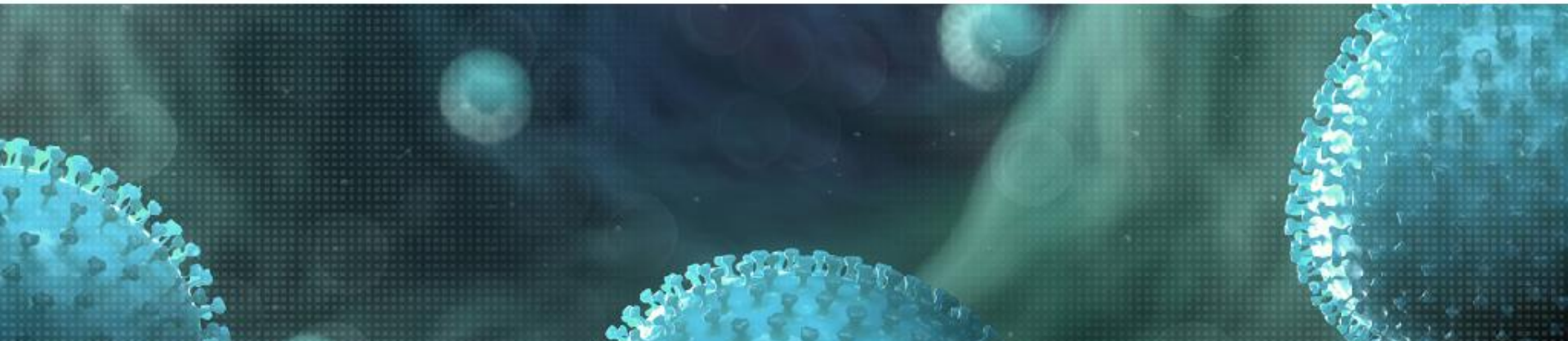


Erasmus MC



Viroscience lab

WHERE SKILLS MEET TO STUDY & PROTECT



Species-specific endotheliotropism of Highly Pathogenic Avian Influenza Virus in avian hosts

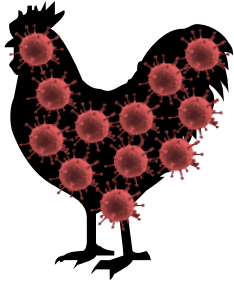
Anja de Bruin

PhD student

Supervised by Mathilde Richard

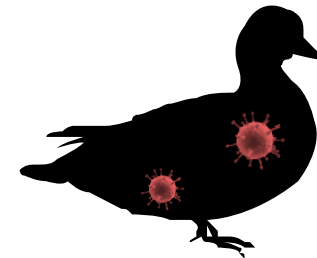
Group of Ron Fouchier

Species-specific pathogenesis of HPAIV



Systemic dissemination
Hemorrhagic disease
Oedema
High mortality (>100%)

Endotheliotropism



Generally mild/no symptoms
Limited/no systemic spread
Pathogenesis depends on:
HPAIV strain
Bird species
Age
Prior exposure

No endotheliotropism

Research question

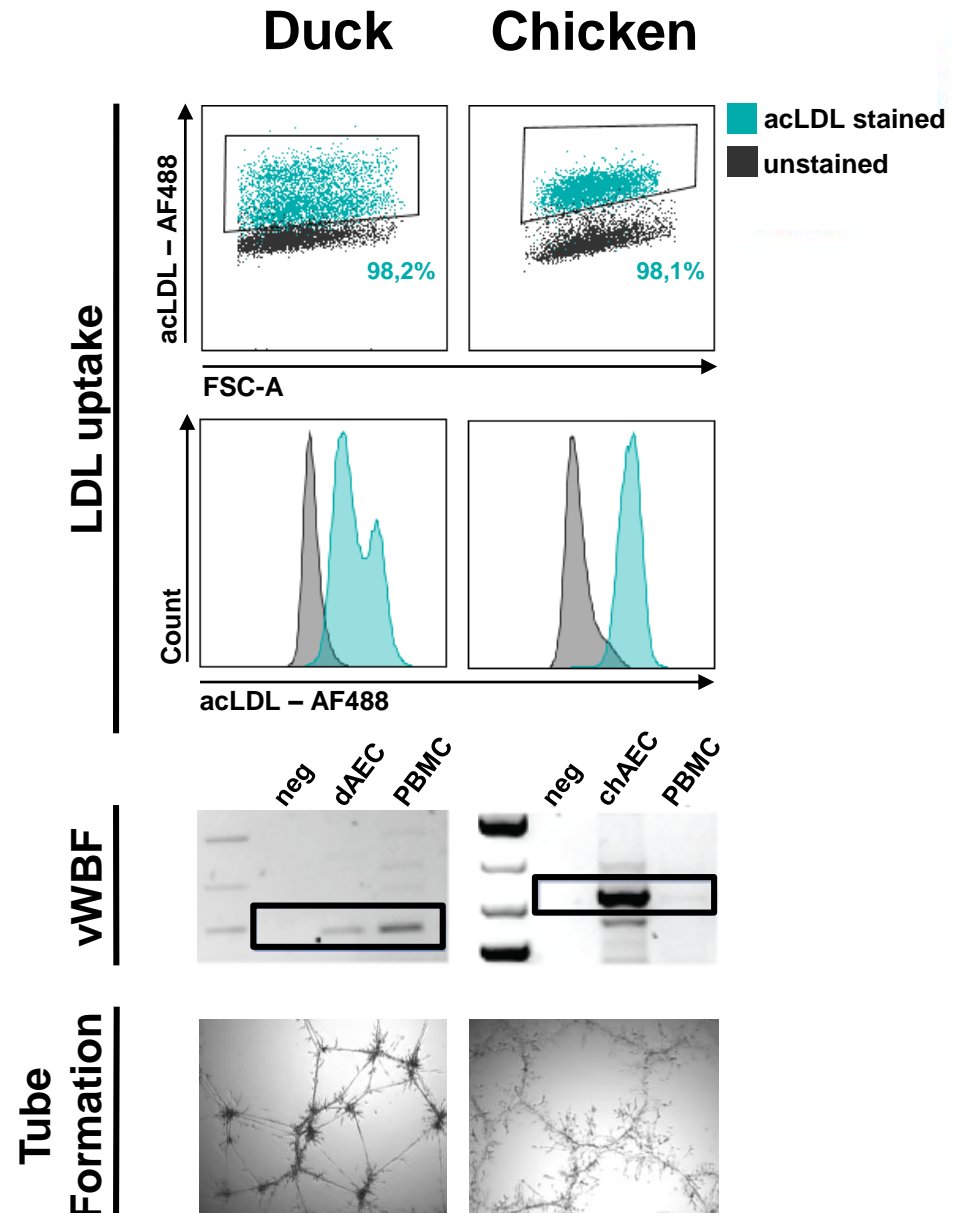
What determines the difference in endotheliotropism between wild birds and poultry?

- Chicken and mallard/pekin duck as model species

Avian primary endothelial cell culture

Aortic endothelial cells (AEC)

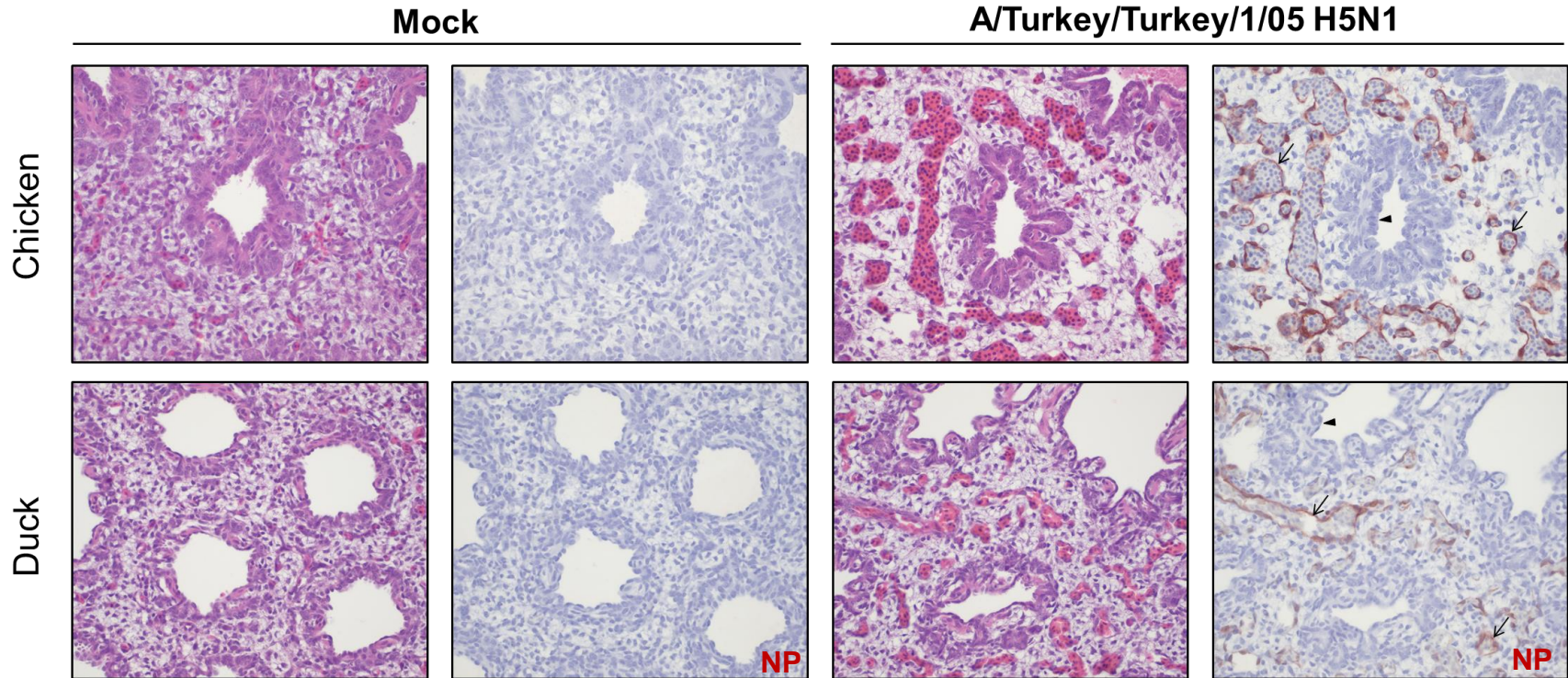
- Embryonic aortic arches
 - Chicken day 18
 - Duck day 21
- Passage 15-23 in Lonza EGM-2MV medium
- Phenotypical and functional characterization



Susceptibility of duck endothelial cells *in ovo*



Allantoic fluid inoculation  Chorio-allantoic membrane  Embryonic vasculature



D18 duck

D14 chicken

1000 TCID₅₀; 24hpi



Conclusions part 1

- (Embryonic) duck endothelial cells *in vitro* and *in ovo* were susceptible to HPAIV infection
- Top differentially upregulated genes in duck endothelial cells included *immune regulation* and *type 1 interferon* related genes
- In contrast, top differentially upregulated genes in chicken endothelial cells did not include genes from these categories, but did include *oxidative stress response* related genes
- Chicken cells mounted a stronger pro-inflammatory response than duck cells upon poly(I:C) stimulation and H5N1 infection

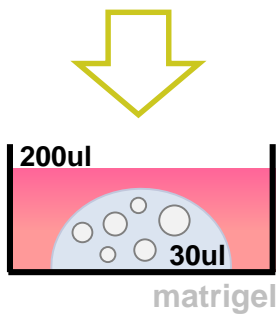
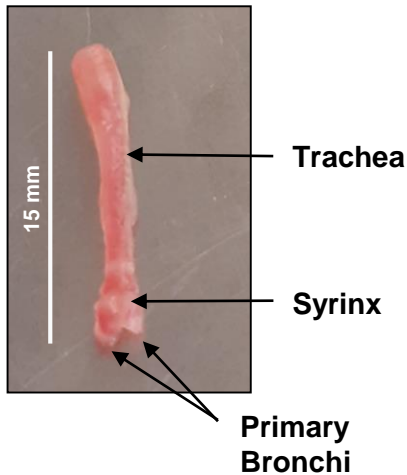
Infection of endothelial cells in monoculture does not recapitulate the natural route of infection via the epithelium:

Moving towards an epithelial/endothelial coculture system

Culture of avian epithelial cells (tracheospheres)

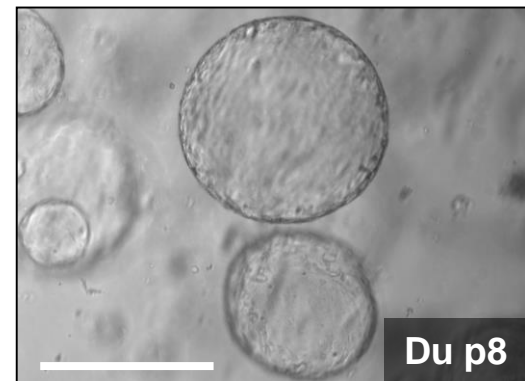
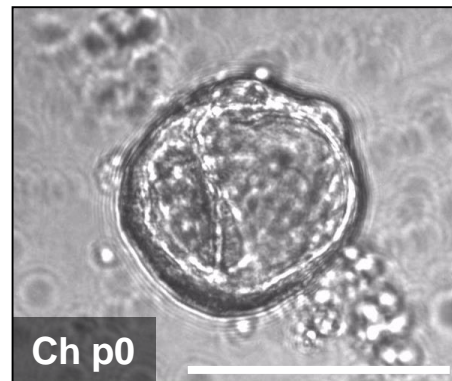
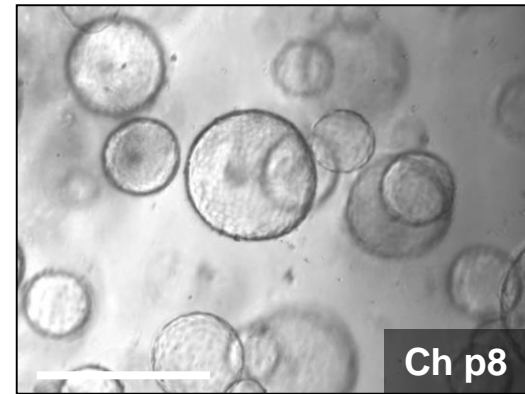
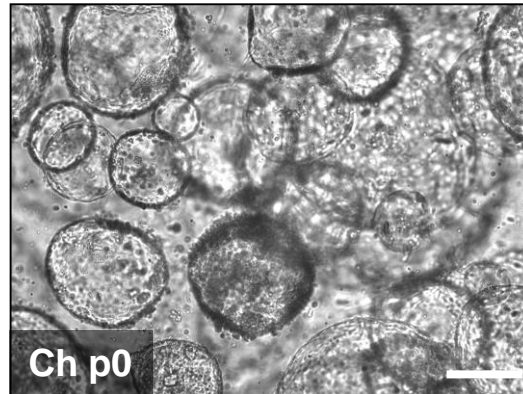


Harvest trachea
from embryos



Early passages

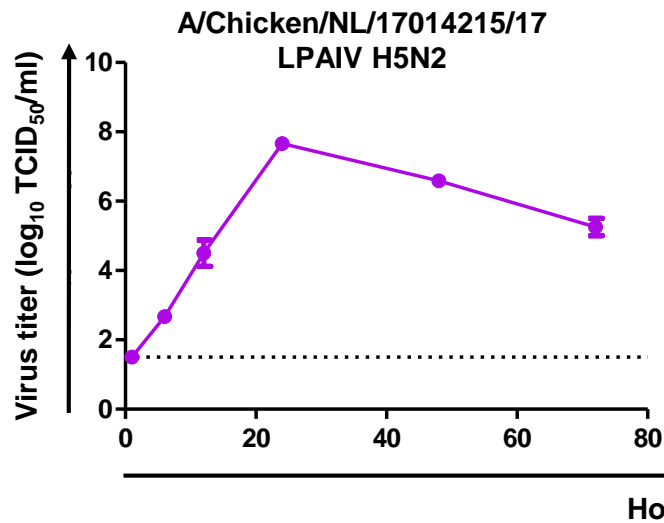
Late passages



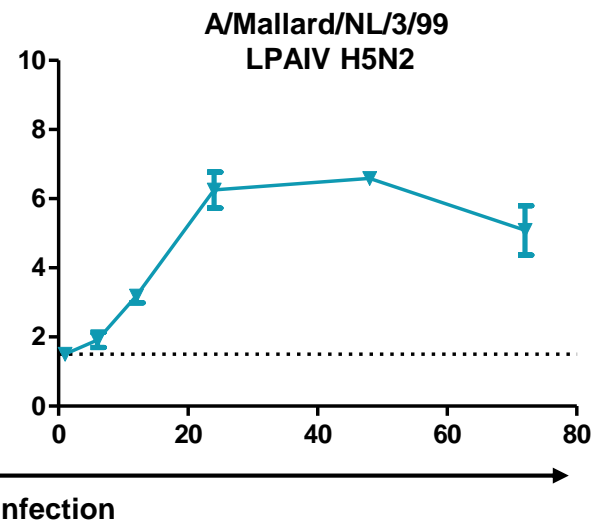
Differentiated avian epithelial cells express trypsin-like proteases



Chicken epithelial cells



Duck epithelial cells





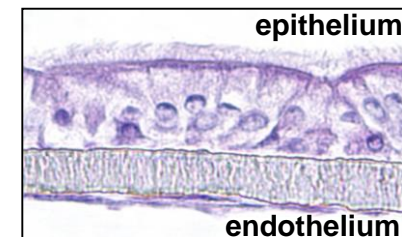
Conclusions part 2

- An avian tracheosphere culture has been set up
 - Long-term culture
 - Capacity to differentiate on air-liquid interface
 - High Trans Epithelial Electrical Resistance (TEER) values
- Differentiated epithelial cells express trypsin-like proteases, allowing for multi-cycle growth of LPAIV

Future perspectives

Use epithelial/endothelial coculture model to study differences between chickens and ducks with regards to:

1. Dissemination to endothelial cell compartment
2. Barrier integrity
3. Polarity of virus release
4. Immunological crosstalk between cell layers



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