

# Pathogenesis of Pulmonary Disease In Ebola Virus-Infected Pigs

**Charles Nfon<sup>1</sup>, Anders Leung<sup>2</sup>, Greg Smith<sup>1</sup>, Carissa Embury-Hyatt<sup>1</sup>, Gary Kobinger<sup>2,3,4</sup> and Hana Weingartl<sup>1,3</sup>**

<sup>1</sup>National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, MB, Canada

<sup>2</sup>Special Pathogens Program, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada

<sup>3</sup>Department of Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada

<sup>4</sup>Department of Immunology, University of Manitoba, Winnipeg, MB, Canada



**Canadian Food  
Inspection Agency**

## Introduction

Family Filoviridae

Species: Ebola virus (EBOV) & Marburg virus

Enveloped, negative-strand RNA viruses

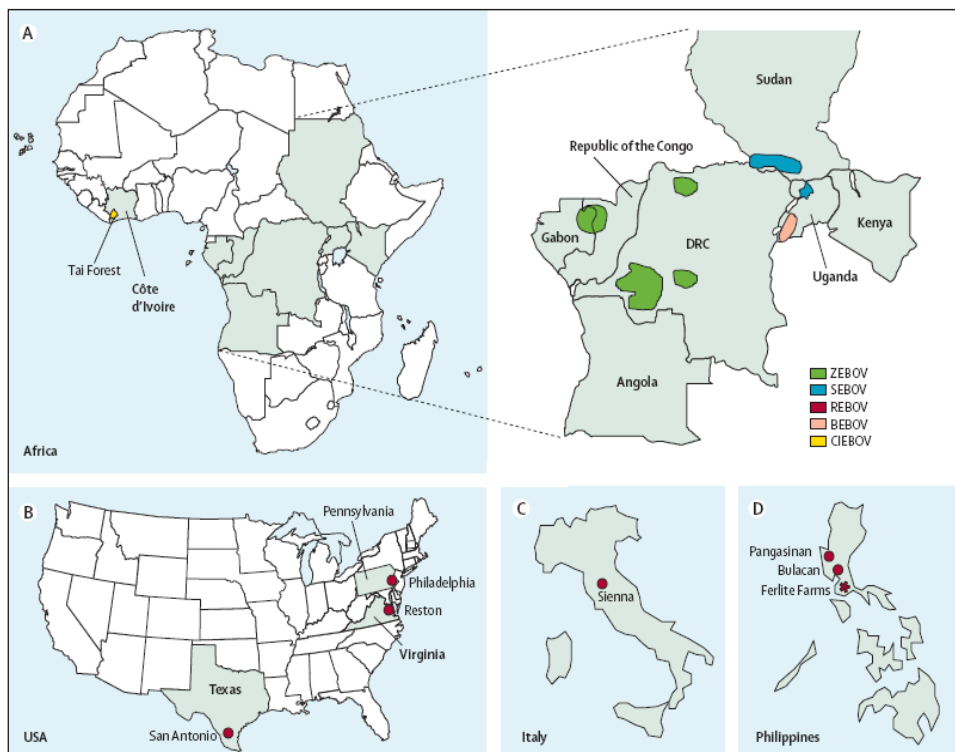
Pleomorphic, filamentous, 80nm diameter,  
varying lengths up to 14,000 nm

EBOV causes ebolavirus hemorrhagic fever  
(EHF)

EHF probably the most severe of viral  
hemorrhagic fevers



## History And Distribution Of Reported EBOV Outbreaks



ZEBOV = Zaire ebola virus (1976)

SEBOV = Sudan ebola virus (1976)

REBOV = Reston ebola virus (1989)

CIEBOV = Cote d'Ivoire ebola virus (1994)

BEBOV = Bundibugyo ebola virus (2007)



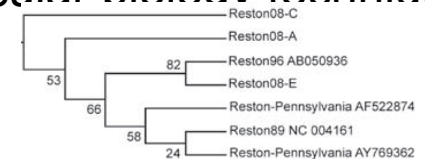
## Ebola In Pigs

Replication, pathogenicity, shedding, and transmission of Zaire ebola virus in pigs. **Kobinger et al**, *The Journal of Infectious Diseases* (2011)

➤ What **possessed** them to perform this seemingly **odd experiment**? **Bausch D.**  
*The Journal of Infectious Diseases* (2011)

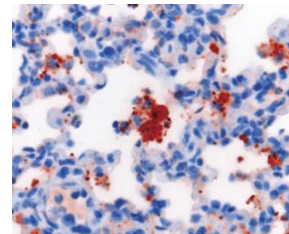
## Ebola In Pigs: Reston Ebola Virus

- Multiple outbreaks of respiratory and abortion syndrome in pigs in 2008
- Detection of porcine reproductive & respiratory syndrome virus (PRRSV)
- CPE in Vero cells suggested presence of another virus other than PRRSV
- The unknown virus identified as REBOV by EM & molecular biology techniques
- REBOV only detected in pigs co-infected with PRRSV



## Reston Ebola Virus (REBOV) In Pigs In The Absence Of Co-infection

- Virus isolated from lungs, lymph nodes, nasal turbinates and muscle
- Gross & histopathology in the respiratory system and lymphoid tissues



- No clinical signs of disease

# Experimental Zaire Ebola Virus (ZEBOV) Infection In Pigs

➤ Pigs infected with  $10^6$  PFUs ZEBOV by oro-nasal routes

➤ Fever starting at dpi 4

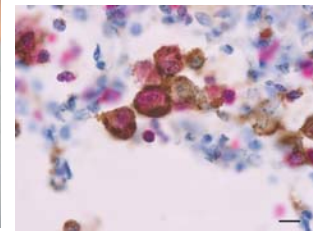
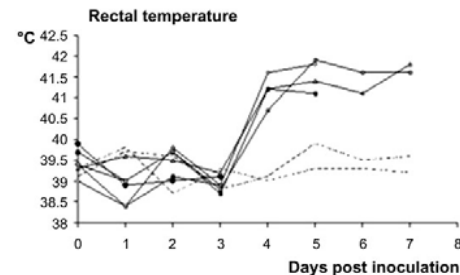
➤ ZEBOV replicated mainly in the lungs

➤ Oral and nasal shedding of virus

➤ Severe pathology in the lungs

➤ Symptoms mainly respiratory

➤ Infected pigs transmit ZEBOV to naïve pigs



## Objective

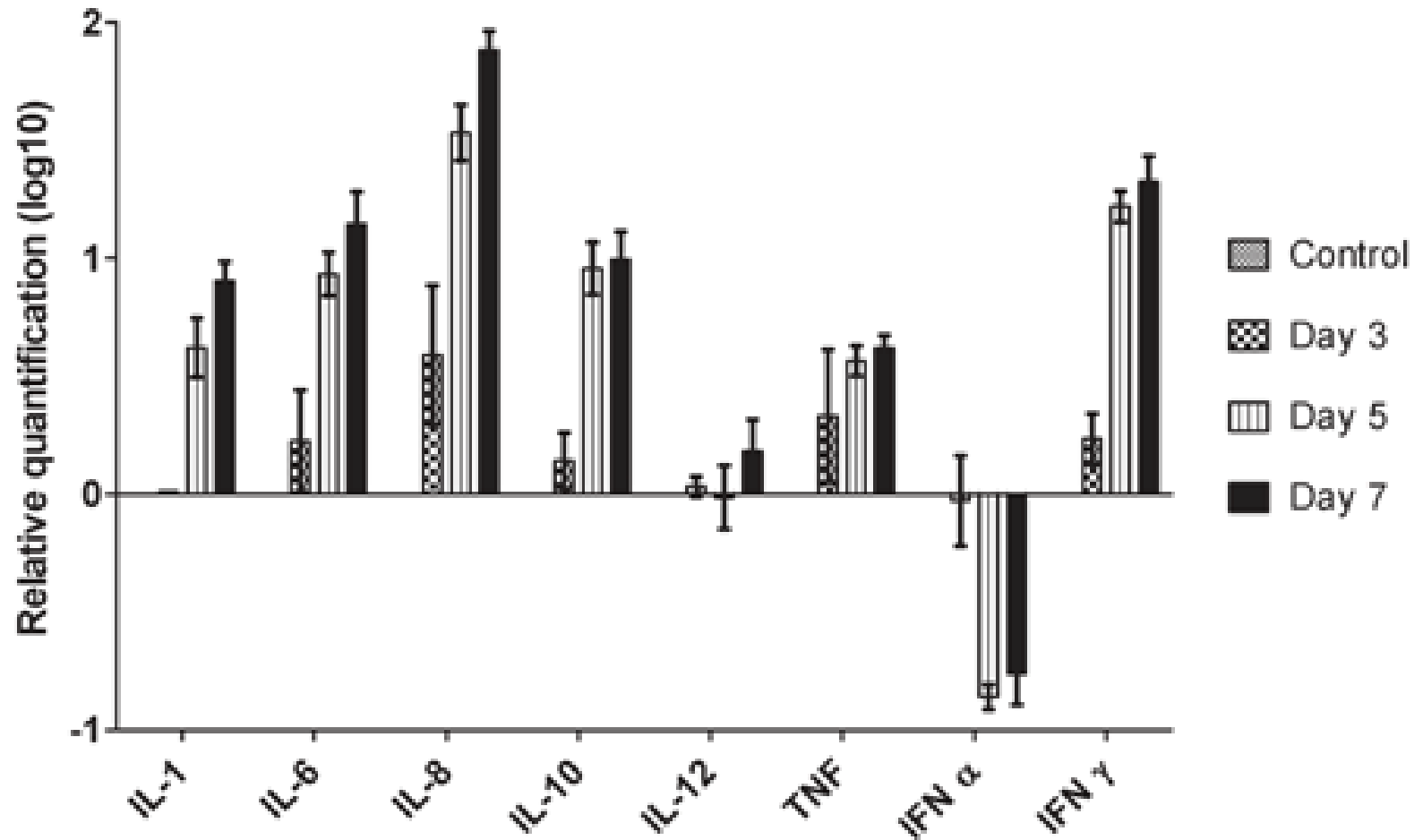
Main clinical sign in ZEBOV infected pigs: respiratory distress (due to pathology in the lungs)

Identify the mechanisms involved the development of pulmonary disease in ZEBOV-infected pigs





## Pulmonary Innate Immune Responses In ZEBOV-infected Pigs



(Kobinger et al, 2011)



## Pulmonary Cytokine Response In ZEBOV Infected Pigs

Gene	Description	Fold change		
		DPI3	DPI5	DPI7
IL-6	Interleukin- 6	2	41	23
IL-8	Interleukin-8	8	106	45
IL-10	Interleukin-10	2	20	18
IL-22	Interleukin-22	1	59	30
IL-26	Interleukin-26	1	10	5
IL-27	Interleukin-27	2	37	48
GM-CSF	Granulocyte monocyte colony stimulating factor	2	25	16
SPP1	Secreted phosphoprotein 1	3	37	36
SELL	Selectin	4	57	97
RETN	Resistin	1	248	185
PLUNC	palate, lung and nasal epithelium associated	66	496	273

Attract monocytes, neutrophils & lymphocytes to sites of infection

Induce the secretion of chemokines & acute phase proteins

Drive differentiation and proliferation of immune cells

Cell adhesion & trafficking

Antinflammatory (IL-10)



## Pulmonary Chemokine Response In ZEBOV-infected Pigs

Gene	Description	Fold change		
		DPI3	DPI5	DPI7
CCL2	Chemokine (C-C motif) ligand 2	1	29	32
CCL3L1	Chemokine (C-C motif) ligand 3-like 1	2	21	22
CCL4	Chemokine (C-C motif) ligand 4	2	33	28
CCL10	Chemokine (C-C motif) ligand 10	10	109	211
CCL19	Chemokine (C-C motif) ligand 19	2	12	9
CCL20	Chemokine (C-C motif) ligand 20	7	12	13
AMCF-II	Alveolar macrophage-derived chemotactic factor	2	478	258
SAA	Serum amyloid A2	4	907	836
C9	Complement component 9	4	48	72

Attract monocytes, neutrophils & lymphocytes to sites of infection



## Upregulation Of Proapoptotic Genes In Lungs Of ZEBOV-infected Pigs

Gene	Description	Fold change		
		DPI3	DPI 5	DPI7
CASP1	Caspase 1	2	13	12
CASP3	Caspase 3	1	3	3
CASP1/4	Caspase 1/3	2	6	5
CASP8	Caspase 8	1	3	2
CASP15	Caspase 15	1	3	5
CARD6	Caspase recruitment domain family	2	4	4
AATYK	Apoptosis-associated tyrosine kinase 2	1	4	4
FADD	Fas (TNFRSF6)-associated via death domain	2	3	3
FAS	TNF receptor superfamily, member 6	2	5	5
TRAF3	TNF receptor-associated factor 3	2	6	7
TNIP3	TNFAIP3 interacting protein 3 isoform 3	3	10	14
AIF1	Allograft inflammatory factor	1	3	3

Induce apoptosis of virus infected cells

Caspases also induce inflammation



# Receptors And Interferon Stimulated Genes In Lungs From ZEBOV-infected Pigs

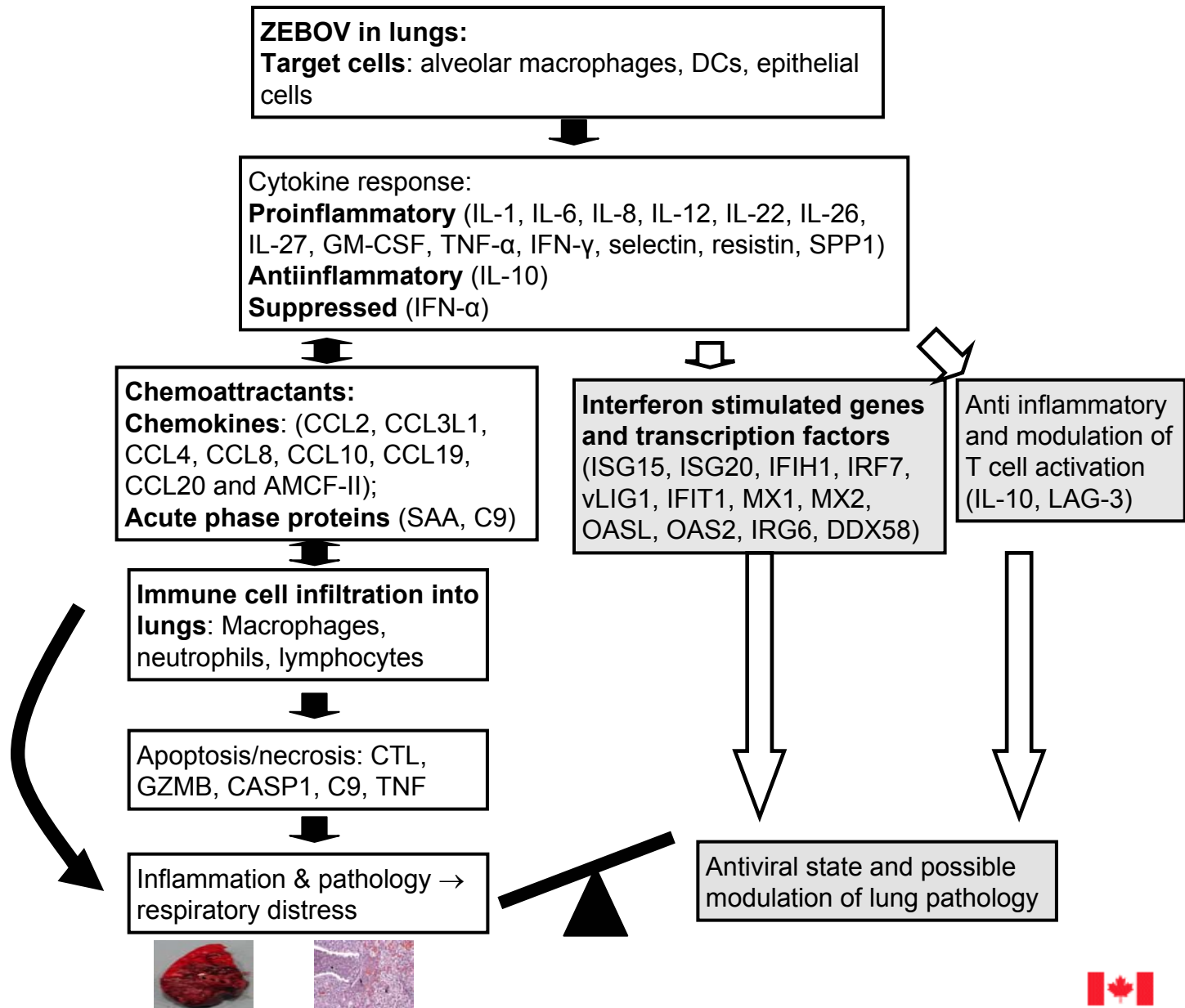
Gene	Description	Fold change		
		DPI3	DPI5	DPI7
CD14	CD14 molecule	1	40	62
CD163	CD163 molecule	8	64	95
TLR2	Toll-like receptor 2	3	36	41
TLR4	Toll-like receptor 4	4	22	21
TLR6	Toll-like receptor 6	2	6	5
RIG-I	Retinoic acid inducible protein I	12	77	253
IRF1	interferon regulatory factor 1	2	6	6
IRF7	interferon regulatory factor 7	4	15	20
ISG15	Interferon-induced 15 kDa protein	20	36	40
ISG20	Interferon-stimulated gene 20 kDa protein	15	126	143
IFIH1	Interferon induced with helicase C domain 1	3	12	18
IFIT-1	Interferon induced with tetratricopeptide 1	8	11	21
MX1	Myxovirus (influenza virus) resistance 1	15	36	71
OAS2	2'5'-oligoadenylate synthetase 2	5	16	47
IRG6	Inflammatory response protein 6	33	25	37

PRR → innate immunity

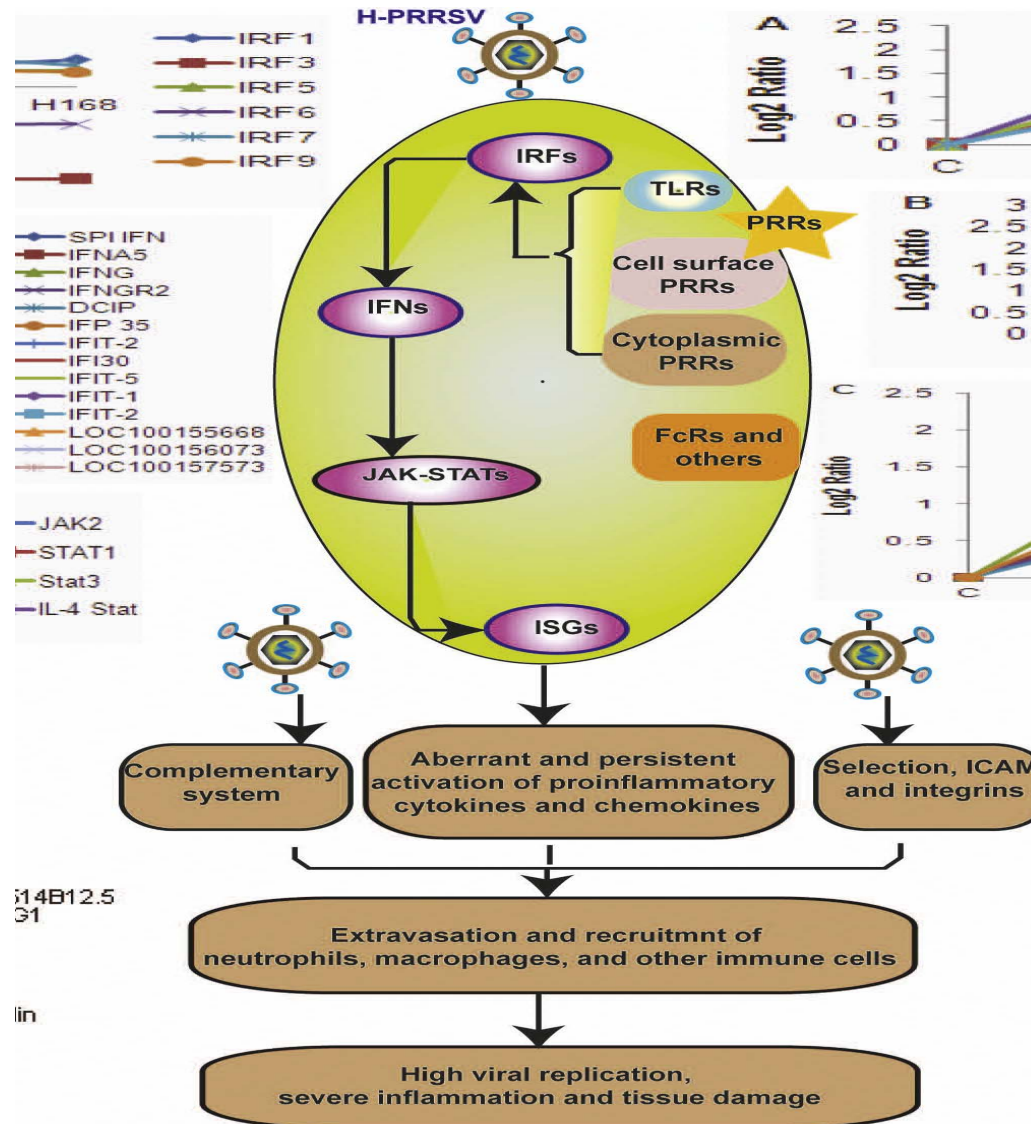
ISG → antiviral response



# Model For The Pathogenesis Of Pulmonary Disease In ZEBOV-infected Pigs



# Similarities In Pathogenesis Of Highly Pathogenic PRRSV & ZEBOV In Pigs



Aberrant host immune response induced by highly virulent PRRSV identified by digital gene expression tag profiling. **Xiao et al. BMC Genomics (2010)**

## Are The Effects Of ZEBOV infection In Pigs Restricted To The lungs?

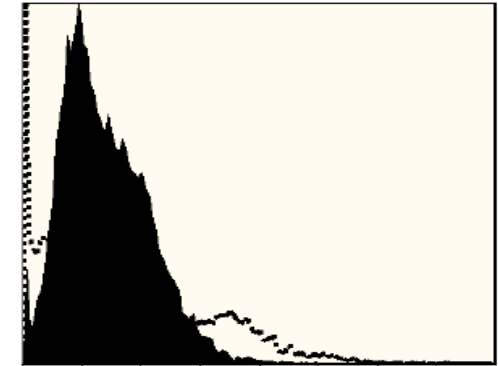
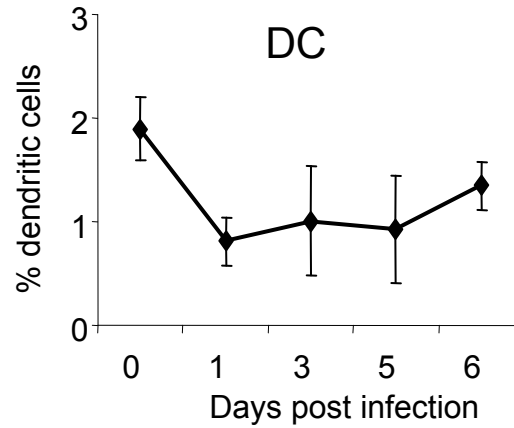
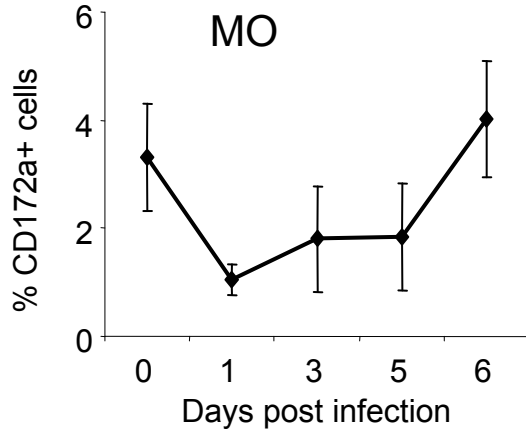
### ZEBOV detection in tissues from infected pigs

	SLN	BLN	MLN	Lung	Liver	Spleen	Trachea	Muscle
Pig 7	4.95	5.99	0	3.73	4.17	0	4.39	0
Pig 8	5.44	9.52	4.47	9.56	5.94	5.92	4.42	0
Pig 9	0	7	0	9.54	4.94	4.39	5.94	0
Pig 10	5.43	7.92	0	9.37	3.62	0	4.94	0
Pig 11	5.5	0	0	0	0	0	0	0
Pig 12	5.94	7.01	0	7.53	5.36	5.53	5.57	0

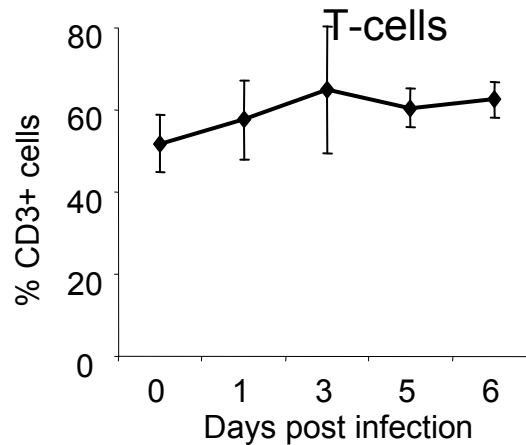
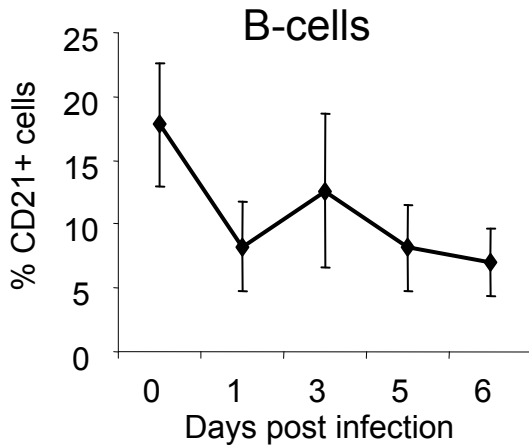




# Changes In PBMC Subsets In ZEBOV-infected Pigs

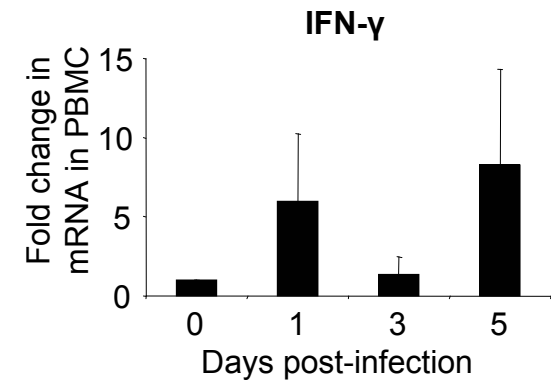
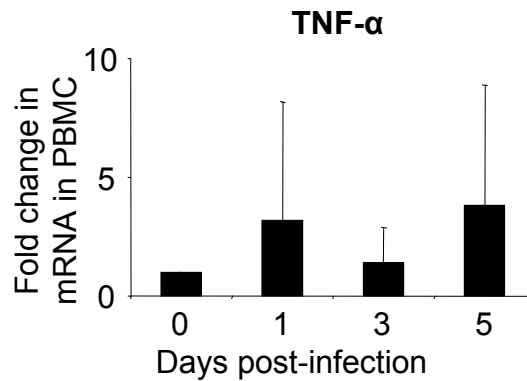
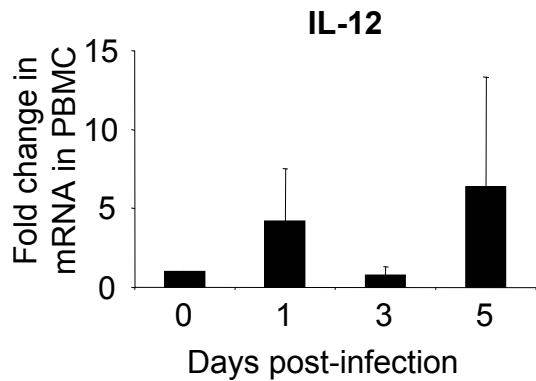
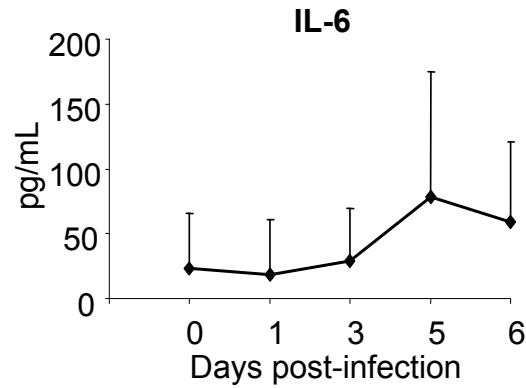
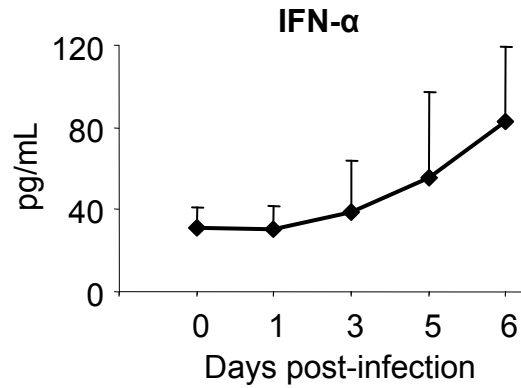


Monocytes infected in vitro

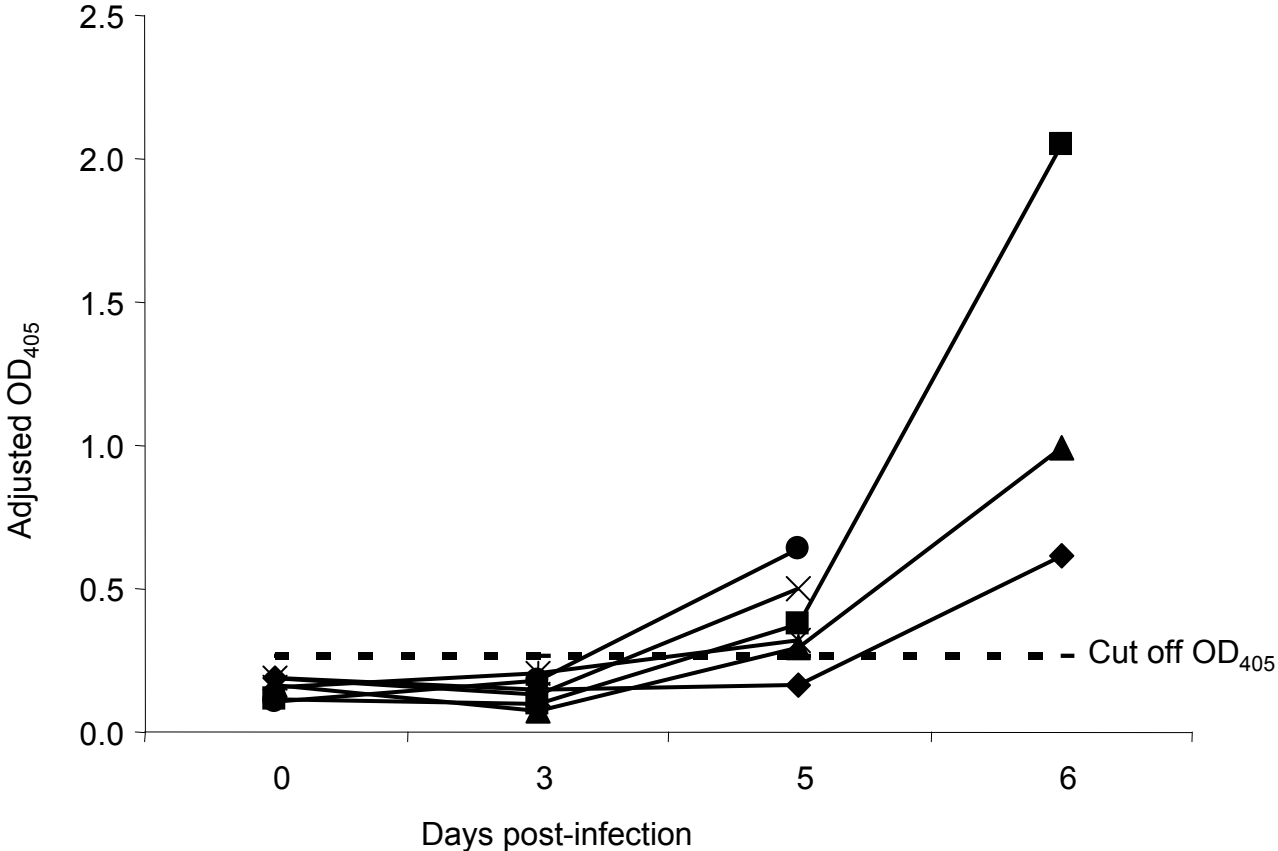


B & T cells not infected

# Systemic Cytokine Response In ZEBOV-infected Pigs



# IgM Antibody Response In ZEBOV-infected Pigs



# Summary

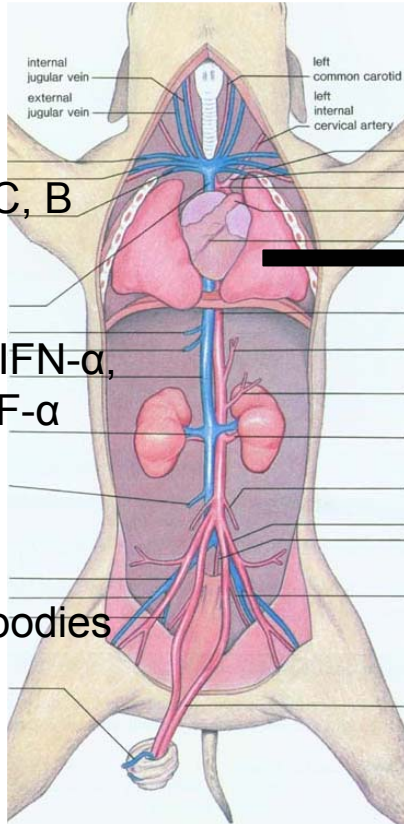
## Systemic effect of ZEBOV

Fever

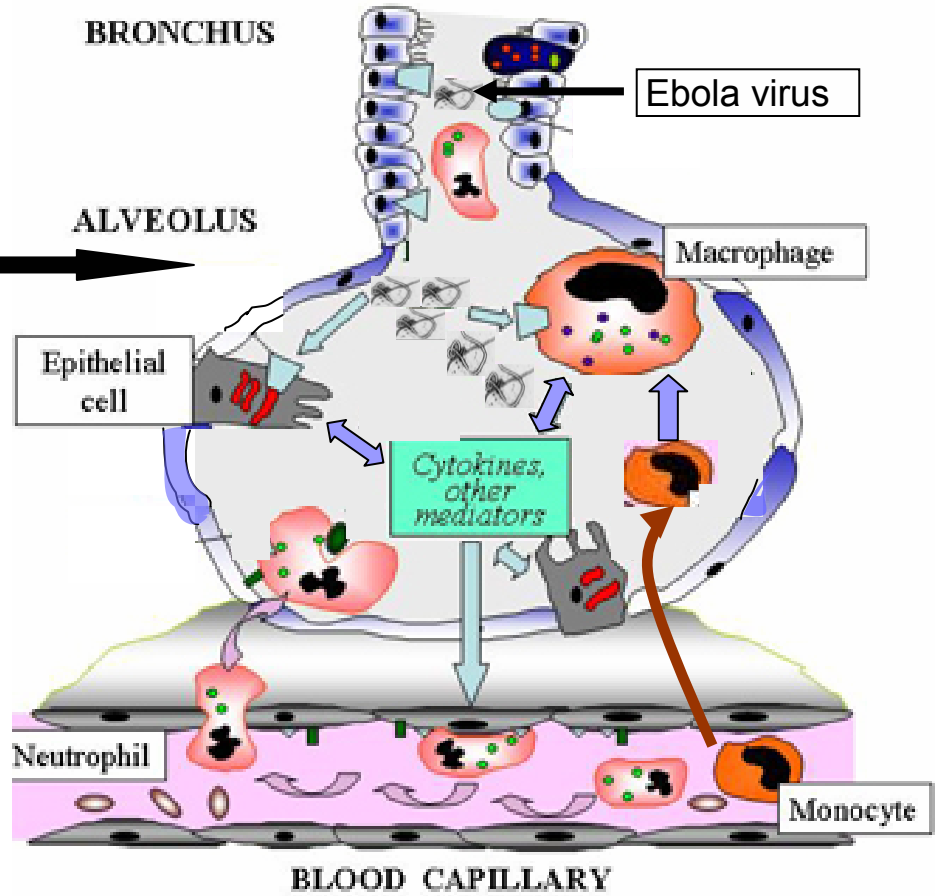
PBMCs: ↓MO/DC, B cells

Cytokines: IL-6, IFN- $\alpha$ , IFN- $\gamma$ , IL-12, TNF- $\alpha$

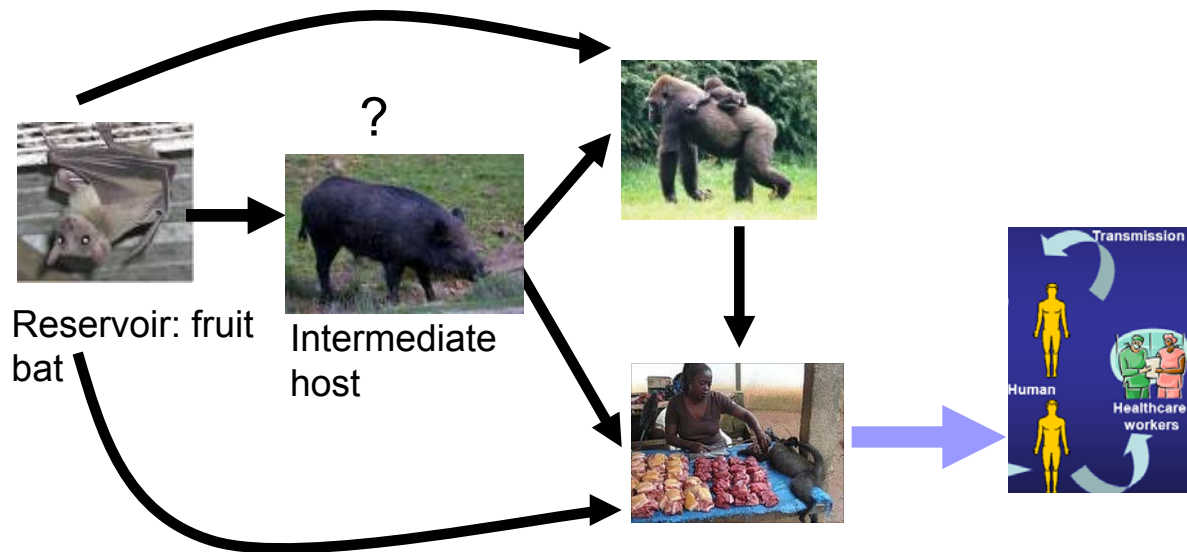
Antibody: IgM, neutralizing antibodies



## Pulmonary effect of ZEBOV



## The Big Picture

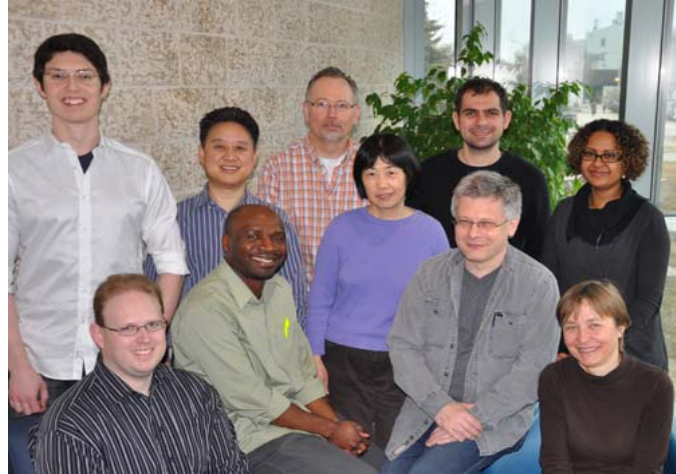


“one should keep an **open mind** for the existence of other reservoir species and a role for **potential amplifying hosts**, especially after the discovery of Reston Ebola virus in **pigs** in the Philippines”: *Feldman H and Geisbert T, 2011*

# Acknowledgements

## Special Pathogens Unit NCFAD, CFIA

Hana Weingartl  
Shawn Babiuk  
Greg Smith  
Shunzhen Zhang  
Peter Marszal  
Thang Truong  
Hani Boshra



## Animal Care Unit

Melanie VanderLoop  
Jamie Bernstein  
Maggie Forbes  
Kevin Tierney  
Cory Nakamura

## Special Pathogens Program NML, PHAC

Gary Kobinger  
Anders Leung  
Gary Wong  
Jason Richardson

## Pathology Unit

Carissa Embury-Hyatt  
Brad Collignon  
Estella Moffat  
Jill Graham

## Microarray Unit, University Health Network, Toronto

