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# HPAI H5N8 outbreak in layers in the Netherlands

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20 November 2014, Ruth Bouwstra



# Avian Influenza Surveillance in the Netherlands

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- Passive surveillance (notification of suspect situation)
  - Acute infections mainly of HPAI
  - LPAI infections that cause mild disease
  - Post-mortem examination of dead birds submitted for diagnosis to (private) laboratories
- Exclusion diagnostics: rule-out AI as possible cause
- Serologic Surveillance programme (safety net)
  - Detection of LPAI introductions that remain subclinical



# Early detection

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- Increased mortality: more than 0.5% mortality for two consecutive days in broiler or egg-producing flocks -> notification of veterinary services
- Reduction of egg production or reduction in water and/or feed intake: > 5% → consultation of practitioner
- Exclusion diagnostics: AI in differential diagnosis, but not first on list -> direct submission to CVI without notification to veterinary services



# Early detection

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- Veterinary Practices and Animal Health Service urged to submit cloacal and throat samples to Ref Lab from birds presented for post-mortem examination in specific circumstances:
  - broilers with severe respiratory problems
  - turkeys with severe respiratory problems or diarrhoea or inflamed intestines
  - layers with severe respiratory problems or mild to severe peritonitis in combination with a dip in egg production
  - all poultry types with no pathological findings during post-mortem, but an anamnesis of slightly increased mortality and/or a dip in production and/or a decrease in feed and/or water intake.



## **Notification 14 November 2014**

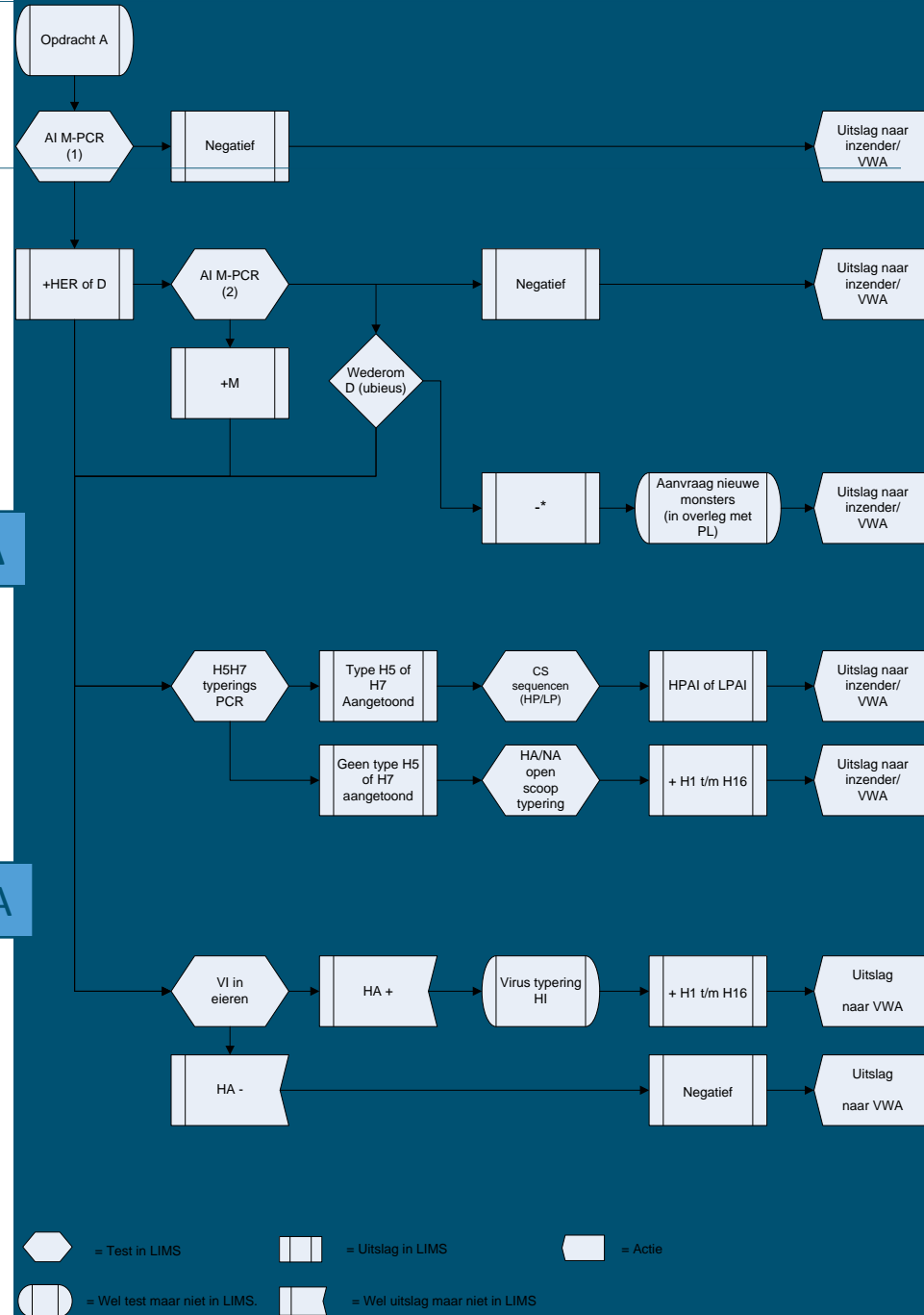
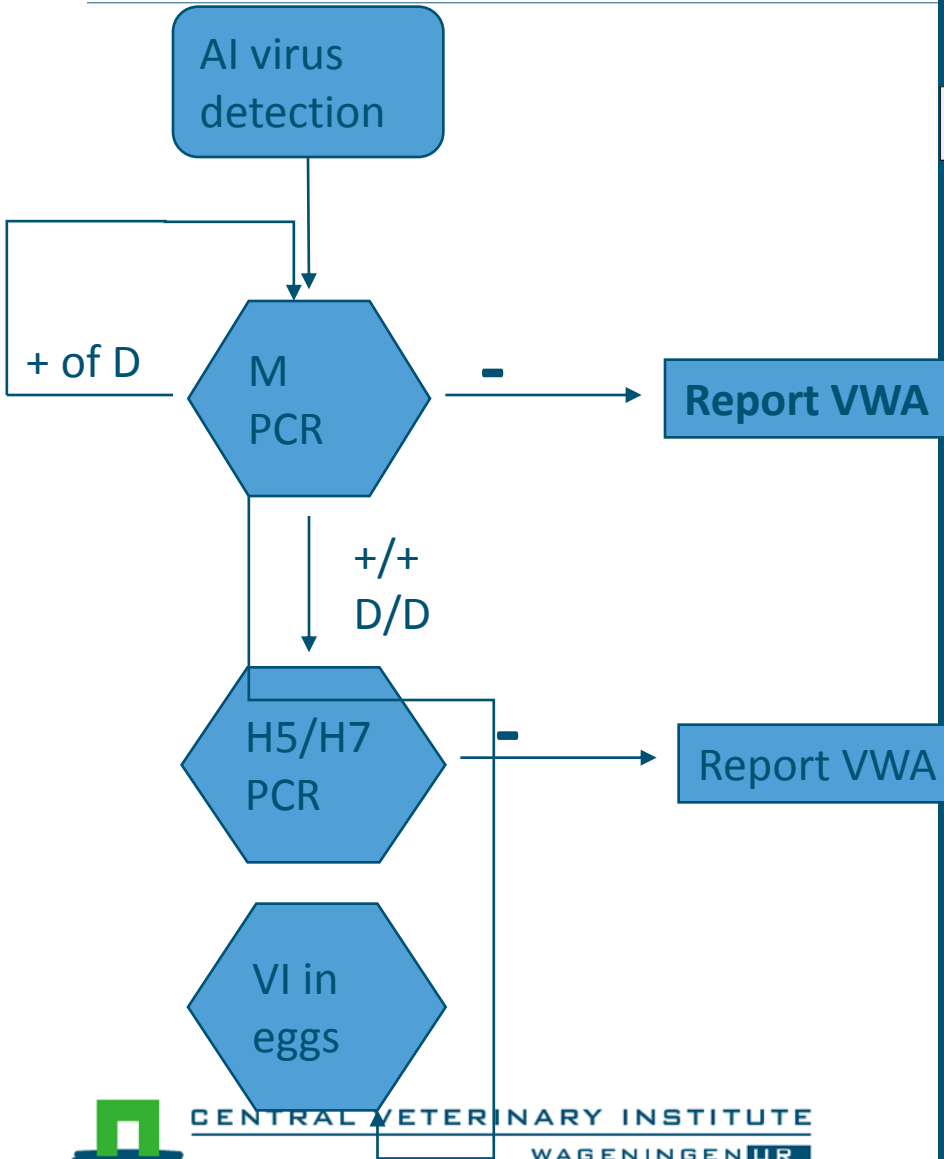
- 5 layer hens submitted to GD for pathology
- no diagnosis + increased mortality-> notification NVWA

## **Sample submission to CVI**

- 5 cloacal swabs + 5 oropharyngeal swabs
- 2 pools in M-PCR



# PCR protocol LIMS



- = Test in LIMS
- = Uitslag in LIMS
- = Wel test maar niet in LIMS.
- = Wel uitslag maar niet in LIMS
- = Actie

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# PCR results

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## **M-PCR**

(15-11-2014 23:59)

- Both pools very strong positive (ct 20)
- Reported to NVWA

## **H5 recommended PCR**

(16-11-2014 6:00)

- Both pools very strong positive (ct 20)
- Reported to NVWA



# Action

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15 November

- NVWA visits the farm for clinical investigation and sample taking (20 trachea/20 cloaca swabs/20 sera per poultry house (6))
- Samples (120 T/120 C/100 Sera) are sent to CVI

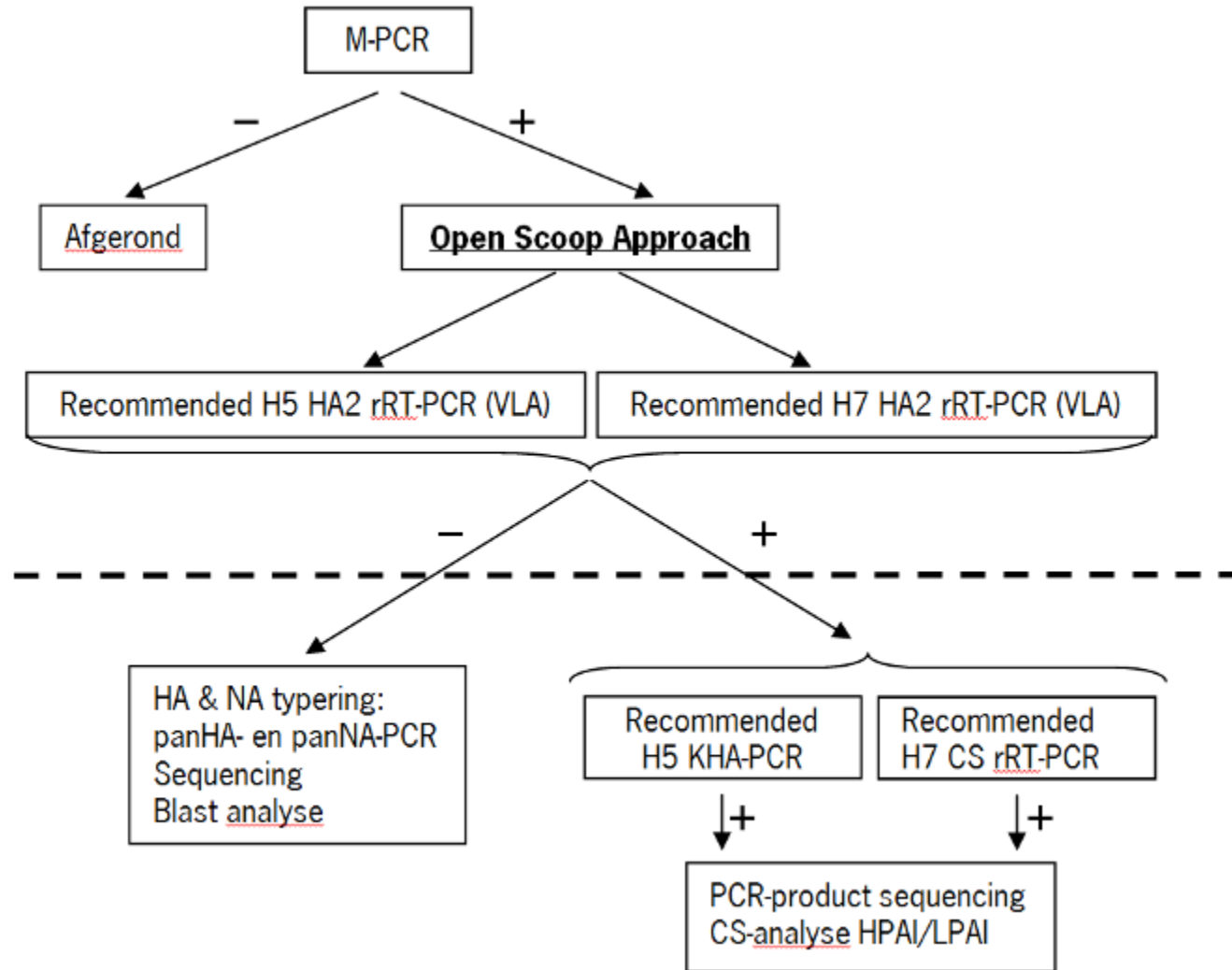
Results

- Severe clinical signs
- Mortality up to 30% in affected poultry house





# At the same time..open scoop approach



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# At the same time..

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## Sequencing

- CS for HP/LP diagnostics
- HA/NA for subtyping

02:00 H5  
confirmed in  
samples  
from nVWA

Results (Sunday 16-11 i)02:00 ii)12:00):

- RNSPLRE**RRRKR**\*GLFGAIA
- **H5N8**



# PCR Results per poultry house

The  
stable  
door

1 out of 8 pools weak positive in H5	No positive samples	No positive samples
7 out 8 pools M-PCR and H5 positive	No positive samples	No positive samples



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# Immediate ACTION

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Based on confirmed  
positive M-PCR AND  
positive H5 PCR

Start of CULLING  
on Sunday 16  
November



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# At the same time...

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Full genome sequencing

*all 8 RNA genome segments of the outbreak virus were amplified by using segment-specific primers and directly sequenced*



# Serology results

Monday 17 November 2014

- 5 out of 6 poultry houses tested (1 house with 1-day old chickens)

The  
stable  
door

All ELISA negative	All ELISA negative	All ELISA negative
5 out 20 sera ELISA very weak + Not confirmed by H5 HI	All ELISA negative	Not tested



# Virus isolation

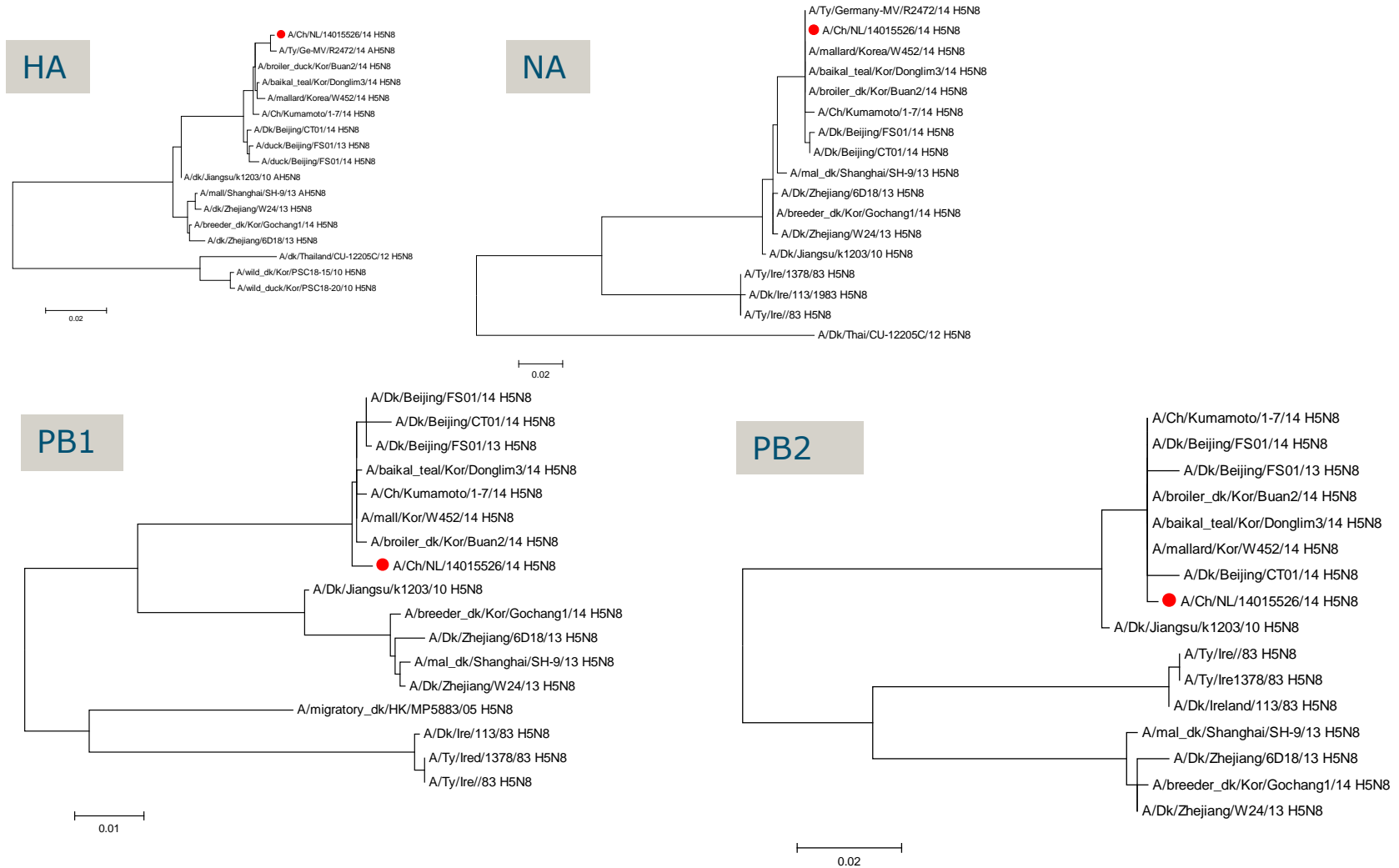


Tuesday 18-11

Positive

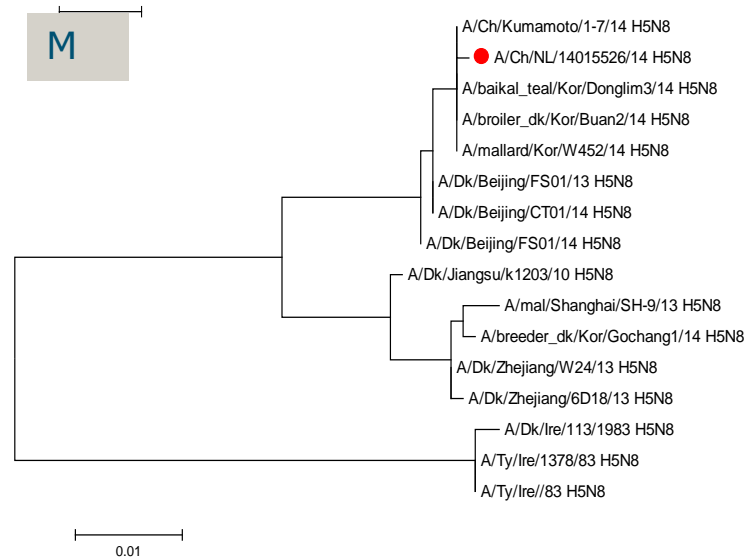
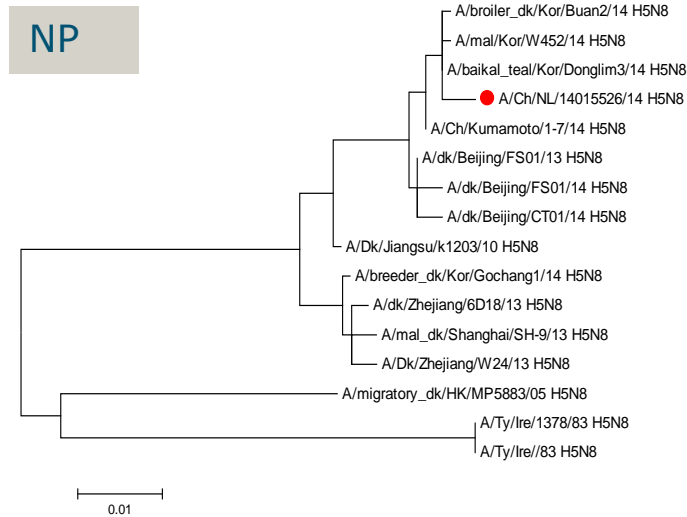
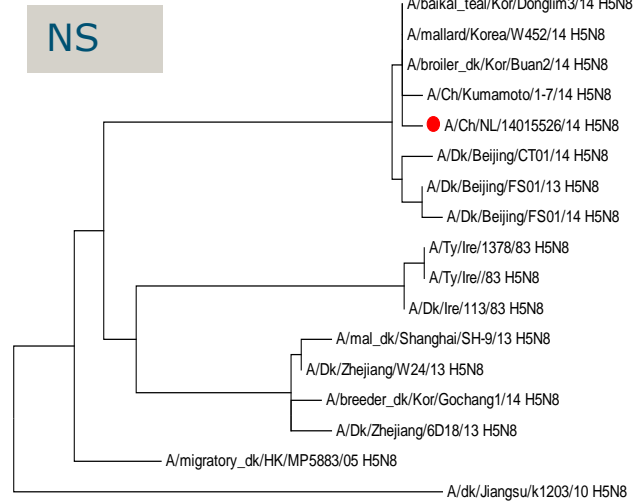
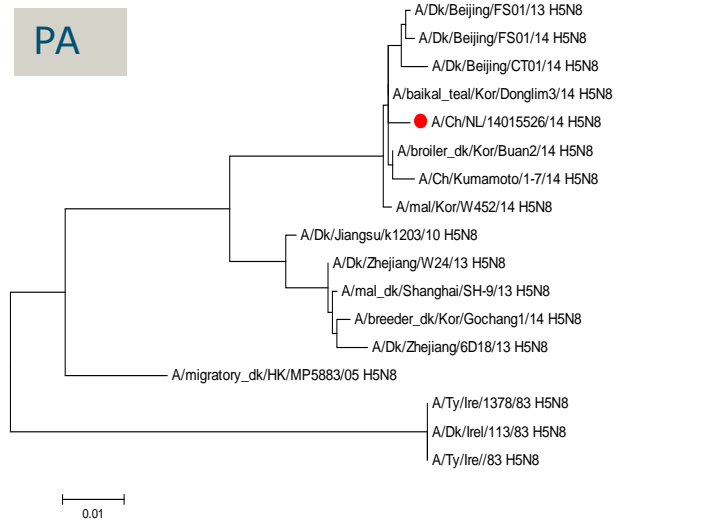


# Full genome sequence results





# Full genome sequence results



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# Conclusion

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Based on available **full genome sequence** data in NCBI Influenza Virus and Epi Flu databases **highest similarity** was found with the **Korean strain A/Baikal\_teal/Donglim3/2014.**

Strong indications that the **Dutch virus strain** has come from **Asia**, maybe with intermediate landing stations on its way to Europe.

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# Conclusion

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## **HPAI introduction?**

- No detection of AI subtype H5N8 strains in the past in the Netherlands neither in commercial poultry nor in wild birds
- No direct trade contacts in time and space with the Netherlands from Asia and Germany that may explain a route of introduction
- Apparent clinical signs ->unlikely that the virus was present in poultry in the Netherlands before the present outbreak was detected

**YES, very likely introduced as HP**



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Thanks for your  
attention!

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Questions?

