

# H5N1 in dogs: virus receptors and susceptibility

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# Influenza in dogs what do we know?

- Question whether dogs are susceptible to  $H_5N_1$  is highly relevant
- Dogs are susceptible to infection with H3N8 influenza USA, 2004
- $H_5N_1$  was isolated from a dead dog in Thailand
- Paper describing dogs with antibodies against  $H_5$
- Mammals are susceptible in particular cats



# Aim of the study

- Do dogs have receptors for AI viruses and if so are dogs susceptible to H5N1 infection?



# Detection of H<sub>5</sub>N<sub>1</sub>-receptors in respiratory tissues of dogs

- Controls SPF-beagle
- Detection of H<sub>5</sub>N<sub>1</sub> receptors in formalin fixed tissue (paraffin slides)
  - Purified inactivated H<sub>5</sub>N<sub>1</sub> virus labelled with FITC
  - FITC-labelled lectin of *Maackia amurensis* which binds  $\alpha$ 2,3-linked sialosides.
  - FITC-labelled lectin of *Sambucus nigra* which binds  $\alpha$ 2,6-linked sialosides.

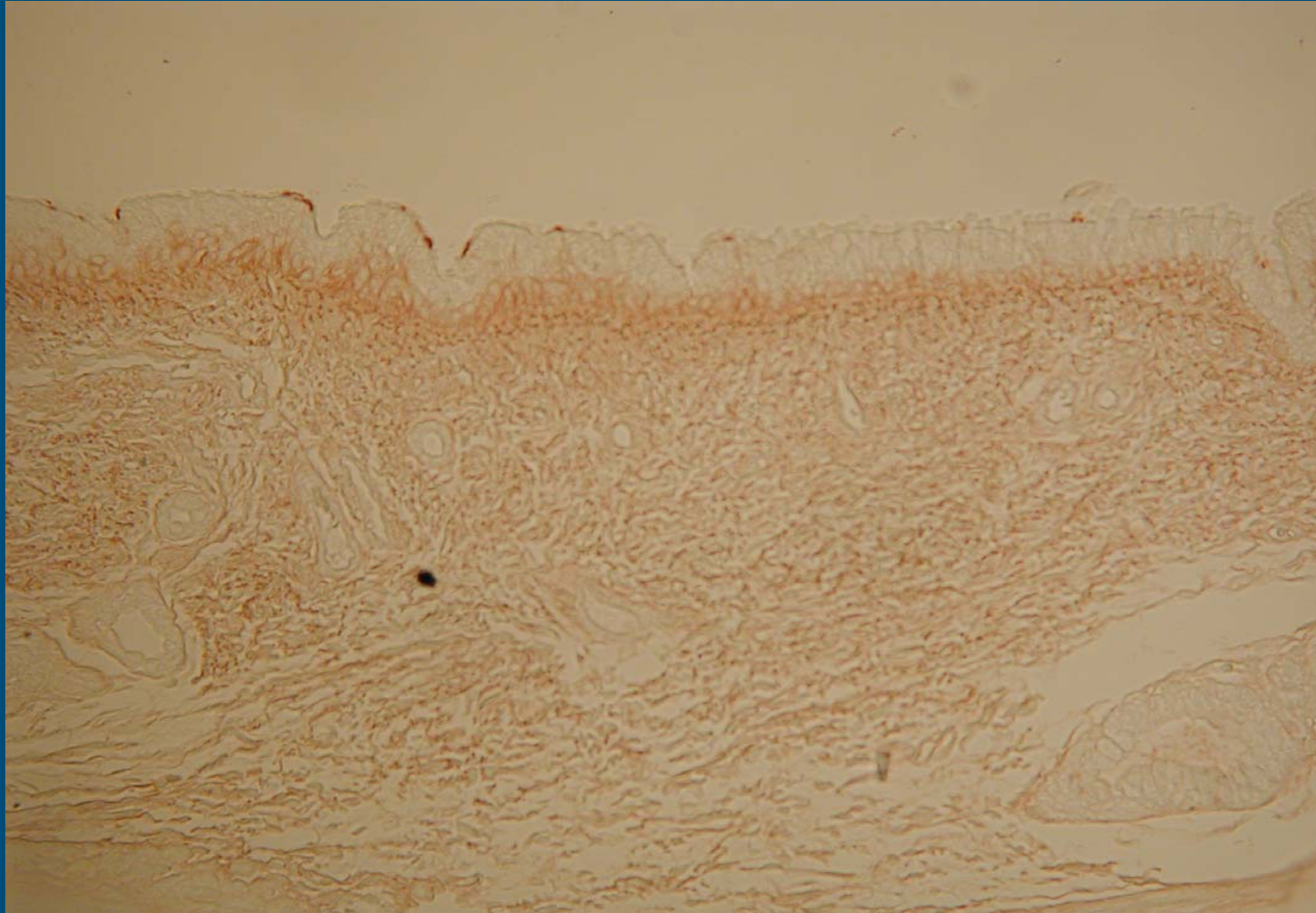


## Detection of H<sub>5</sub>N<sub>1</sub>-receptors in respiratory tissues of dogs 2.

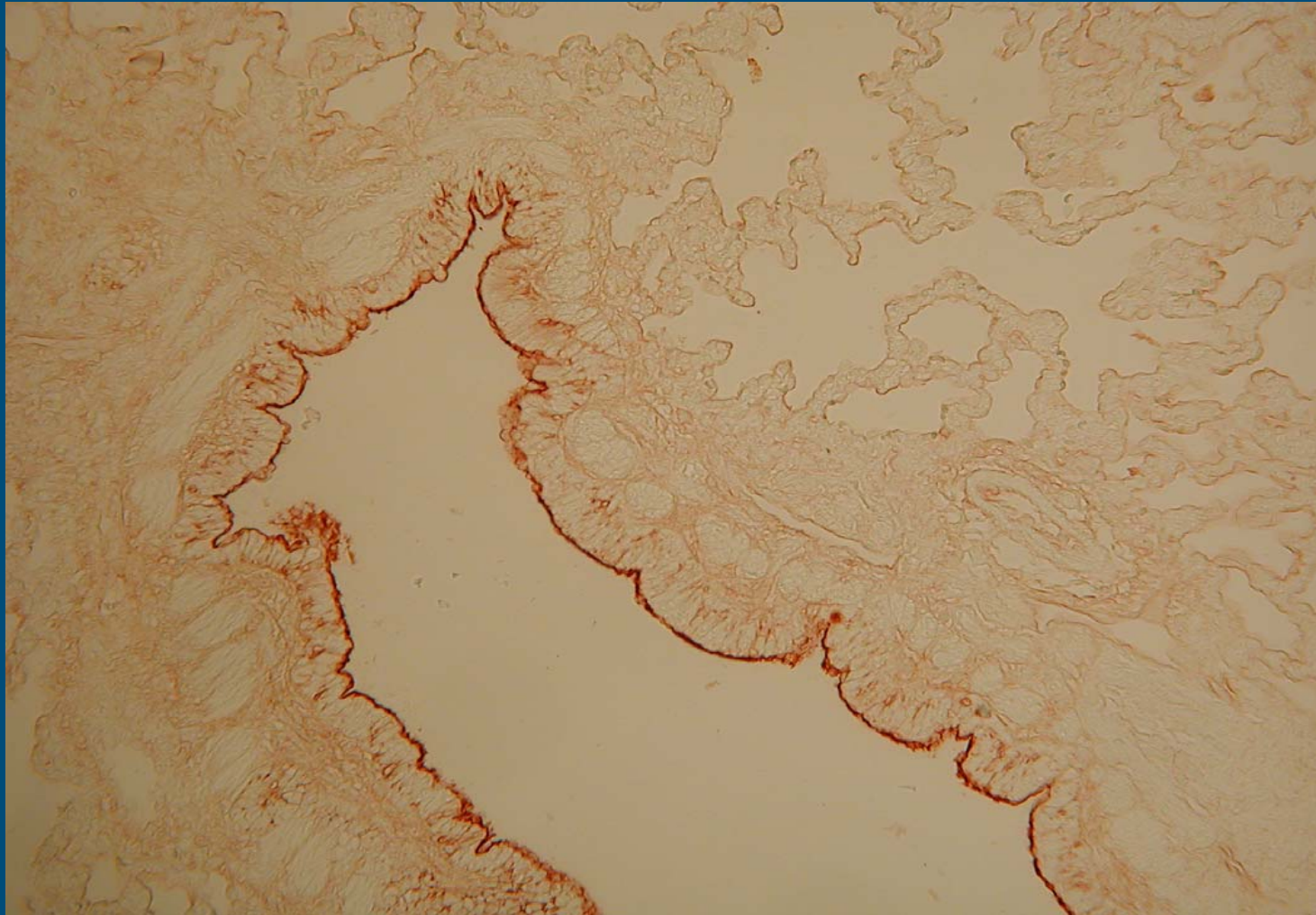
- Staining using anti-FITC-peroxidase labelled antibody (method of van Riel ad Kuiken)
- Controls were stained with antibody conjugate but omitting the incubation with FITC labelled virus or lectin
- Incubation with FITC-labelled virus after pre-incubation with non-labelled lectin



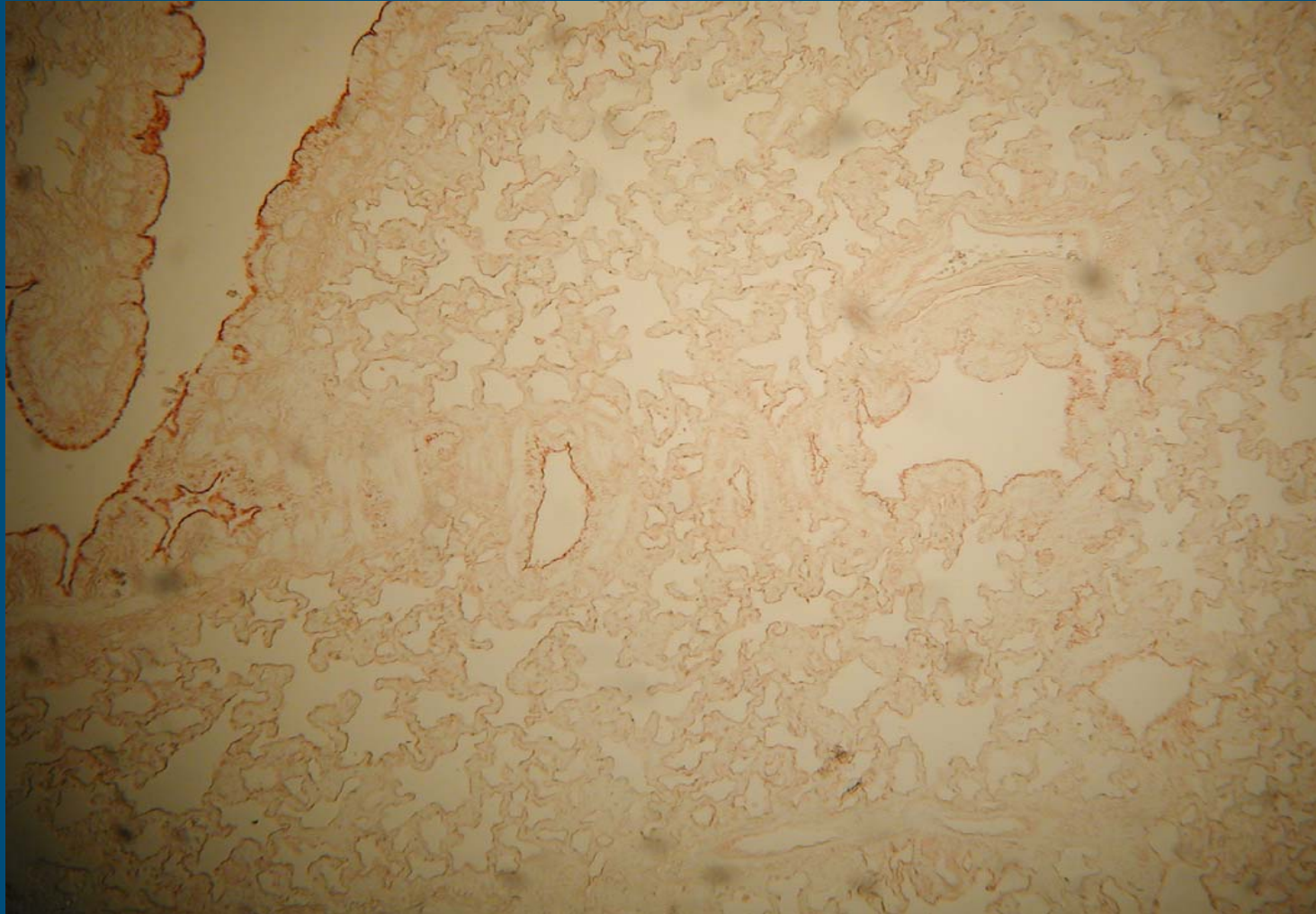
# Trachea of dog $\alpha$ 3,2-linked sialosides specific lectin



# Bronchus of dog $\alpha$ 3,2-linked sialosides specific lectin



# Bronchus of dog $\alpha$ 3,2-linked sialosides specific lectin

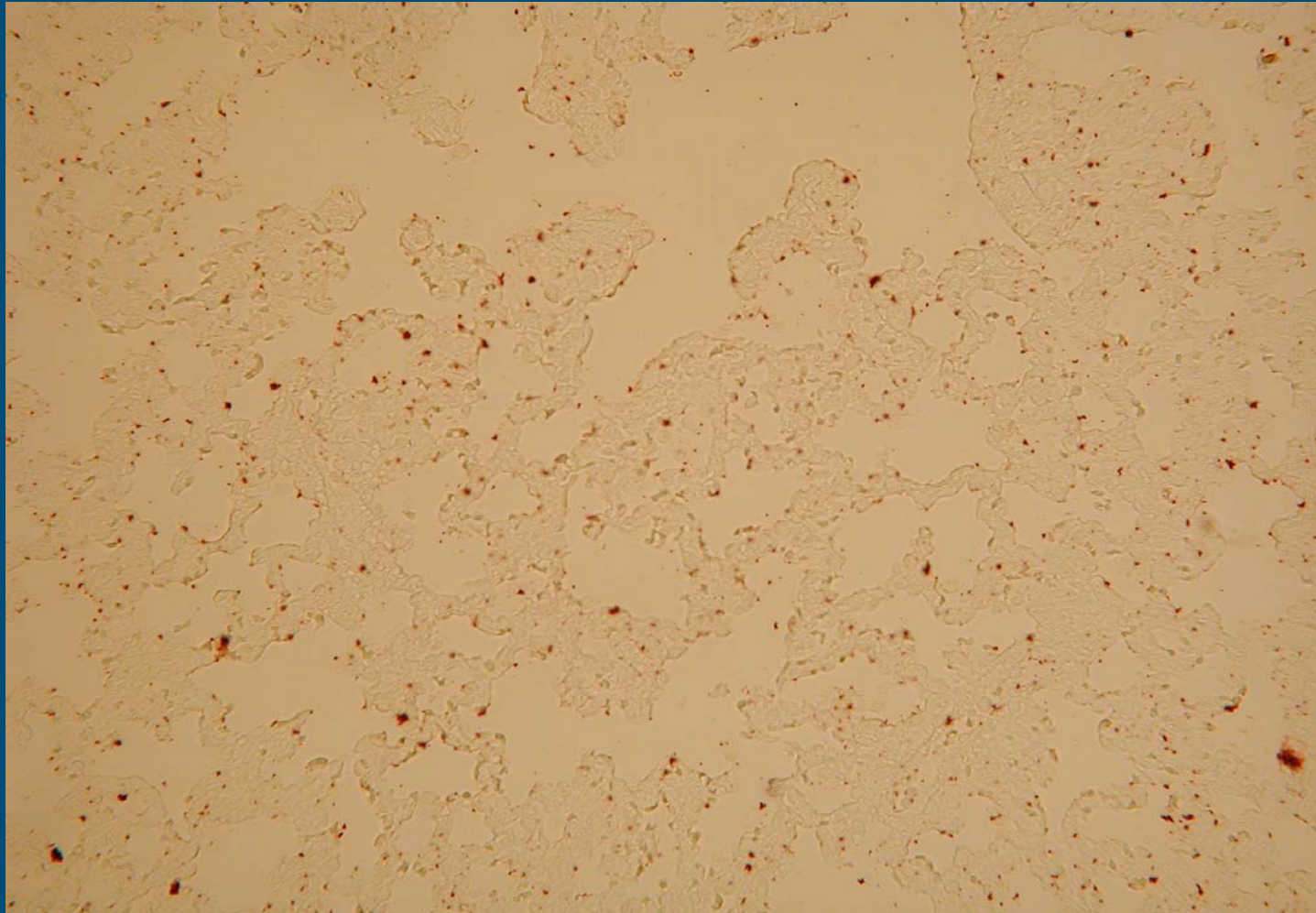




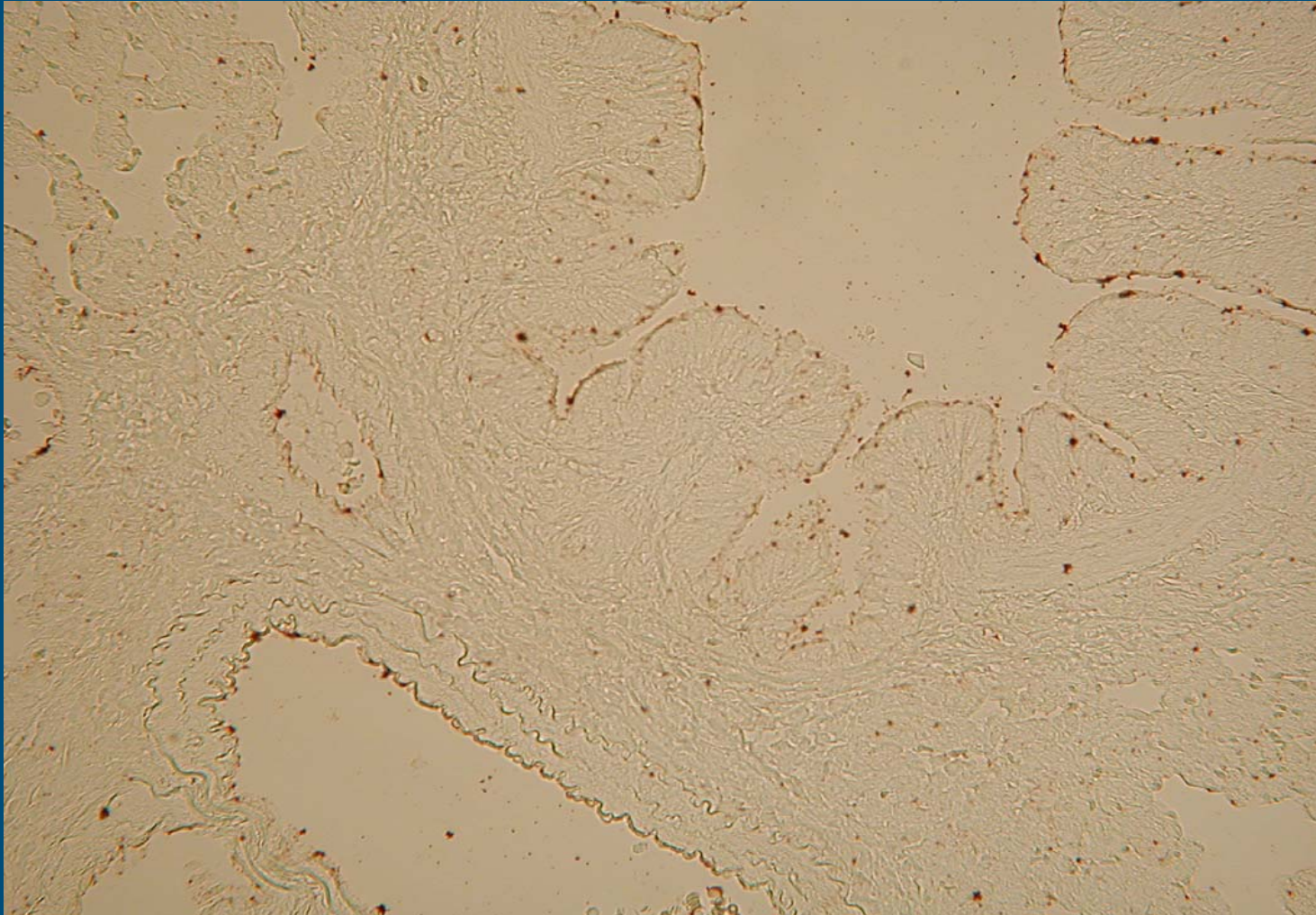
# Trachea of dog stained with FITC labelled $H_5N_1$



# Aveoli of dog stained with labelled H5N1

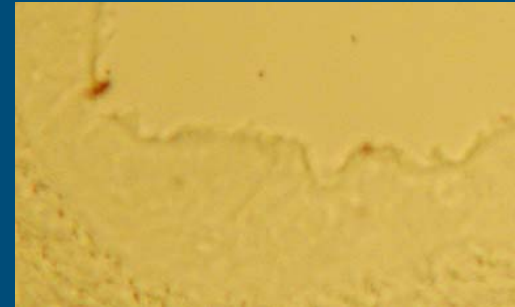
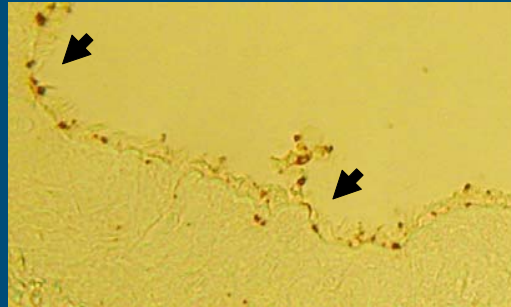


# Lung of dog stained with FITC labelled H<sub>5</sub>N<sub>1</sub>

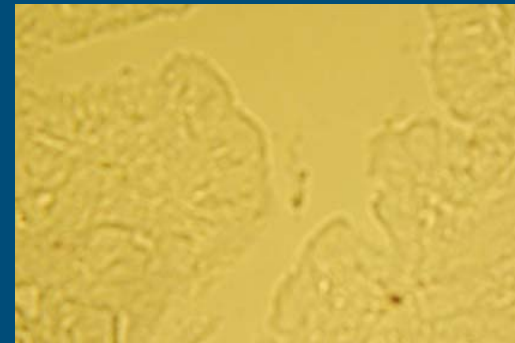
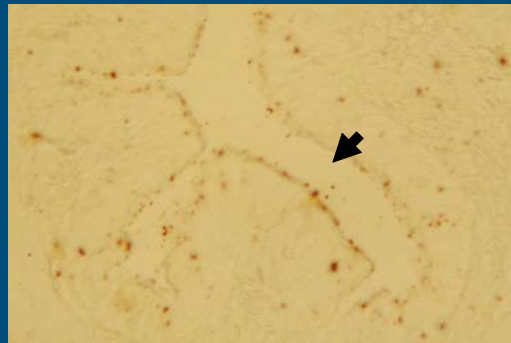


# Blocking of H<sub>5</sub>N<sub>1</sub> binding using MAA-lectin

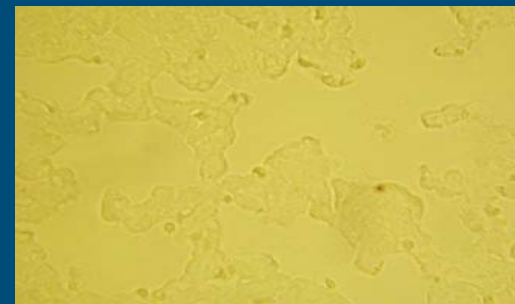
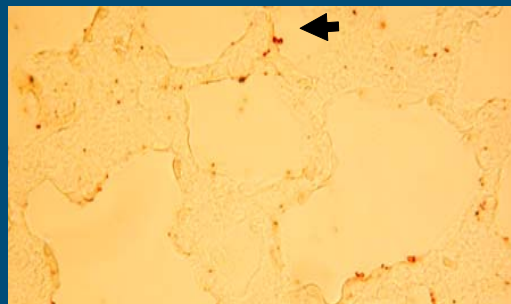
Trachea



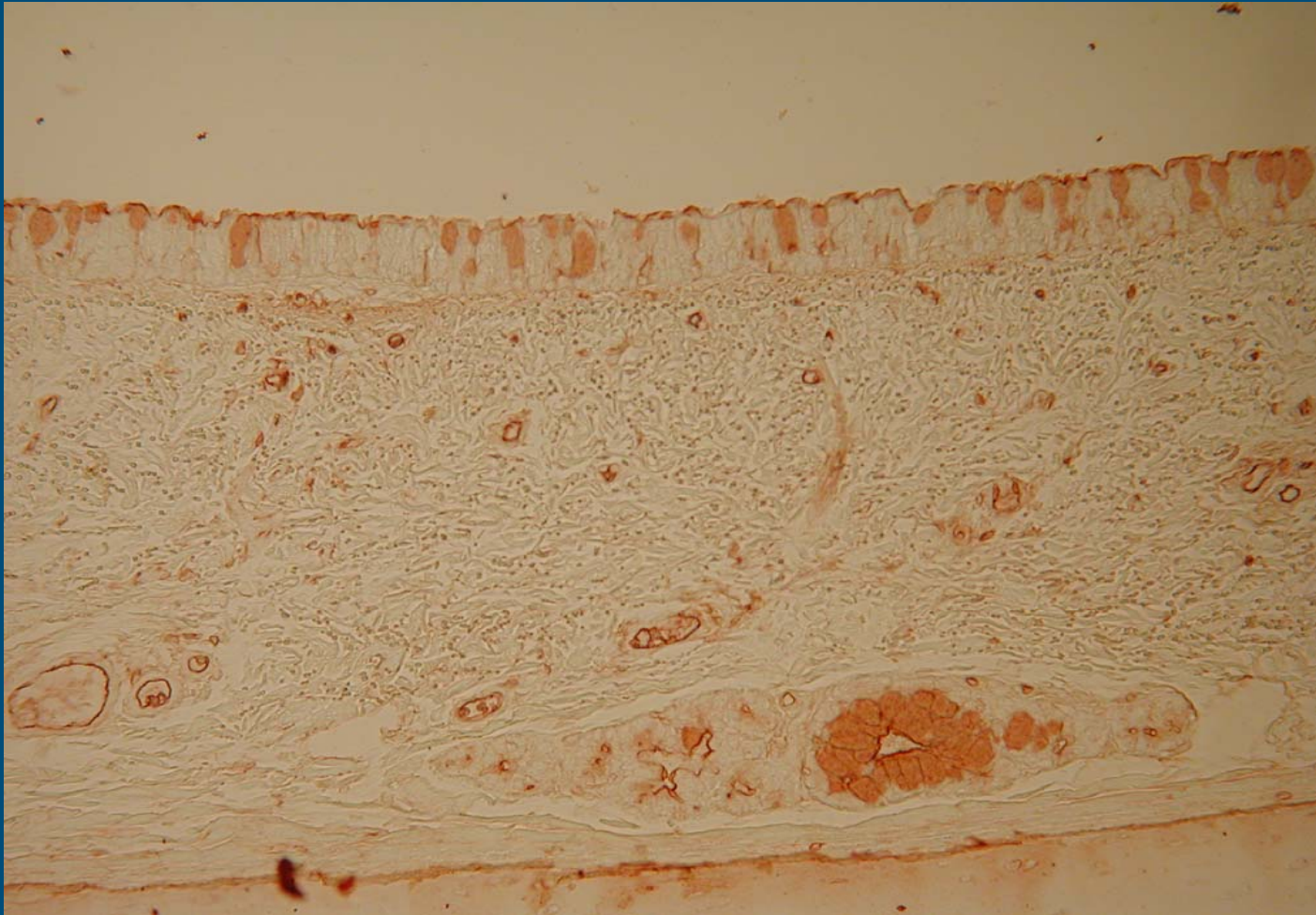
Bronchi



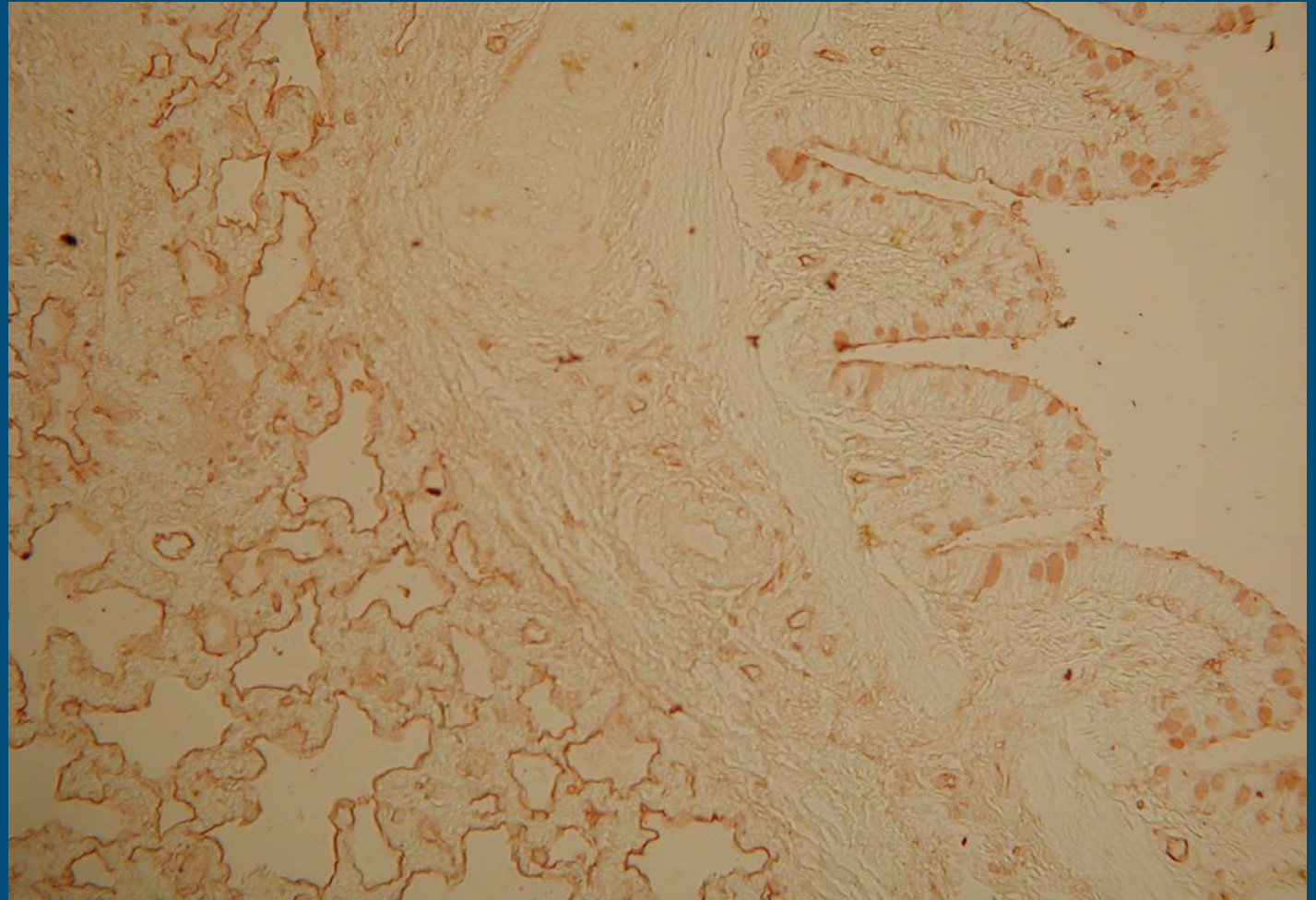
Alveoli



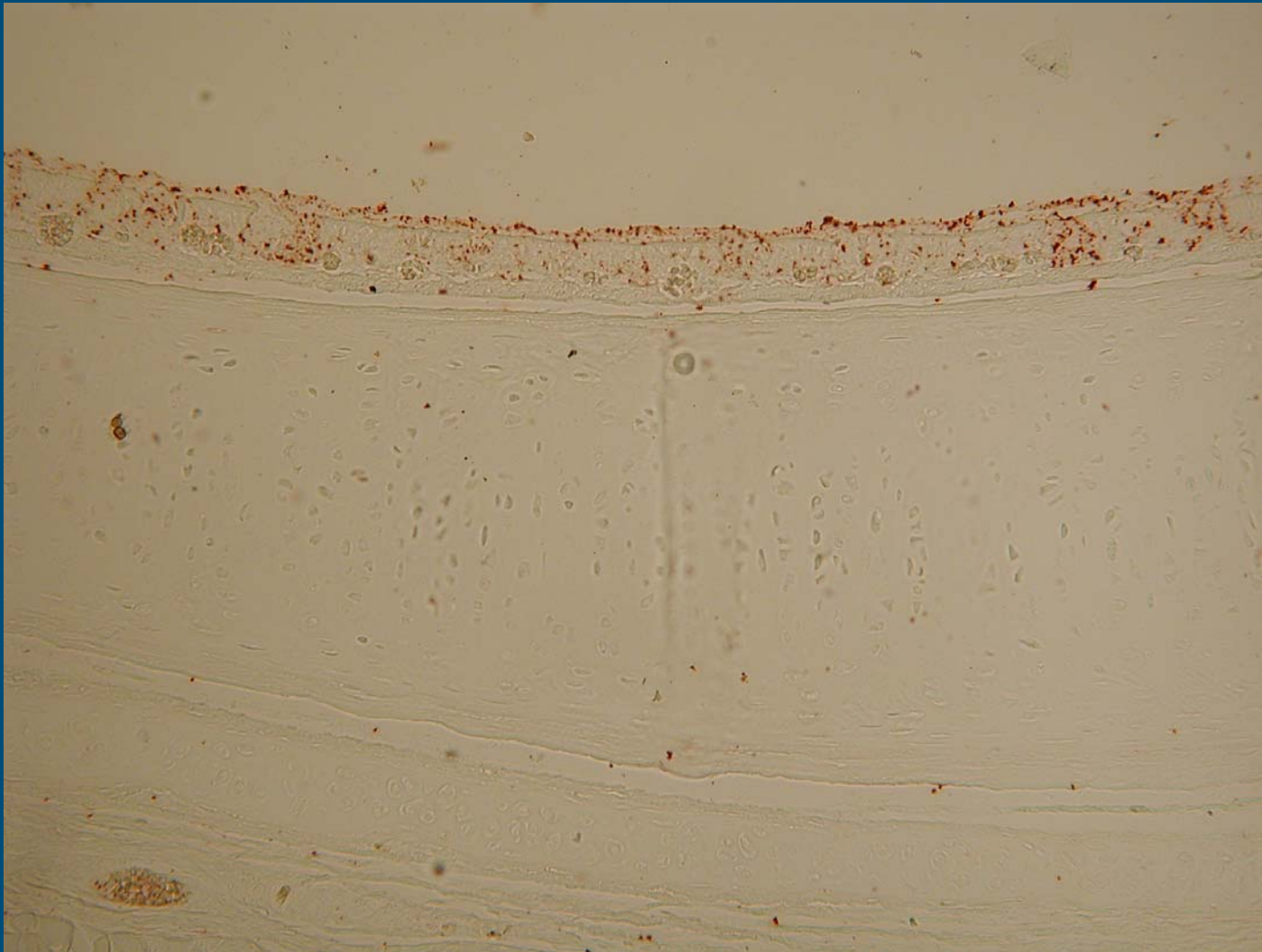
# Trachea of dog stained with $\alpha$ 2,6 sialosides specific lectin



# Bronchus and alveoli of dogs stained with $\alpha$ 2,6 sialosides specific lectin



# H5N1 binding to trachea epithelial cells of chicken



# Summary of AI receptors in humans, cats and dogs

Tissue	Presence of $\alpha$ 2,3-sialosides-linked virus receptors in			
	humans	cats	dogs	chicken
Trachea	-	-	+	++
Bronchi	-/+	-/+	++	++
Alveoli	+	+	+	++





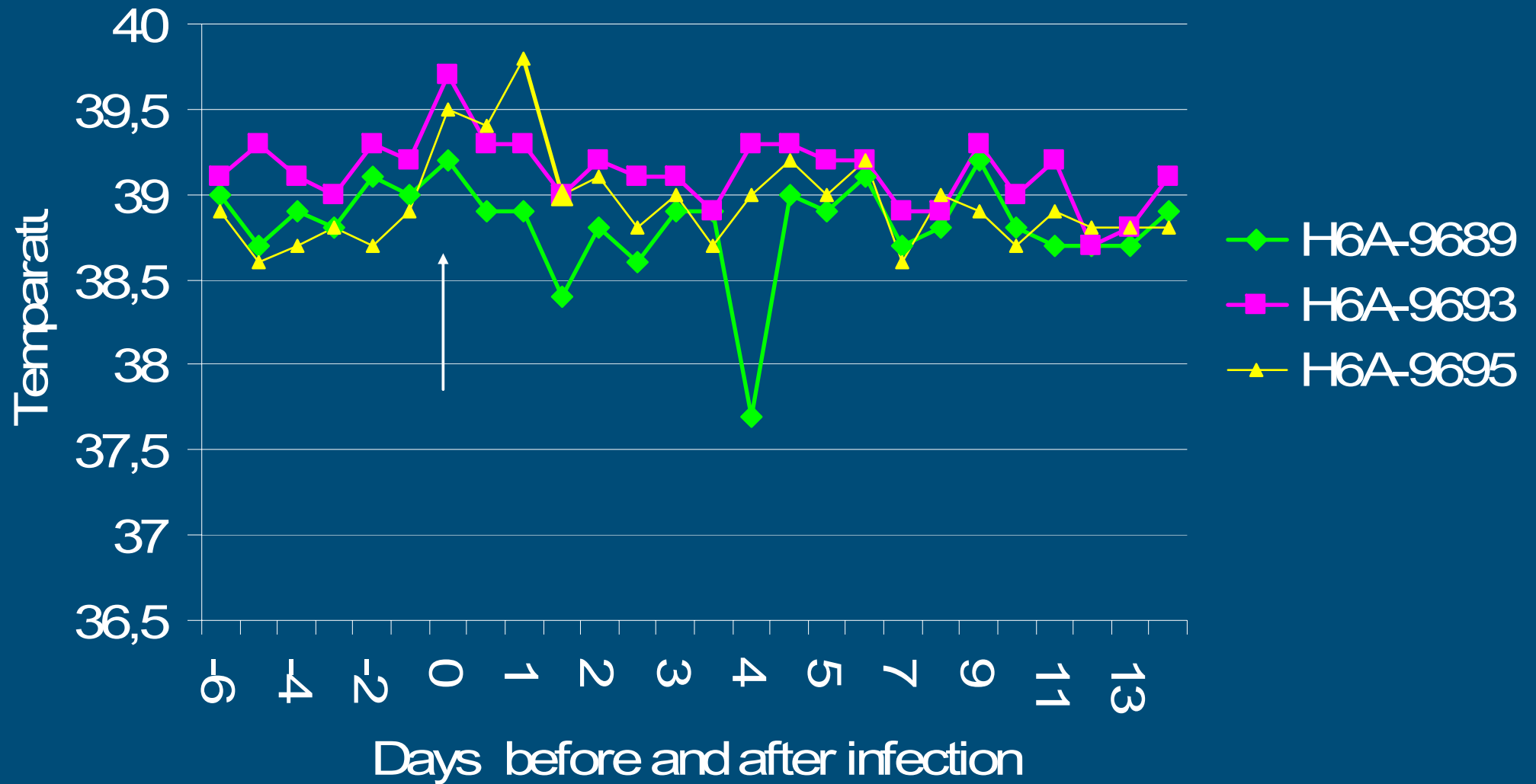
- Dogs have receptors required for infection.

# H5N1 infection of dogs

- 3 SPF-beagles (20 weeks of age)
- Each dog intratracheally and intranasally infected with  $10^6$  CCID50 A/Chicken/GxLA/1204/04
- Daily observation and recording of temperature daily once or twice a day
- Nasal, buccal and rectal swab at days 1 – 14
- Blood samples at days 0, 7, 14
- Necropsy of dead dogs and at end of experiment on day 14



# Body temperature of dogs



# Results of infection experiment in dogs (1)

- No fever
- No clinical signs
- No abnormalities observed at necropsy



# Results of infection experiment in dogs (2)

## Antibodies against influenza at

Dog	Day 0		Day 7		Day 14	
	ELISA	HI	ELISA	HI	ELISA	HI
1	-	<2	-	<2	+	16
2	-	<2	-	<2	+	16
3	-	<2	+	16	+	32



# Results of infection experiment in dogs (3)

	Virus detection at day							
	1		2		3		4	
	PCR	Egg	PCR	Egg	PCR	Egg	PCR	Egg
Dog 1*	10 <sup>3.2</sup>	-	10 <sup>2.0</sup>	+	10 <sup>2.9</sup>	+	10 <sup>2.8</sup>	-
Dog 2	-	-	-	-	-	-	-	-
Dog 3	-	-	-	-	-	-	-	-

\* Nasal swabs



# Summary

- No clinical signs in dogs
- Dogs do seroconvert
- Sub-clinical infection can result in virus shedding by SPF-beagles



## Discussion: H5N1 isolated from dog in Thailand

- H5N1 was isolated from a dog in 2004 (Songserm *et al* Emerg. Inf. Dis. 2006)
- Dog consumed dead duck in region with H5N1 cases
- After 5 days developed high fever and lethargy and died the next day
- Severe pulmonary congestion and oedema
- H5N1 detected in lung, liver, kidney and urine by inoculation of embryonated eggs and by RT-PCR.





# Influenza in dogs in USA

- January 2004: respiratory problems of 22 greyhounds on race track in Florida.
- 14/22 dogs developed fever and coughing recovered after 10-14 days
- 8/22 dogs: deadly pneumonia with haemorrhages in the respiratory tract
- CPE of MDCK cells inoculated with lung homogenate
- Antibodies detected in Influenza A NP-ELISA and in HI test using H3 virus.

# Influenza in dogs:H3N8

- Virus was sequence: A/canine/Florida/43/2004 H3N8.
- All 8 genes have >96% homology to recent H3N8 horse influenza strains.
- 5 mutations in HA gene: N54K, N83S, W222L, I328T, N483T
- In 73 dogs that were sick or had no symptoms 93% had antibodies against H3
- Till Augustus 2004 respiratory infections in greyhounds on 14 race tracks in 6 states and positive serology
- Currently 20 states have positive cases in greyhounds and other races.



# Experimental infection of dogs with H<sub>3</sub>N<sub>8</sub>

- 4 SPF-beagles (6 month old)
- Infected with  $10^{6,6}$  TCID<sub>50</sub>
- Fever of 39°C for 2 days
- No clinical signs
- Seroconversion
- 2 of 4 dogs showed virus shedding one dog on days 1-2 and other dog on days 1-4
- At post mortem studies at day 5 tracheitis and bronchitis, but no pneumonia or haemorrhages. H3 antigen was detected by immunohistochemistry



# Summary of H3N8 infection in dogs

- H<sub>3</sub>N<sub>8</sub> infection in dogs is very contagious
- Experimental H<sub>3</sub>N<sub>8</sub> infection in SPF-dogs show:
  - mild pathology
  - Virus shedding in 2 of 4 infected dogs
- Similarity of H<sub>5</sub>N<sub>1</sub> and H<sub>3</sub>N<sub>8</sub> experimental infection of SPF-dogs.



# Influenza virus receptors

- Receptors of influenza virus are sialosides oligosacharides comprising sialic acid  $\alpha$ 2,3 or  $\alpha$ 2,6 linked to galactose
- Receptor Avian influenza:  $\alpha$ 2,3 linked sialosides
- Receptor Humane influenza:  $\alpha$ 2,6 linked sialosides



# Receptor binding of H5N1-isolaten of humans

- 18 humans H5N1 isolates (Hong Kong):
  - Bind to  $\alpha 2,3$  sialosides receptors
  - Do not bind to  $\alpha 2,6$  sialosides receptors
- Thus receptor specificity is not required for H5N1-infection of humans (Matrosowich et al, J.Virol. 1999)



# Conclusions

- In dogs H5N1-receptors are present in the lower and upper respiratory tract
- Difference with situation in humans and cats
- Can explain the efficient transmission of H<sub>3</sub>N<sub>8</sub> in dogs in the USA
- Dogs can be infected sub-clinically and can shed virus
- Contact between dogs and H<sub>5</sub>N<sub>1</sub> infected poultry should be avoided
- Strain of dogs and the virus strain used may have played part in the outcome