

H5 and H7 avian influenza A virus pathotyping

Joe James¹, Nicola Lewis¹, Eric Niqueux², Nancy Beerens³, Charlotte Hjulsager⁴, Mats Isaksson⁵, James Seekings¹, Marek J. Slomka¹, Ben Mollett¹, Sahar Mahmood¹, Sharon M. Brookes¹, Ian H. Brown¹

Next-Generation Sequencing for Genetic Characterisation of Swine Influenza A Viruses in Europe

Scott M. Reid¹, Gaele Simon², Willie Loeffen³, Lars Larsen⁴, Siamak Zohari⁵, Chiara Chiapponi⁶, Timm Harder⁷, Stéphane Gorin², Jesper Schak Krog⁴, Emanuela Foni⁷, Sharon M. Brookes¹, Ian H. Brown¹

¹APHA-Weybridge (UK), ²Anses, Ploufragan (France), ³Wageningen Bioveterinary Research Laboratory (WBVRI), Lelystad, The Netherlands, ⁴Technical University of Denmark, Copenhagen (Denmark), ⁵Statens veterinärmedicinska anstalt (SVA), Sweden, ⁶IZSLER, Parma (Italy), ⁷Friedrich-Loeffler-Institute (Germany)

H5 and H7 avian influenza virus A pathotyping

Aim to develop RRT-PCR pathotyping assays to differentiate subtypes H5 and H7 based on presence/absence of a multi-basic cleavage site. Two separate H5 and H7 assays were validated.

RESEARCH ARTICLE

Novel real-time PCR-based patho- and phylotyping of potentially zoonotic avian influenza A subtype H5 viruses at risk of incursion into Europe in 2017

MM Naguib ^{1,2}, A Graaf ¹, A Fortin ³, C Luttermann ⁴, U Wernery ⁵, N Amarin ⁶, HA Hussein ⁷, H Sultan ⁸, B Al Adhahd ⁹, MK Hassan ², M Beer ¹, I Monne ³, TC Harder ¹

1. Institute of Diagnostic Virology, Friedrich Loeffler Institute, Greifswald-Riems, Germany
2. National Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Giza, Egypt
3. Istituto Zooprofilattico Sperimentale delle Venezie, Padua, Italy
4. Institute of Immunology, Friedrich Loeffler Institute, Greifswald-Riems, Germany
5. Central Veterinary Research Laboratory (CVRL), Dubai, United Arab Emirates
6. Boehringer Ingelheim, Dubai, United Arab Emirates
7. Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
8. Birds and Rabbits Medicine Department, Faculty of Veterinary Medicine, Sadat City University, Egypt
9. Central Veterinary Laboratory, Ministry of Agriculture, Baghdad, Iraq

Correspondence: Timm Harder (timm.harder@fli.bund.de)

Graaf et al. *Virology Journal* (2017) 14:137
DOI 10.1186/s12985-017-0808-3

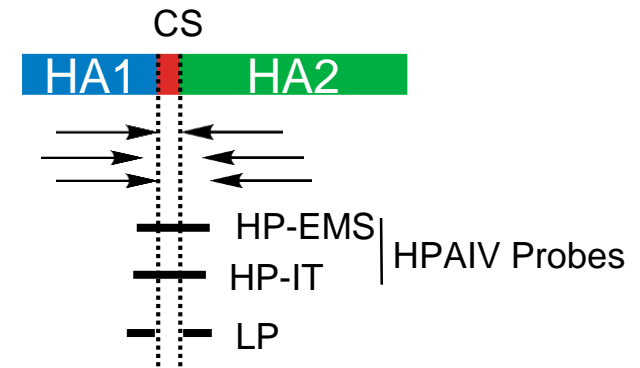
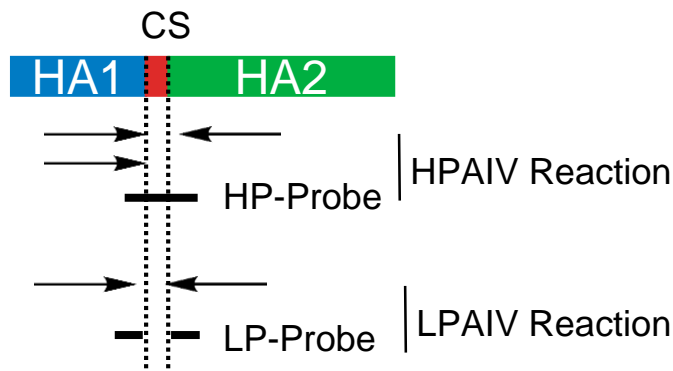
Virology Journal

SHORT REPORT

Open Access

Real-time reverse transcription PCR-based sequencing-independent pathotyping of Eurasian avian influenza A viruses of subtype H7

Annika Graaf, Martin Beer and Timm Harder*





H5 Pathotyping Assay Results

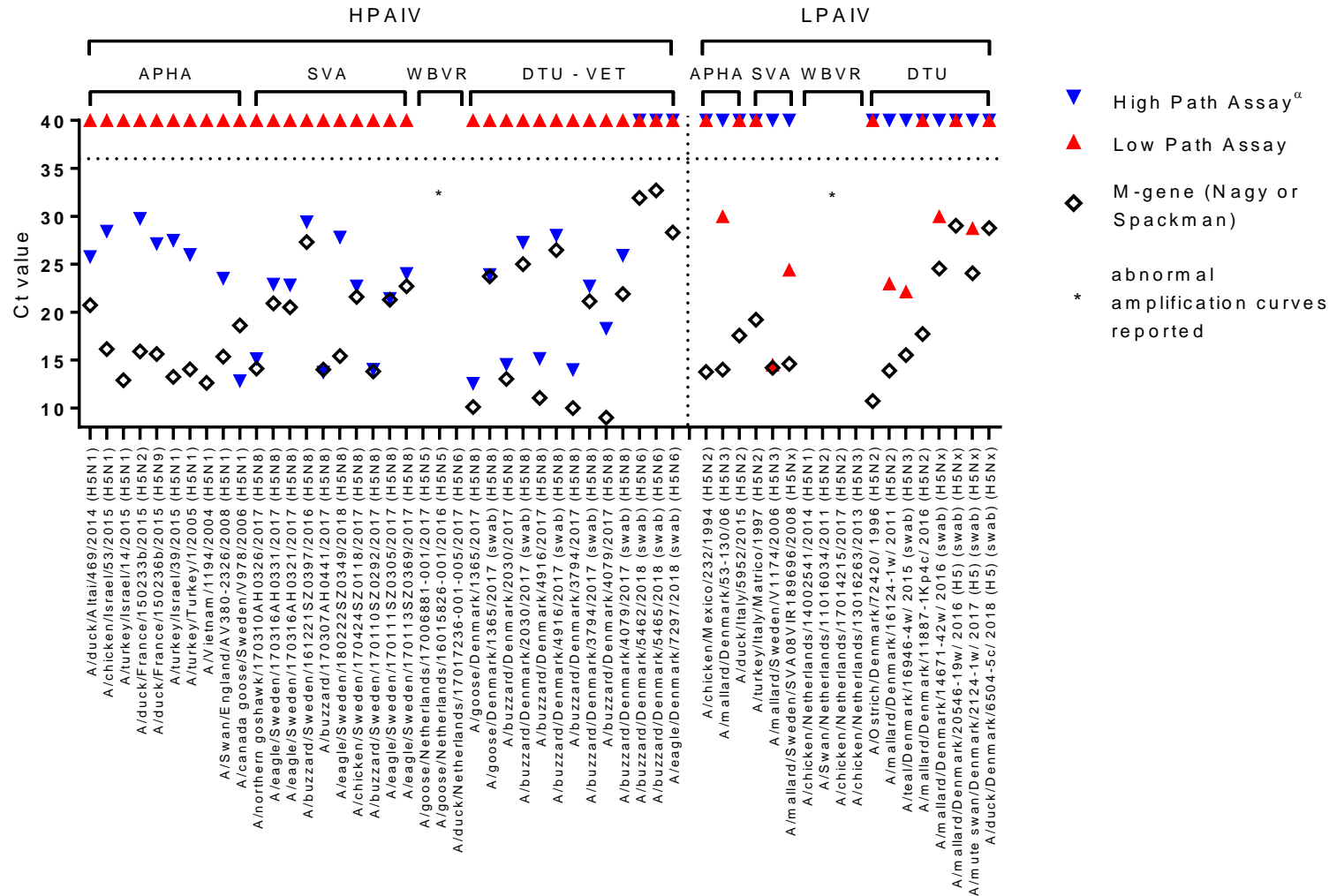
All 54 H5Nx RNA tested positive by M-gene RRT-PCR

H5 HPAIV Assay

- For the HPAIV assay 28 of the 33 HPAIV samples tested positive (23 out of 28 different H5Nx HPAIV strains)
- HP assay had 4.14 mean higher Ct values compared to M-gene

LPAIV Assay

- 8 out of 14 LPAIV RNA samples detected
- 6.07 mean higher Ct values





Animal &
Plant Health
Agency

H7 Pathotyping Assay Results

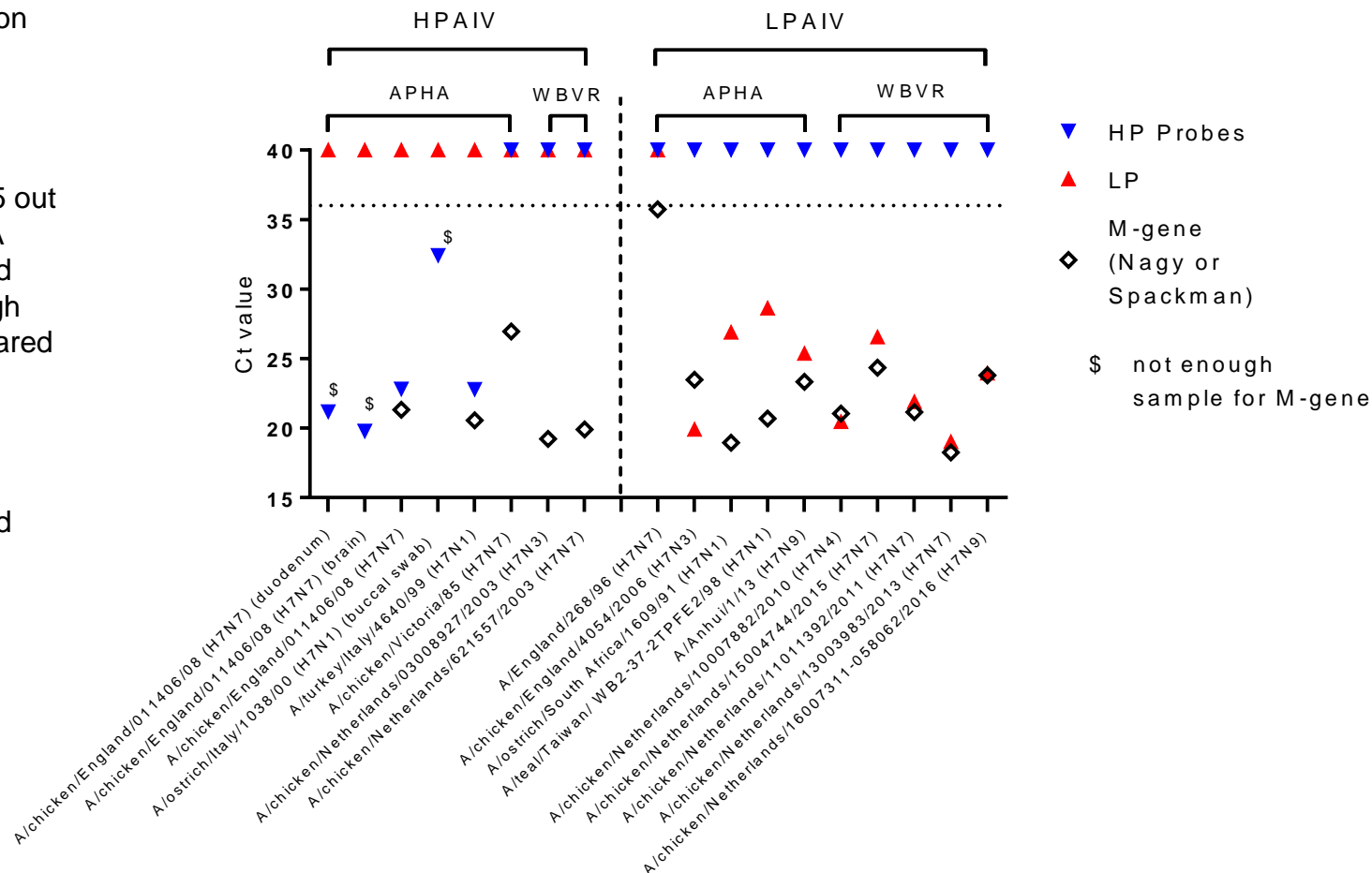
18 different RNA samples were used for the validation (consisting of 16 different H7Nx virus isolates)

H7 HPAIV Assay

- For the HPAIV assay 5 out of 8 of the HPAIV RNA samples were detected
- HP assay had 1.85 high mean Ct values compared to M-gene

LPAIV Assay

- 10 of 11 LPAIV RNA samples were detected
- 6.07 mean higher Ct values





H5 and H7 pathotyping: Summary / Conclusions

- The H5 HPAIV assay detects 82.14% of HPAIV H5Nx virus strains sampled (n=28). The H5 LPAIV assay detects 57.14% of LPAIV H5Nx virus strains sampled (n=14).
- Both H5 assays produce higher Cts, compared to M-gene (Nagy or Spackman) (4.14 and 6.07 mean higher Ct values for the HPAIV and LPAIV assay respectively).
- Performance of the H5 HPAIV pathotyping assay was similar against clinical vs egg amplified material (swab samples produced 1.26 mean higher Ct values [n=5 pairs]).
- The H7 HPAIV assay detects 50% of HPAIV H7Nx virus strains sampled (n=6). The H7 LPAIV assay detects 90% of LPAIV H7Nx virus strains tested (n=10).
- Historic H5/H7 LP and HPAIVs are more likely to be **undetected** by both pathotyping RRT-PCRs. Mismatches in primer/probe sequences are a possible reason for some viral RNA samples not being detected.
- Both H5 and H7 pathotyping RRT-PCRs may benefit from **optimisation** of the conditions to possibly improve its sensitivity.
- Routine pathotyping by these H5/H7 RRT-PCRs may be highly dependent on prior knowledge of a particular MBCS.
- **Pathotyping assays can be used as pre-screening tools for policy.**
- **Useful tools for detection of HP or LPAIVs in ongoing outbreaks.**

NGS for Genetic Characterisation of Swine Influenza Viruses in Europe: Work plan / Milestones

1. Share existing sample preparation and NGS protocols across partners. **YES**
2. Initial parallel evaluation of the existing protocols on a representative panel of swIV samples (including reassortants).
3. Optimisation of test protocol/improve test design.
4. Organise and conduct full-scale ring trial amongst partners for validation of NGS protocol for sequencing 10 isolates per partner. **YES**
5. Dissemination of the technology.
6. Contribution to validation studies and documentation for quality assurance of NGS for influenza A viruses e.g. UKAS 17025 in the UK. **YES**



NGS for Genetic Characterisation of Swine Influenza Viruses in Europe

1. Share existing sample preparation and NGS protocols across partners > QUESTIONNAIRE

Virus enrichment method used (gDNA and rRNA removal or PCR of the virus)	
RNA extraction method	
Removal of carrier RNA (yes or no)	
Method used if "yes"	
NGS Instrument/Supplier (MiSeq etc.)	
Sequencing protocol(s)	
Methods:	
1	
2	
3	
4	
Additional assay information:	
Analysis methods/software used	



NGS for Genetic Characterisation of Swine Influenza Viruses in Europe

4. Organise and conduct full-scale ring trial amongst partners for validation of NGS protocol for sequencing 10 isolates per partner (n=5, plus Italy) **MARCH 2018**

	10 egg-grown samples blind coded as below:			
Isolate	Strain	Subtype / strain	Country of origin	Blind panel code
1	A/swine/England/04202/2013	pdm09	UK	10
2	A/swine/England/024079/13	H1 _{av} N1	UK	2
3	A/swine/England/000304/2009	H1N2	UK	7
4	A/swine/Denmark/10-1310-1/2011	H1N1	Denmark	8
5	A/swine/Denmark/10345-1/2012	H1N2	Denmark	9
6	A/swine/Netherlands/Ysselsteyn-CV18864A/2012	H3N2	The Netherlands	3
7	A/swine/France/59/120031/2012	H3N2	France	5
8	A/swine/France/Cotesd'Armor-0198/2011	H1N2	France	6
9	A/swine/England/453/2006	H1N1	UK	1
10	A/swine/England/453/2006 at 1:100 dilution	H1N1	UK	4



NGS for Genetic Characterisation of Swine Influenza Viruses in Europe: Results from ring trial

- **APHA** - Good quality NGS data was obtained from 10 blind panel viral isolates that enabled the correct identification of the virus strain and subtype
 - Illumina MiSeq, Nextera XT kit
- **ANSES** – Very strong data with number of reads correctly obtained for each sample and the mean coverage provided – can circulate the report
 - Ion proton instrument, NucleoSpin RNA kit
- **THE NETHERLANDS** – Good data – 10/10 identified
 - Illumina, High Pure Viral RNA Kit (Roche)
- **SWEDEN** – Good data – 10/10 identified
 - Illumina MiSeq, Trizol and Rneasy Mini Kit
- **ITALY (associate par CoVetLab)** – Good data – 10/10 identified
 - MiSeq, One-for-one Kit (Qiagen)
- **DENMARK** – Awaiting results

5. Dissemination of the best and most effective technology across the partners

Acknowledgements

- Richard Ellis – CSU
- ESNIP 3 partners for donating panel viruses; Chris Russell, Jayne Cooper, Alex Holland (Avian Virology, APHA) for disseminating panel
- CoVetLab project – Development and validation of a rapid pathotyping assay for avian influenza viruses by real-time PCR
- CoVetLab project – NGS for genetic characterisation of swine influenza viruses in Europe
- CoVetLab project – Development and validation of molecular tools for sub-typing swine influenza viruses
- Defra project SV3041: Monitoring of influenza A viruses in the UK pig population