

# Avian cathelicidins: paradigms for the development of anti-infectives

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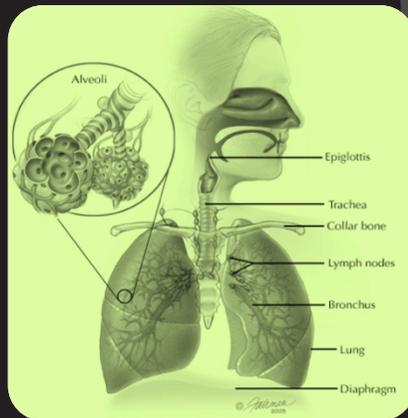
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# Organization of the lecture

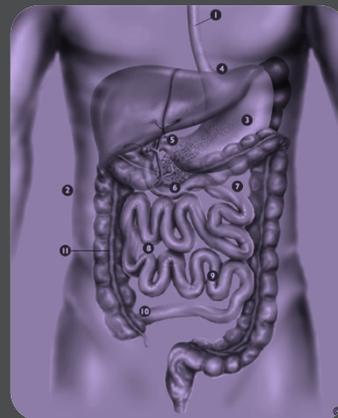
- Introduction
- Host defense peptides (HDPs)
- Chicken cathelicidin 2 (CATH-2)
- CATH-2 based anti-infectives
- Discussion



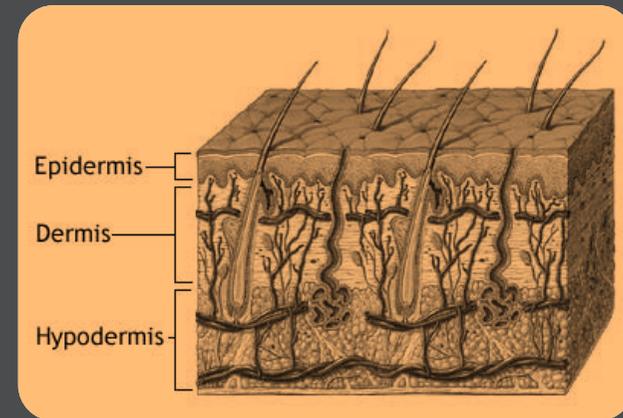
# Ports of entry of microorganisms



150 m<sup>2</sup>



400 m<sup>2</sup>



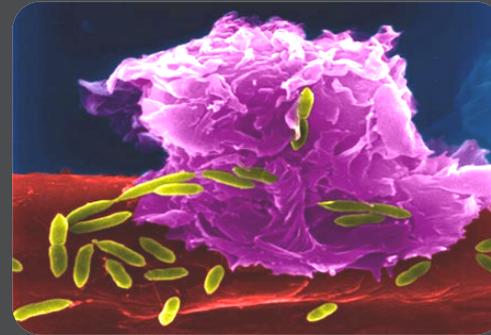
2 m<sup>2</sup>



# Innate Host Defence

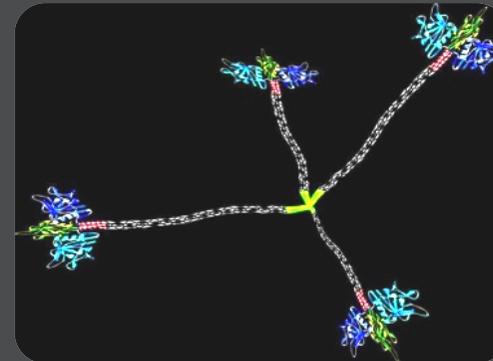
- Present in all organisms
- Limited repertoire of molecules
- Rapid
- Broad specificity
- Ancient

**The first line of defence against infection**



## Cellular defences

Neutrophils (heterophils)  
Macrophages

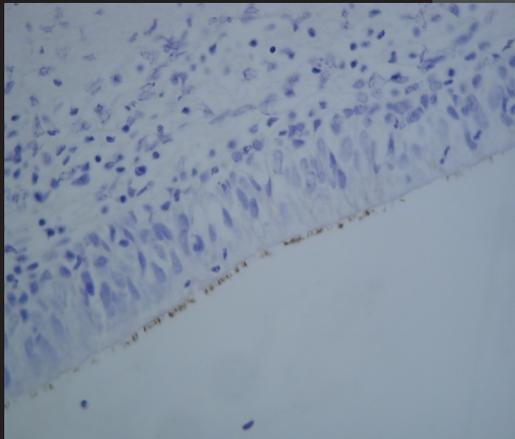


## Effector molecules

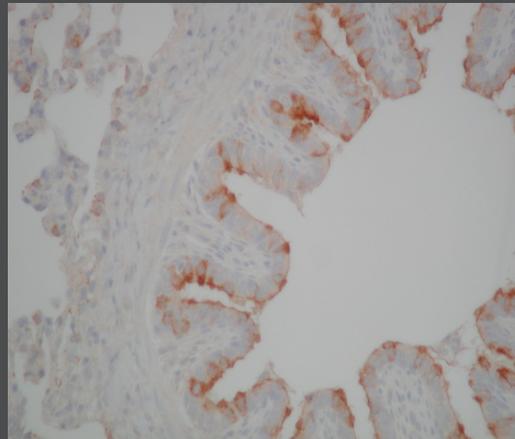
Enzymes, HDPs  
Collectins



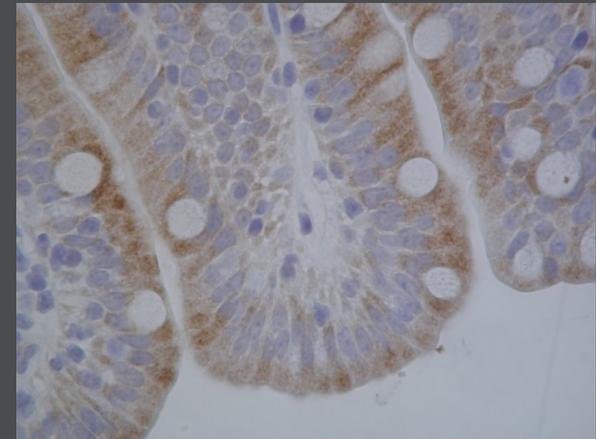
# Molecular host defence at the interface



Lung, trachea



Lung, bronchioli



Small intestine

Herias et al., 2007



# Effector molecules

- **Host Defence Peptides (HDPs)**
  - Defensins
  - Cathelicidins
- **Large soluble pattern recognition molecules**
  - Collectins

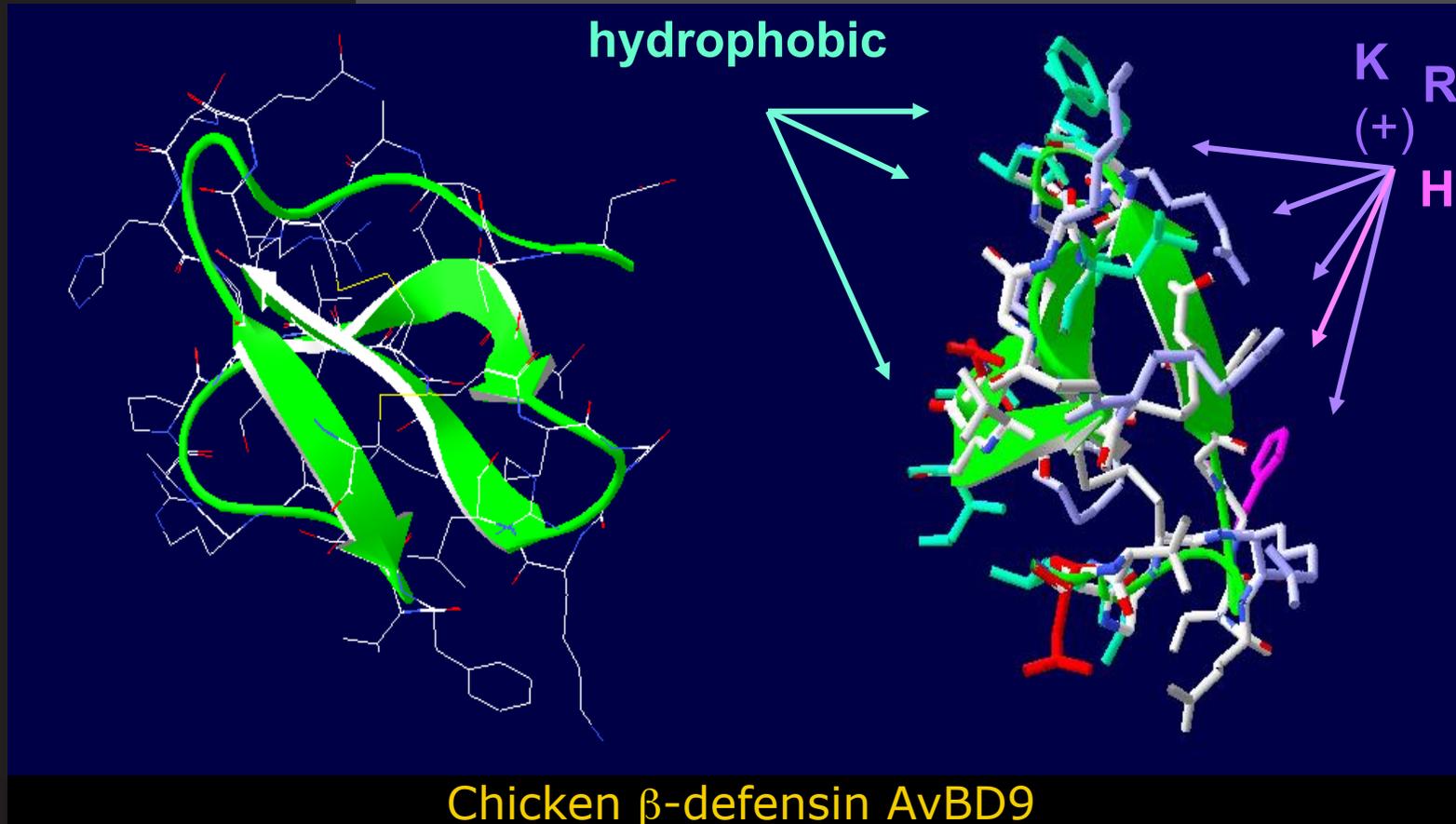


# Host defence peptides

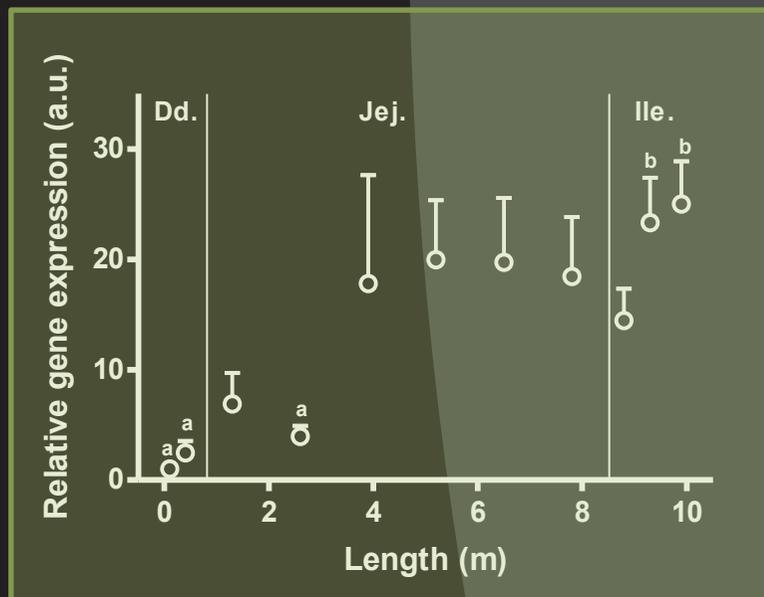
- Between 10 and 50 amino acids
- Positive charge
- Amphipathic
- Rapid acting; potent; broad spectrum of antimicrobial activity
- Immunomodulatory effects
- Constitutive and stimulated secretion



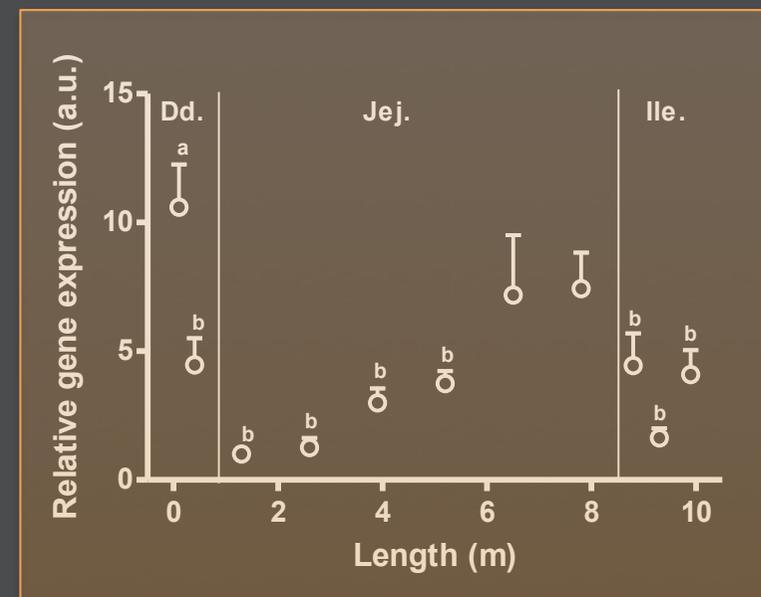
# Defensins



# Differential expression of 2 $\beta$ -defensin genes in porcine intestine



pBD-1



pBD-2

Veldhuizen et al., 2007



# ***S. typhimurium* adheres to (and invades) porcine intestinal IPI-2 cells**

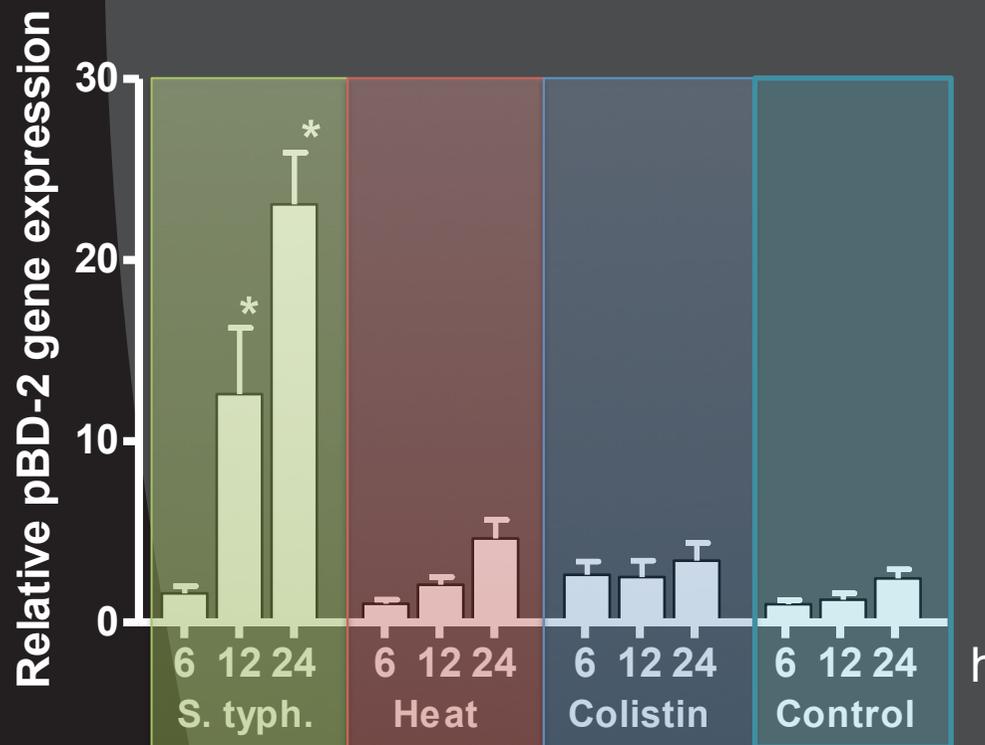


Veldhuizen et al., 2009



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# *S. typhimurium* induces pBD-2 in porcine intestinal epithelial cells



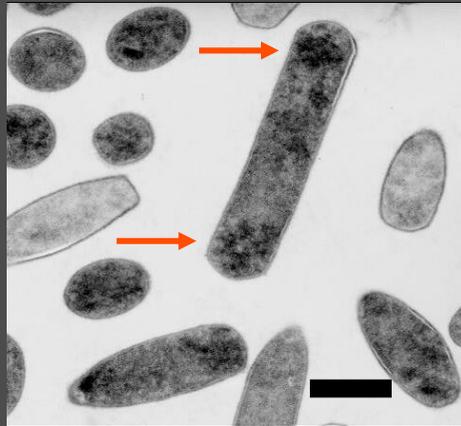
Veldhuizen et al., 2009



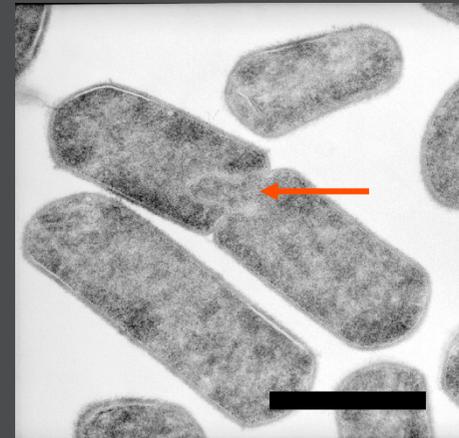
# AvBD-9 induces morphological changes in *C. perfringens*



Control



1.5 µg/ml



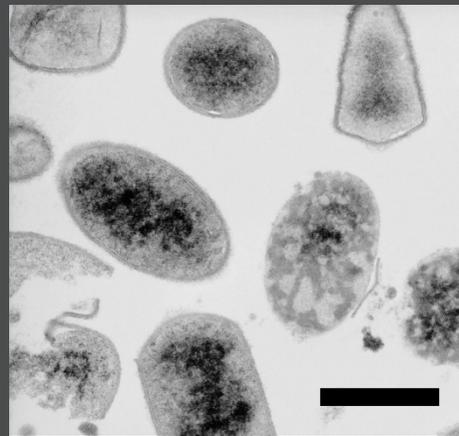
6.2 µg/ml



12.5 µg/ml



12.5 µg/ml



25 µg/ml



# Conclusions 1

- Defensins are differentially expressed in porcine digestive tract
- Upregulation of porcine  $\beta$ -defensin 2 in epithelial cells requires uptake of live bacteria
- HDPs may exert intracellular antimicrobial effects at low concentrations



# Chicken cathelicidin 2 (Cath-2)

- Cationic (+9)
- 26 amino acids
- Amphipathic

RFGRLRKRIRRFKVTITIQGSARF-NH<sub>2</sub>

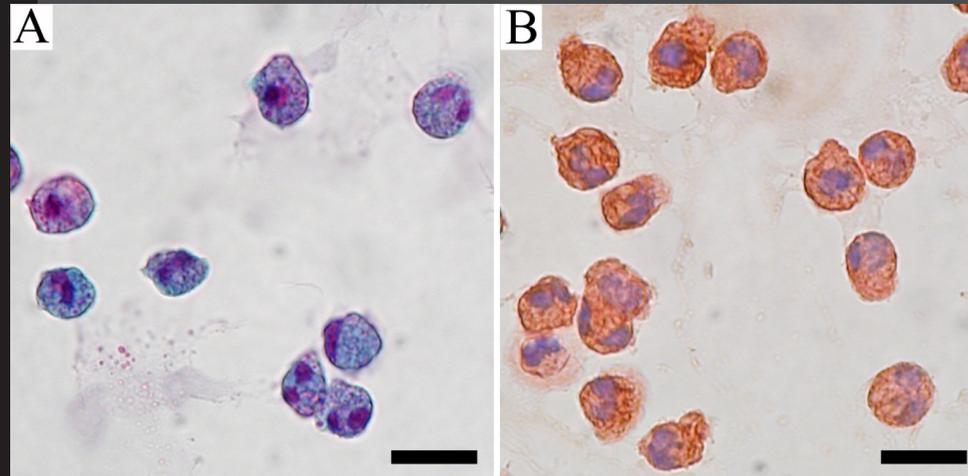


$\alpha$  Hinge region  $\alpha$

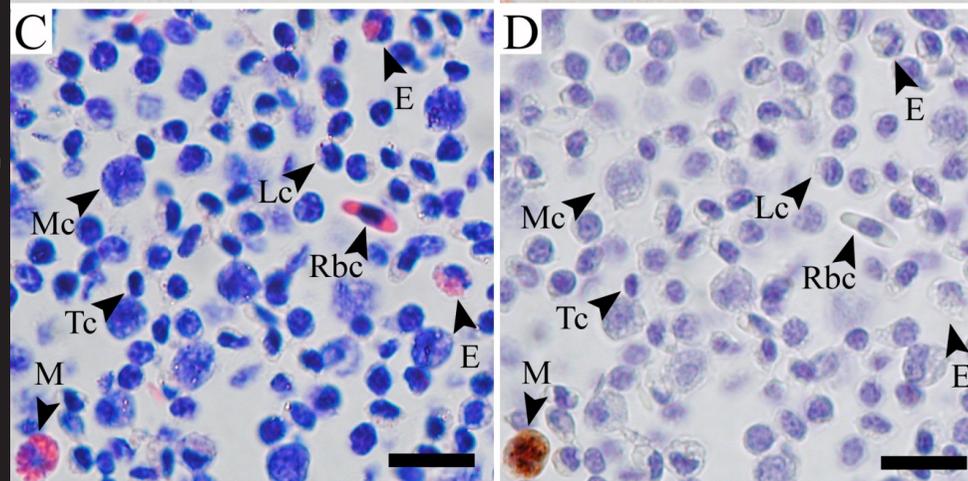


# CATH-2 is produced by chicken heterophils

Heterophils



Mononuclear cells



Giemsa

Anti-CATH-2

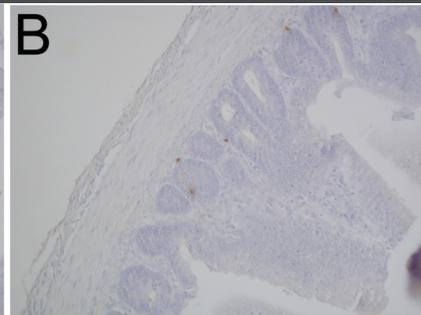
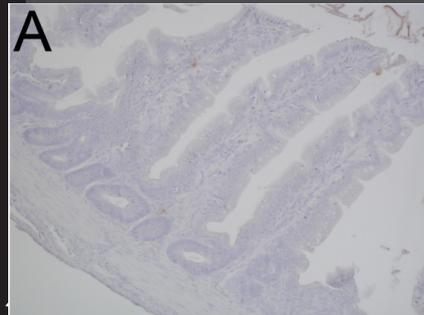


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Van Dijk et al., 2009a

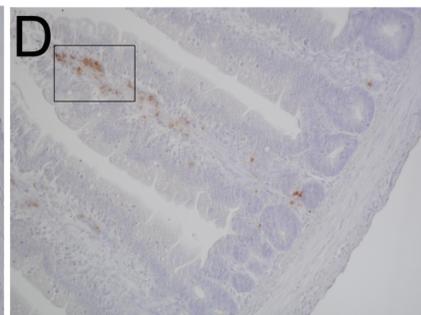
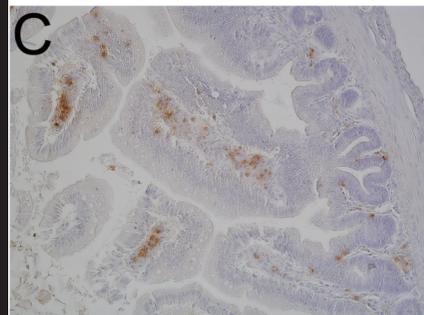
# *Salmonella enteritidis* challenge results in recruitment of CATH-2 containing heterophils

Control  
8h p.i.



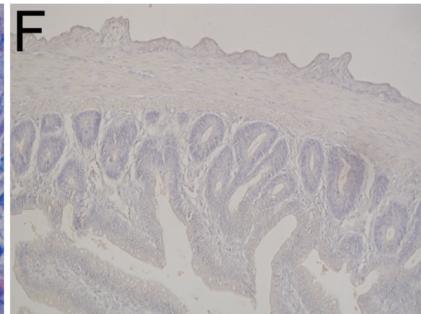
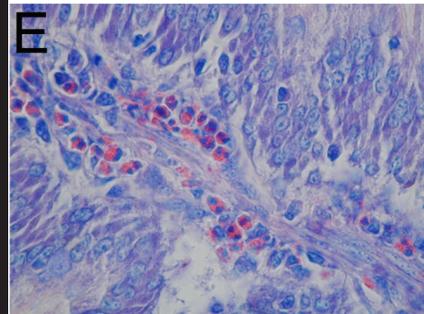
Control  
48h p.i.

*S. enteritidis*  
8h p.i.



*S. enteritidis*  
48h p.i.

*S. enteritidis*  
48h p.i.  
(Giemsa)



*S. enteritidis*  
48h p.i.  
(preimmune)

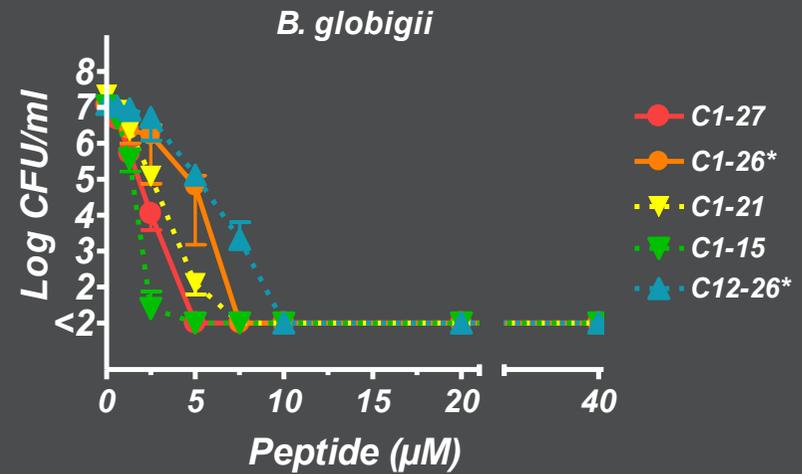
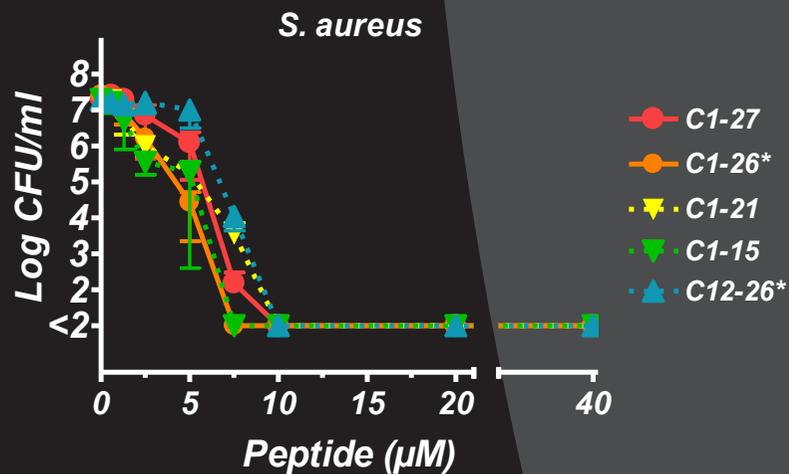
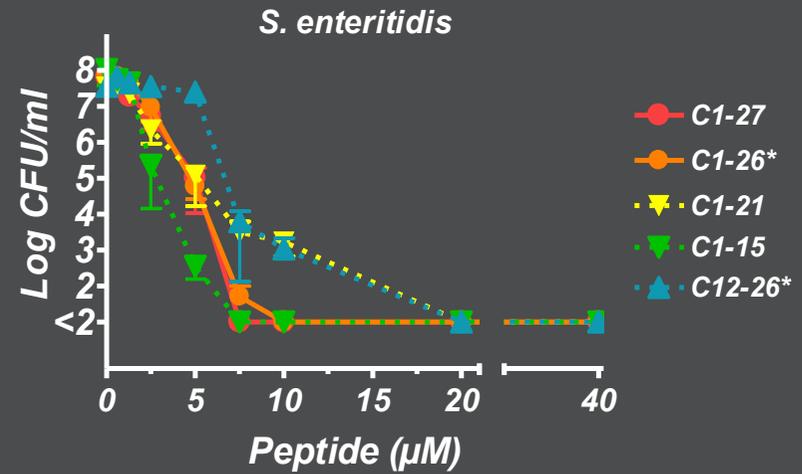
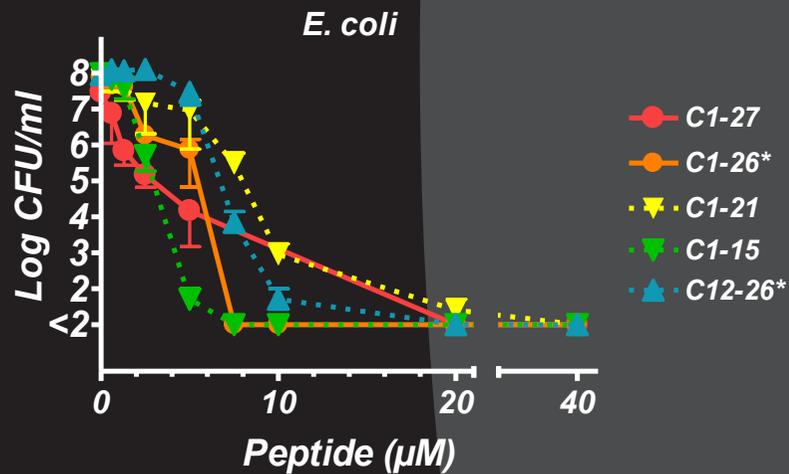


## Conclusions 2

- **CATH-2 is produced by heterophils but not by monocytes, lymphocytes, thrombocytes or red blood cells**
- ***Salmonella enteritidis* infections of chickens result in accumulation of CATH-2 positive heterophils at the site of infection**

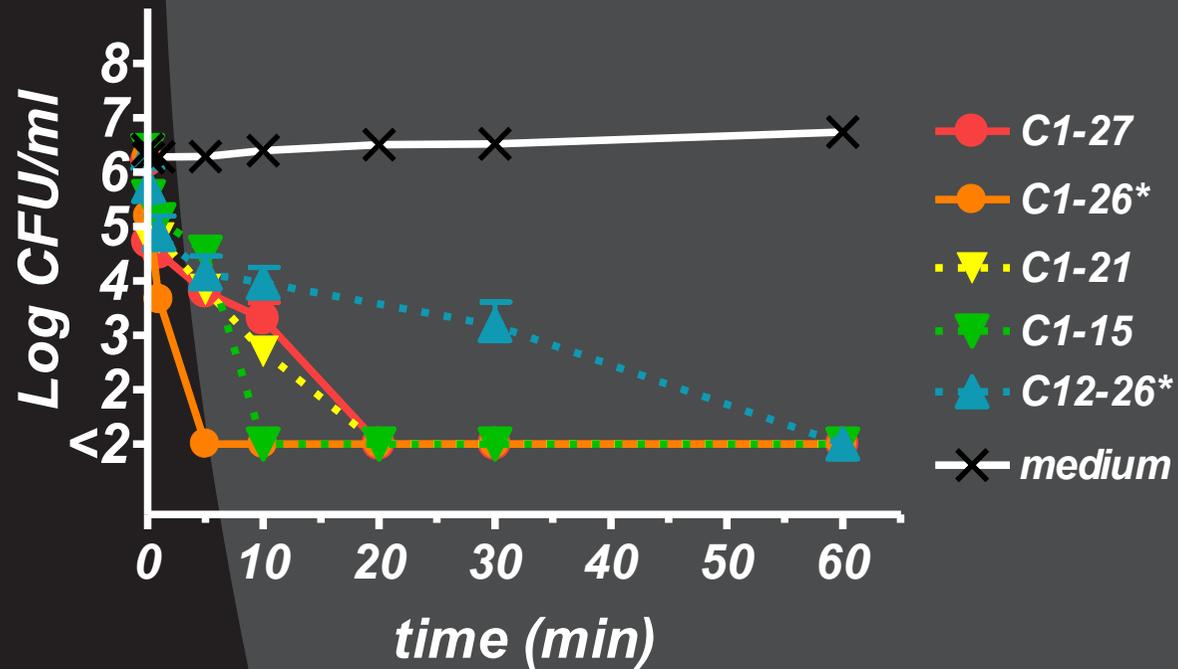


# Antibacterial activities of truncated CATH-2 analogs



# Killing curves of truncated CATH-2 analogs

*S. enteritidis* 13367



Van Dijk et al., 2009b



# Conclusions 3

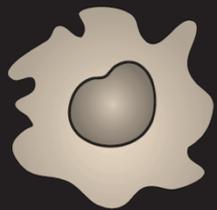
- **CATH-2 is microbicidal against Gram (-), Gram (+) bacteria, yeasts and fungi**
- **Truncated CATH-2 analogs are antimicrobial**



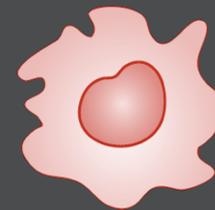
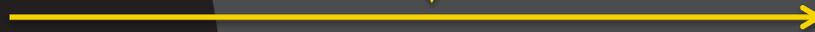
# Direct effects of CATH-2 on chemokine expression in HD11 cells

## Experimental set-up

HD11 cell line



CATH-2



chemokine expression (qPCR)

4h   
24h 



## Conclusions 4

- CATH-2 and derived peptides induce cytokine production in a chicken macrophage cell line
- CATH-2 dampens the LPS-induced inflammatory response



# General conclusions

- Based on host defense peptides of chicken (and pig), small peptides were produced that exhibit strong, broad-range, antibacterial activity
- Peptides were produced that are very active towards bacteria but are not toxic to host cells
- Peptides were produced that show immunomodulatory activities
- Prophylactic or therapeutic treatment of chickens by CATH-2 significantly reduced Salmonella survival in the crop



# Challenges (discussion)

- Improve specific activities of compounds
- Improve stability of lead structures
- Development carrier/delivery systems
- Pharmacokinetics
- Cost-effective large scale production



# Division Molecular Host Defence



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