRNA) in pen-water and oral and cloacal swabs. The notable delay in VN Ab development in the in-contact animals is suggestive of a different virus-host dynamic in these animals compared to hatchlings inoculated directly with WNV_{KUN}.