

Development of diagnostic tools to reduce antimicrobial (mis)use

Novel identified biomarkers and available biobanked samples

*Elise Schieck and Sonal Henson
Animal and Human Health, ILRI*

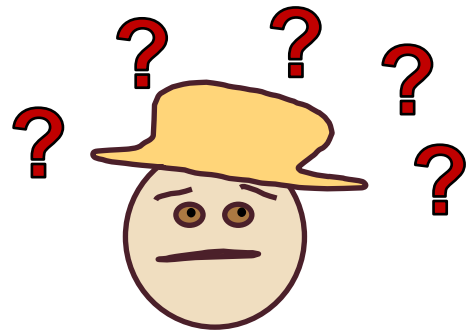
Scientific seminars
ILRI Nairobi, 8 November 2021



The idea...



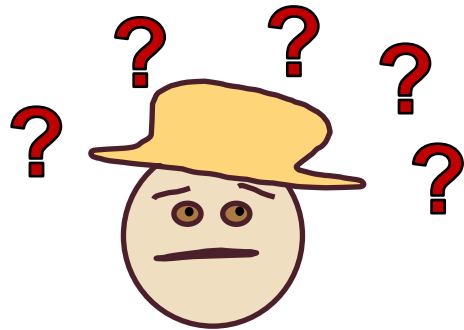
to treat OR not to treat



The idea...



to treat OR not to treat

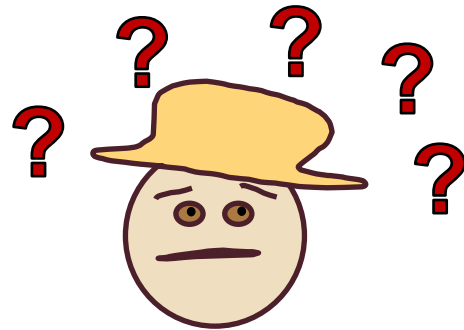


Today, sick animals are often treated with antimicrobials, regardless of the cause of disease. A diagnostic that differentiates between viral and bacterial infections could potentially reduce this overuse.

The idea...



to treat OR not to treat



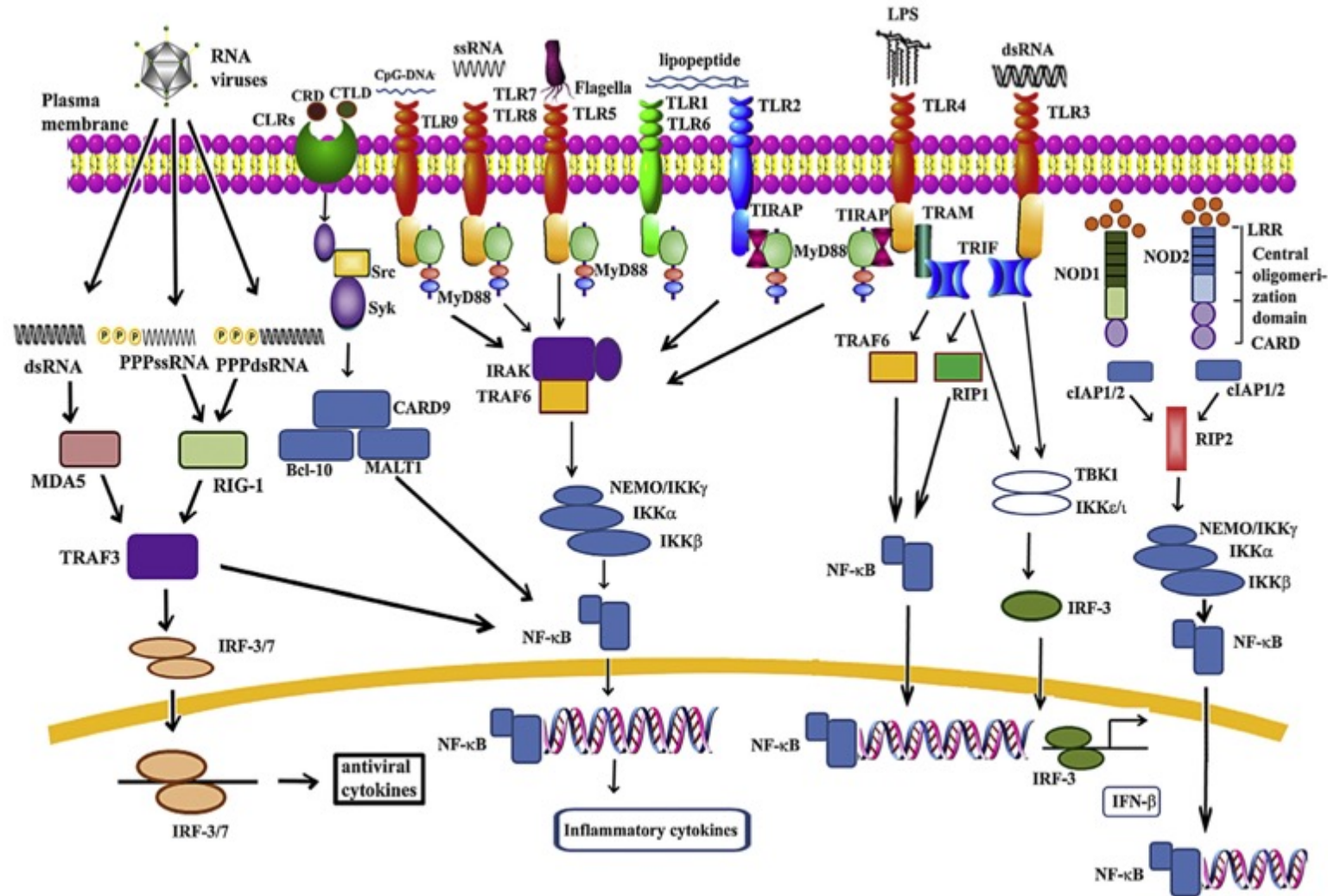
To test the possibility of developing an easy-to-use and cheap diagnostic test that can differentiate between bacterial and viral infections.

Bacterial and viral infections typically induce slightly different responses in the hosts

We are testing the possibility of using these host markers to develop a quick and easy-to-use field test.

This may reduce the use of antibiotics to animals infected with viruses.

Responses induced by pathogen associated molecular patterns



JAMA | Preliminary Communication | INNOVATIONS IN HEALTH CARE DELIVERY

Diagnostic Test Accuracy of a 2-Transcript Host RNA Signature for Discriminating Bacterial vs Viral Infection in Febrile Children

Jethro A. Herberg, PhD; Myrsini Kaforou, PhD; Victoria J. Wright, PhD; Hannah Shailes, BSc; Hariklia Eleftherohorinou, PhD; Clive J. Hoggart, PhD; Miriam Cebey-López, MSc; Michael J. Carter, MRCPCH; Victoria A. Janes, MD; Stuart Gormley, MRes; Chisato Shimizu, MD; Adriana H. Tremoulet, MD; Anouk M. Barendregt, BSc; Antonio Salas, PhD; John Kanegaye, MD; Andrew J. Pollard, PhD; Saul N. Faust, PhD; Sanjay Patel, FRCPCH; Taco Kuijpers, PhD; Federico Martín-Torres, PhD; Jane C. Burns, MD; Lachlan J. M. Coin, PhD; Michael Levin, FRCPCH; for the IRIS Consortium

Discovery group: 240 children
 52 definite bacterial infection,
 92 definite viral infection,
 96 indeterminate infection.

Validation group: 130 children
 23 definite bacterial,
 28 definite viral,
 79 indeterminate infections

JAMA | Preliminary Communication | INNOVATIONS IN HEALTH CARE DELIVERY

Diagnostic Test Accuracy of a 2-Transcript Host RNA Signature for Discriminating Bacterial vs Viral Infection in Febrile Children

Jethro A. Herberg, PhD; Myrsini Kaforou, PhD; Victoria J. Wright, PhD; Hannah Shailes, BSc; Hariklia Eleftherohorinou, PhD; Clive J. Hoggart, PhD; Miriam Cebey-López, MSc; Michael J. Carter, MRCPCH; Victoria A. Janes, MD; Stuart Gormley, MRes; Chisato Shimizu, MD; Adriana H. Tremoulet, MD; Anouk M. Barendregt, BSc; Antonio Salas, PhD; John Kanegaye, MD; Andrew J. Pollard, PhD; Saul N. Faust, PhD; Sanjay Patel, FRCPCH; Taco Kuijpers, PhD; Federico Martínón-Torres, PhD; Jane C. Burns, MD; Lachlan J. M. Coin, PhD; Michael Levin, FRCPCH; for the IRIS Consortium

- Identified 38 transcripts differentially expressed
- Narrowed down to 2-transcript ratio to discriminate bacterial vs. viral infections

Setting up...

- Homologues of top candidates identified in pigs
- Candidate reference genes identified
- Primers for qPCR designed
- qPCR set up (normalized to reference genes)

Category	Gene
Putative viral markers	<i>IFNα</i>
	<i>IFNβ</i>
	<i>IFITM3</i>
	<i>STING</i>
	<i>IFI44L</i>
	<i>IFIT3</i>
	<i>MxA</i>
Putative Bacterial markers	<i>RSAD2</i>
	<i>FAM89A</i>
	<i>S100PBP</i>
	<i>SLPI</i>
Pro-inflam-matory cytokines	<i>UPB1</i>
	<i>IL-1β</i>
	<i>IL-6</i>
	<i>IL-8</i>
	<i>TNF-α</i>

Test material

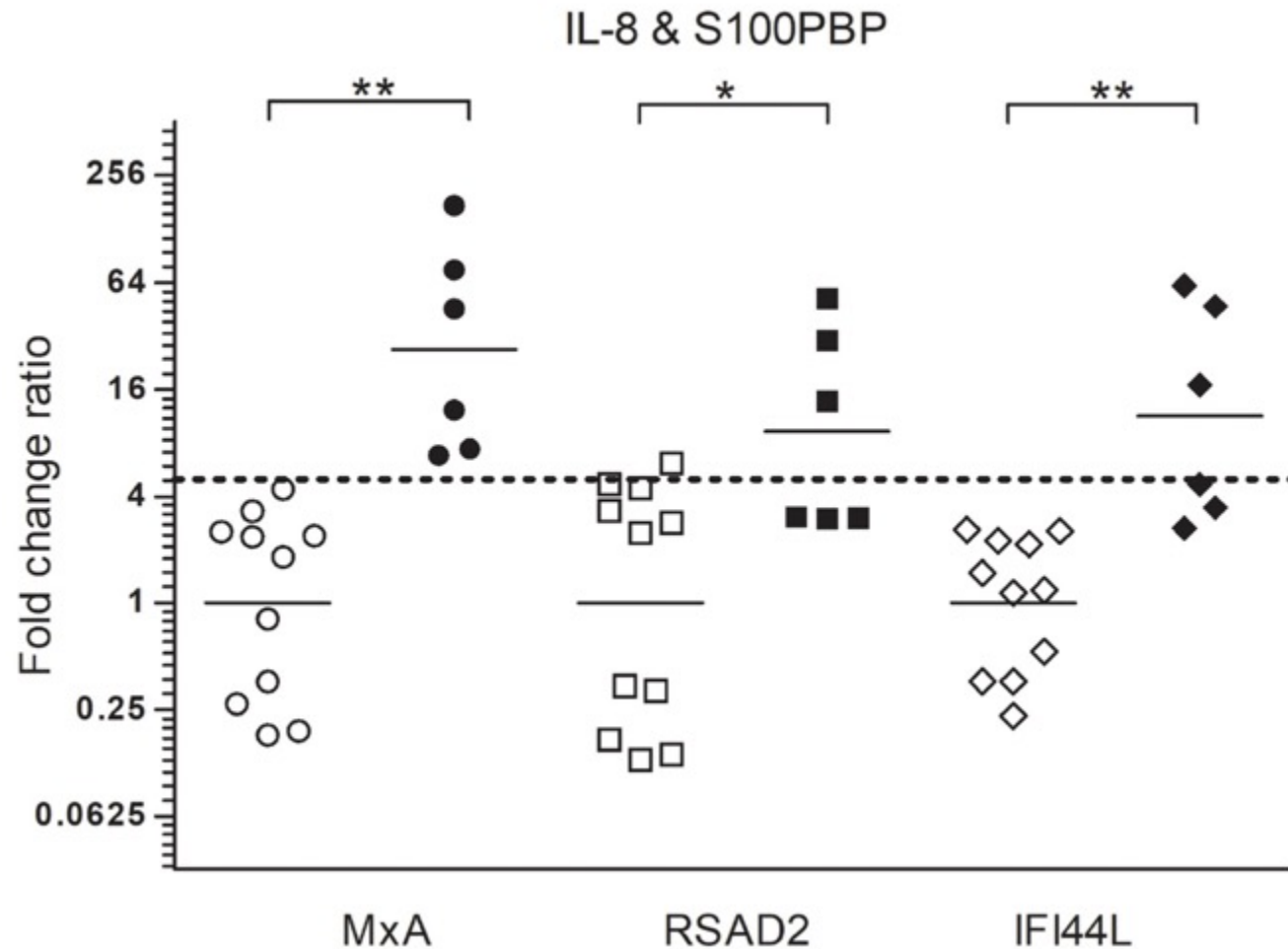
- Pig PBMCs stimulated with agonists
- Pig PBMCs stimulated with split influenza virus or inactivated *Actinobacillus pleuropneumoniae*

Category	Gene
Putative viral markers	<i>IFNα</i>
	<i>IFNβ</i>
	<i>IFITM3</i>
	<i>STING</i>
	<i>IFI44L</i>
	<i>IFIT3</i>
	<i>MxA</i>
	<i>RSAD2</i>
Putative Bacterial markers	<i>FAM89A</i>
	<i>S100PBP</i>
	<i>SLPI</i>
	<i>UPB1</i>
Pro-inflammatory cytokines	<i>IL-1β</i>
	<i>IL-6</i>
	<i>IL-8</i>
	<i>TNF-α</i>

Category	Gene	Viral mimics			Bacterial mimics			Inactivated microbes	
		ODN 2216 (TLR9)	R848 (TLR7/8)	poly (I:C) (TLR3)	Pam3CSK4 (TLR2/1)	LPS (TLR4)	FLiC (TLR5)	Split Influenza	<i>A. pleuro</i> (HI)
Putative viral markers	<i>IFNα</i>	3.4	0	1.0	0.2	0.3	0.3	1.7	0.4
	<i>IFNβ</i>	409.2	1.8	1992.0	3.0	0.5	1.7	70.9	1.4
	<i>IFITM3</i>	19.9	11.9	5.7	2.7	0.9	1.3	22.5	0.7
	<i>STING</i>	0.6	0.2	0.6	0.5	0.5	0.7	1.3	0.4
	<i>IFI44L</i>	7.5	3.4	3.3	1.4	0.7	1.1	8.0	0.5
	<i>IFIT3</i>	41.7	12.4	7.0	1.5	46.2	0.7	95.9	0.2
	<i>MxA</i>	72.6	39.5	17.7	7.9	0.5	1.0	63.0	0.7
	<i>RSAD2</i>	206.7	73.3	30.7	7.6	0.8	1.5	113.5	0,5
Putative Bacterial markers	<i>FAM89A</i>	0.6	0.1	0.5	0.5	0.8	0.8	0.6	0.6
	<i>S100PBP</i>	0.6	0.2	0.7	0.6	0.5	0.6	0.7	0.4
	<i>SLPI</i>	72.6	5.3	28.1	5.0	0.2	2.0	233.8	0.3
	<i>UPB1</i>	25.5	78.5	5.9	66.5	38.8	20.1	2.2	30.8
Pro-inflam-matory cytokines	<i>IL-1β</i>	1.0	1.8	10.3	67.2	40.7	30.0	0.7	17.7
	<i>IL-6</i>	20.7	96.7	19.7	72.3	51.7	24.5	3.7	12.2
	<i>IL-8</i>	1.9	9.0	44.0	119.8	66.6	32.0	0.3	24.3
	<i>TNF-α</i>	2.6	0.9	2.3	1.9	2.4	2.5	2.1	1.6

≤ 0.0625	$> 0.0625 - 0.125$	$> 0.125 - 0.25$	$> 0.25 - 0.5$	$> 0.5 - < 2$	2 to < 4	4 to < 8	8 to < 16	≥ 16
---------------	--------------------	------------------	----------------	---------------	------------	------------	-------------	-----------

Testing on blood from *Actinobacillus pleuropneumoniae* infected pigs



Phase 2

Goal: To expand the list of candidate genes to test
On more samples

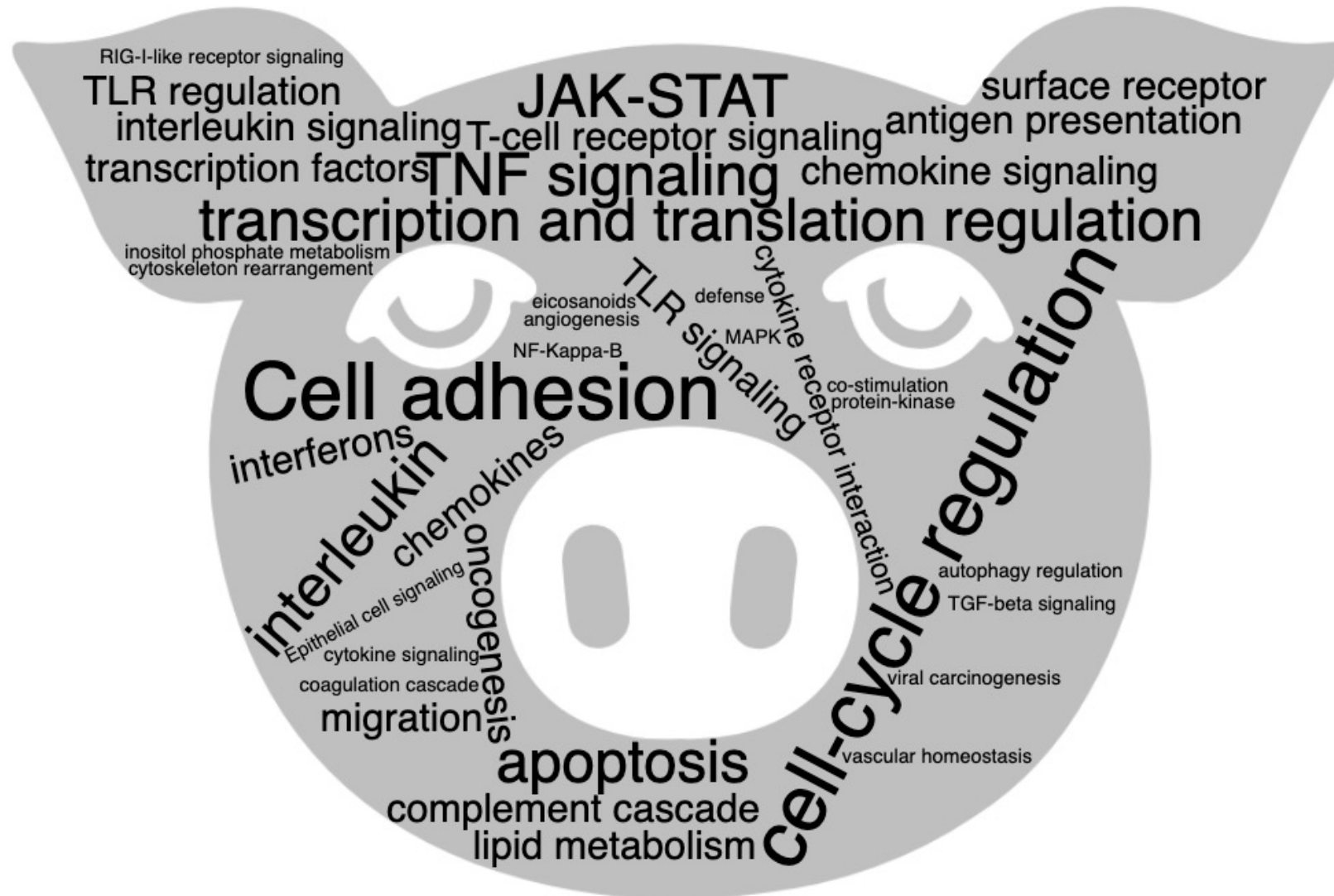
Method: Identify genes from published
transcriptome studies in pigs.



Transcriptome studies included in analysis

Viral-infection	Bacterial-infection	Virus-Bacterial co-infection
<p>Cruz-Pulido et al. (2021) - Comparative Transcriptome Profiling of Human and Pig Intestinal Epithelial Cells after Porcine Deltacoronavirus (<u>PDCov</u>) Infection. - RNASeq</p>	<p>Kamminga et al. (2020) - Combined Transcriptome Sequencing of <u>Mycoplasma hyopneumoniae</u> and Infected Pig Lung Tissue Reveals Up-Regulation of Bacterial F1-Like ATPase and Down-Regulation of the P102 Cilium Adhesin in vivo - RNASeq</p>	<p>Dang et al. (2014) - Transcriptional approach to study porcine tracheal epithelial cells individually or dually infected with <u>swine influenza virus</u> and <u>S. suis</u> - Microarray</p>
<p>Miller et al. (2020) - Comparison of the transcriptome response within the swine tracheobronchial lymph node following infection with <u>PRRSV, PCV-2</u> or <u>IAV-S</u>. - Digital Gene Expression Tag Profiling (DGETP)</p>	<p>Ni et al. (2019) - RNA-seq transcriptome profiling of porcine lung from two pig breeds in response to <u>Mycoplasma hyopneumoniae</u> infection. - RNASeq</p>	<p>Lin et al. (2015) Investigation of Pathogenesis of <u>H1N1 Influenza Virus</u> and <u>Swine Streptococcus suis</u> Serotype 2 Co-Infection in Pigs by <u>Microarray</u> Analysis</p>
<p>Liang et al. (2017) – PRRSV-infected PAMs Dong et al. (2021) – PRRSV-infected tonsils Hu et al. (2020) - PEDV-infected IPEC</p>	<p>Yan et al. (2019) - Histological and comparative transcriptome analyses provide insights into small intestine health in diarrheal piglets after infection with <u>Clostridium perfringens</u> type C. - RNASeq</p>	<p>Auray et al. (2016) - Transcriptional Analysis of <u>PRRSV</u>-Infected Porcine Dendritic Cell Response to <u>Streptococcus suis</u> Infection Reveals Up-Regulation of Inflammatory-Related Genes Expression - Microarray</p>

Pathways upregulated in the studies



The analysis

1. Clean and merge data from all selected studies
 - There are many gaps in the data, e.g there are no genes where we have data from all pathogen infections.
 - Different methods used for measuring gene expression
2. Identify genes with similar expression profiles by clustering
 - UR in viral-infected but DR in bacterial-infected
 - UR in bacterial-infected but DR in viral-infected

Final datasets chosen for comparison

1. Microarray datasets

1. Dang et al., 2014

- Infection with H1N1, *Streptococcus suis*, and co-infection with both in porcine tracheal epithelial cells

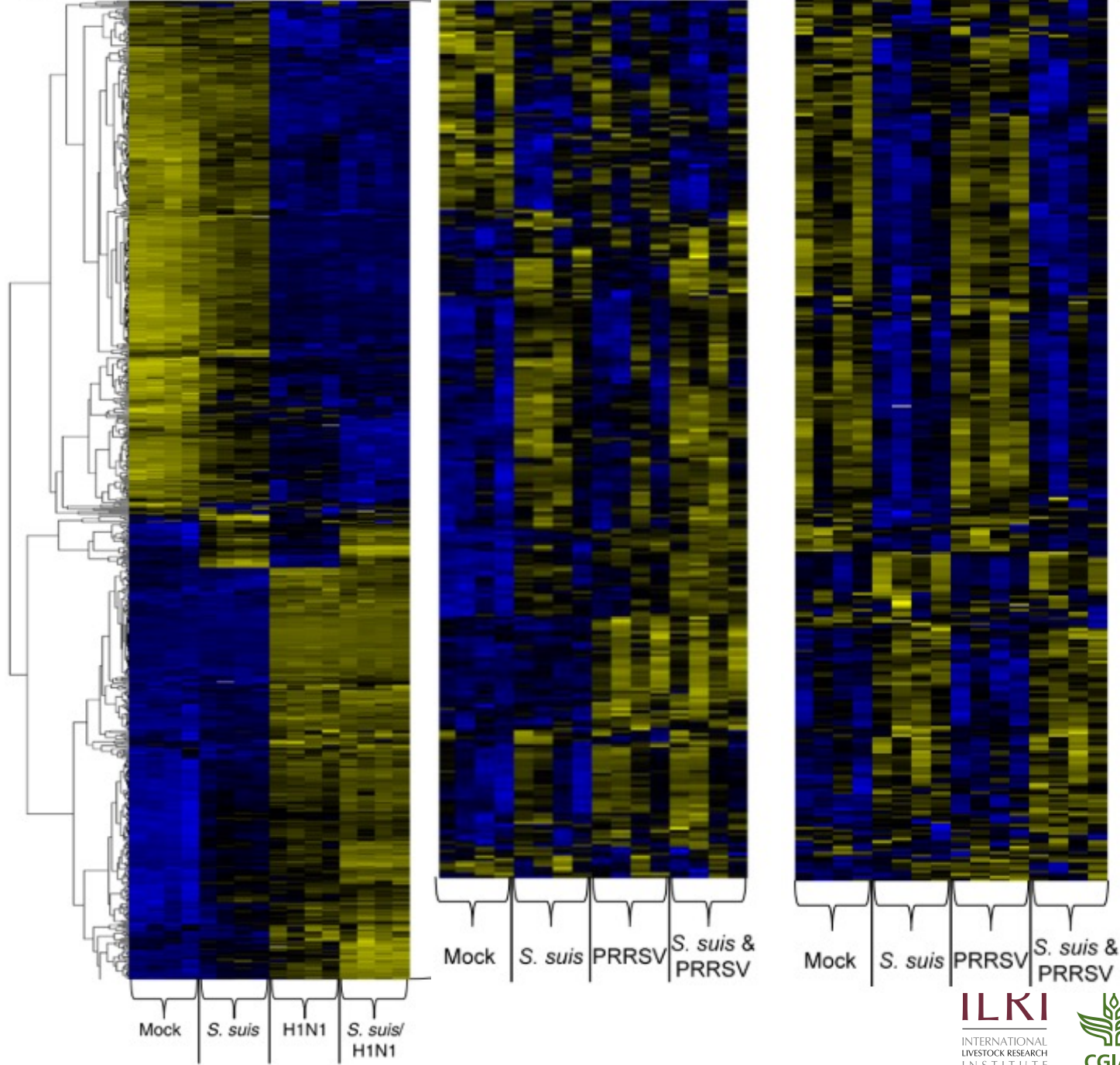
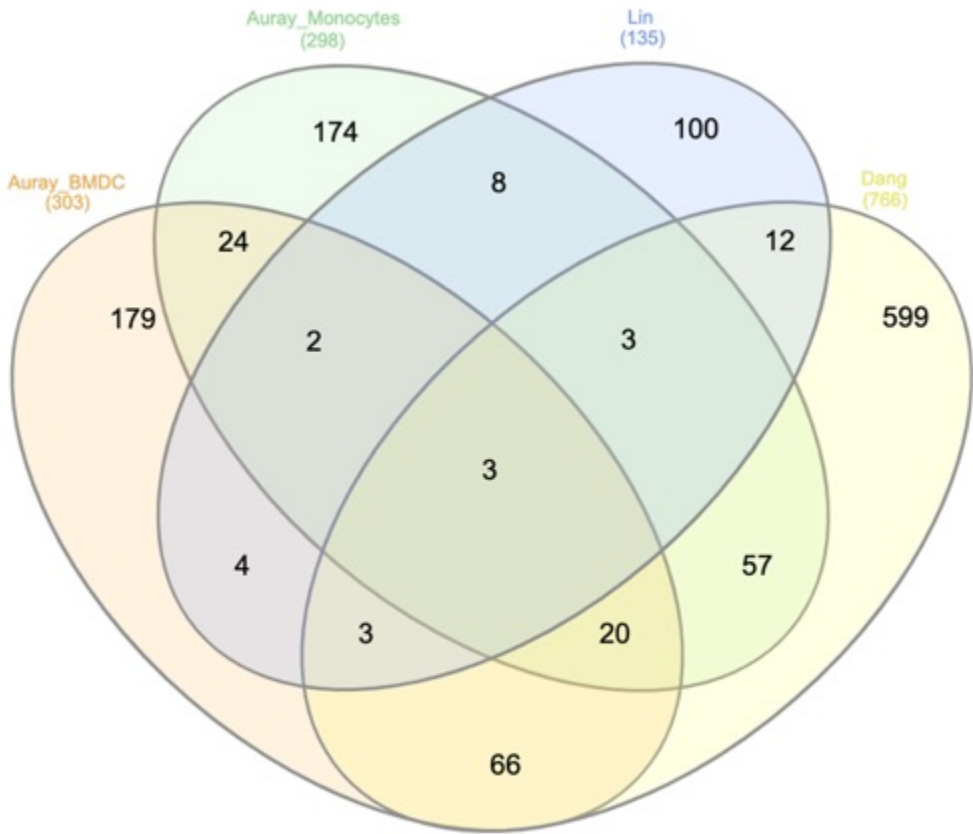
2. Lin et al., 2015

- Pigs infected with H1N1, *S. suis* and co-infection with both.
- Expression in lung tissue.

3. Auray et al., 2016

- Infection of dendritic cells and monocytes with *S. suis* and PRRSV.

Some results



Genes UR by bacterial infection in at least 2 studies

Function	SYMBOL	Gene description
Biological and metabolic processes	HK2	Hexokinase 2
	PLAT	plasminogen activator, tissue
	SLC2A1	Solute carrier family 2 (facilitated glucose transporter), member 1
Cytokines, chemokines, and related receptors	CSF2	colony stimulating factor 2
	SPP1	Secreted phosphoprotein 1
Lipid metabolism	LDLR	Low density lipoprotein receptor
Transcriptional and translational regulation	AGO2	argonaute RISC catalytic component 2

Genes UR by viral infection in at least 2 studies

Function	SYMBOL	Gene description
Biological & metabolic processes	USP18	ubiquitin specific peptidase 18
	ZBP1	Z-DNA binding protein 1
	BCR	BCR activator of RhoGEF and GTPase
Cytokine signalling	EIF2AK2	eukaryotic translation initiation factor 2 alpha kinase 2
	TRIM21	tripartite motif containing 21
Cytokines, chemokines, & related receptors	CCL4	C-C motif chemokine ligand 4
	TNFSF10	TNF superfamily member 10
	IFNB1	interferon beta 1
Cytoskeleton/actin rearrangement	TMOD4	tropomodulin 4
Defence response	RSAD2	radical S-adenosyl methionine domain containing 2
	IFIT3	interferon-induced protein with tetratricopeptide repeats 3
	IFIT1	interferon-induced protein with tetratricopeptide repeats 1
	MX1	MX dynamin like GTPase 1
	MX2	myxovirus (influenza virus) resistance 2 (mouse)
	DDX58	DEXD/H-box helicase 58
	OAS2	2'-5'-oligoadenylate synthetase 2
	GBP1	guanylate binding protein 1, interferon-inducible
	IFIH1	interferon induced with helicase C domain 1
Transcriptional & translational regulation	PARP12	poly(ADP-ribose) polymerase family member 12
	PARP14	poly(ADP-ribose) polymerase family member 14

What's next?

Short term:

- Test more biomarkers
- Collect more samples

Long term:

- Expand to other livestock
- Transfer to penside format


Samples collected for future use

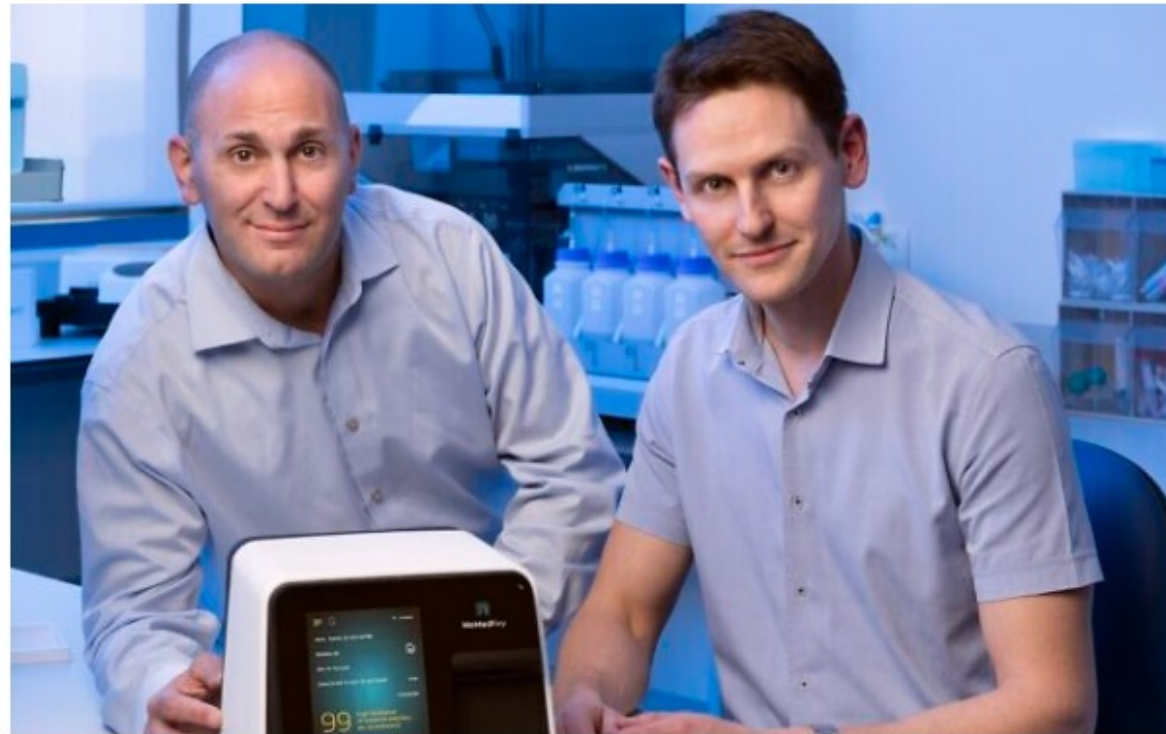
disease	species	healthy	disease	sample type (full blood)
CBPP	cattle	15	15	paxgene
CCPP	goats	40	28	paxgene
CBPP	cattle	31	9	RNAlater
AFS	pigs	17	5	RNAlater
BRSV	cattle	54		RNAlater

Israel's MeMed gets FDA approval for 'breakthrough' infection test

Purpose of test is to tell physicians whether body is waging war on bacteria or virus, and make decisions about whether to treat with antibiotics

By **RICKY BEN-DAVID** ✓

20 September 2021, 5:14 pm | 



MeMed Diagnostics founders Dr. Kfir Oved and Dr. Eran Eden. (Courtesy)

Acknowledgements

ILRI Team

Elise Schieck
Sonal Henson
Benjamin Nzau

SLU Team

Caroline Fossum
Bernt Hjertner
Claudia Lützenschwab
Ulf Magnusson

SVA

Marie Sjölund



ILRI
INTERNATIONAL
LIVESTOCK RESEARCH
INSTITUTE



The International Livestock Research Institute (ILRI) is a non-profit institution helping people in low- and middle-income countries to improve their lives, livelihoods and lands through the animals that remain the backbone of small-scale agriculture and enterprise across the developing world. ILRI belongs to CGIAR, a global research-for-development partnership working for a food-secure future. ILRI's funders, through the [CGIAR Trust Fund](#), and its many partners make ILRI's work possible and its mission a reality. Australian animal scientist and Nobel Laureate Peter Doherty serves as ILRI's patron. You are free to use and share this material under the Creative Commons Attribution 4.0 International Licence © ⓘ.

*better lives
through
livestock*

ilri.org