Animal & Plant Health Agency

DELTA-FLU

Using Sediment, Environmental and Wild Bird Samples as Tools for Avian Influenza Virus Surveillance

Alex Byrne, PhD

Avian Influenza Research Team,

Avian Virology and Mammalian Influenza Workgroup

Animal and Plant Health Agency, United Kingdom

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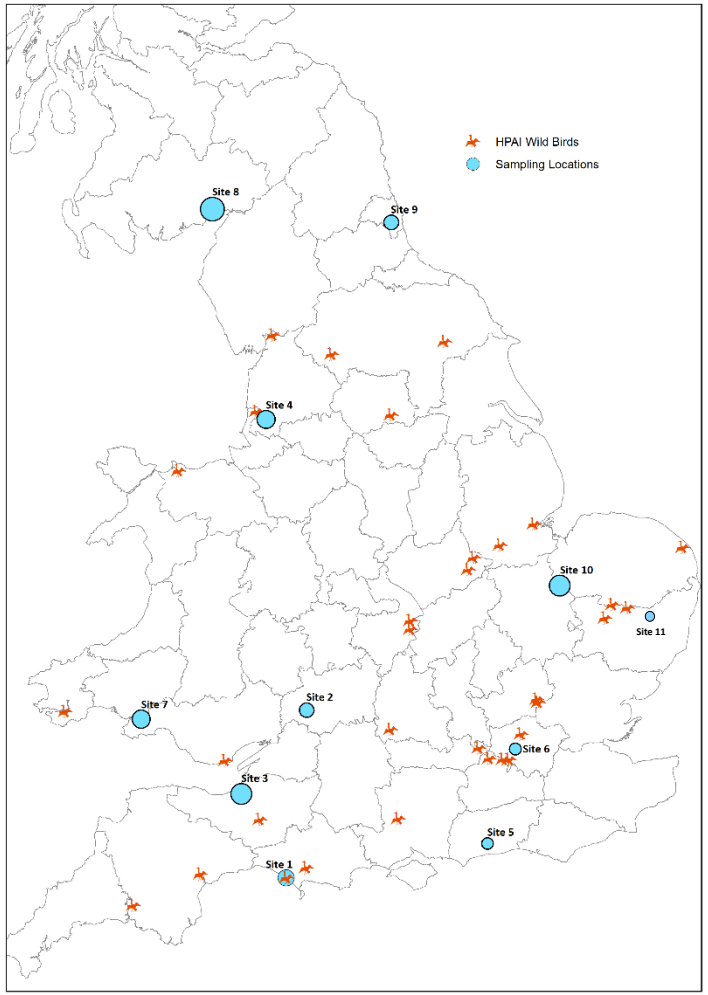
Environmental samples from UK Wetland Sites



- Four samples:
  - **Water** – from pond/lake
  - **Sediment** – taken from under the water <1 m from water's edge
  - **Mud and Faeces** – complex taken from land <1 m from water
  - **Feather** – if any, < 6 cm

Environmental samples from UK Wetland Sites

Sediment and Environmental Samples - Report, Collection and Submission Form

Contents of the Sample Collection Kit

Each kit will contain the following items:

Item	Quantity
Sediment and Environmental Samples - Report, Collection and Submission Form	1
Sediment and Environmental Samples - Report, Collection and Submission Form Completion Guidance Notes (This Document)	1
Small Bio Bottle	1
UN3373 Box For Bio Bottle	1
Large Labelled Freezer Safe Tube	3
Small Labelled Freezer Safe Tube	1
Disposable forceps	1
Disposable weighing boat	1
Grip Lock Bags – Large	1
Grip Lock Bags – Small	4
Nitrile gloves	2

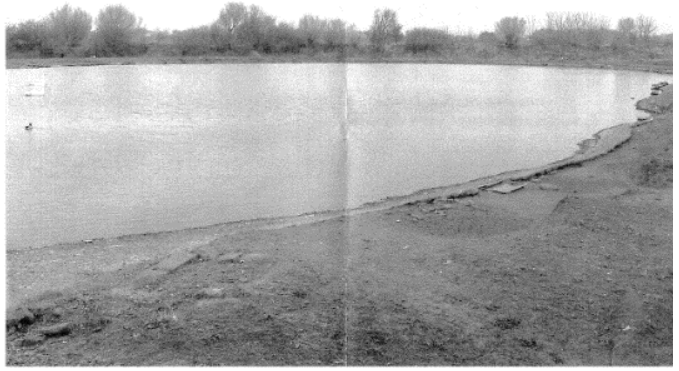
Please check the contents of the kit and if anything is missing, then please contact APHA as soon as possible.

An electronic copy of the Sediment and Environmental Samples - Report, Collection and Submission Form and Sediment and Environmental Samples - Report, Collection and Submission Form Completion Guidance Notes will be provided as well.

- Collection form and guidance:
  - Kit contents
  - Lab contact details
  - Advice on site selection
  - Location (longitude and latitude)
  - Time of collection
  - Photo of collection site
  - Signs of animal activity and species (if known)
  - Location description (river, canal, lake reservoir, coastal, urban, agricultural etc.)

Environmental samples from UK Wetland Sites

Site No.	Result	No. of avian species observed
1	Negative	7
2	Negative	3
3	Negative	1
4	Negative	*Not given*
5	*Not sampled*	*Not sampled*
6	Negative	7
7	Negative	*Not given*
8	Negative	9
9	Negative	5
10	Negative	2
11	Negative	1



Sediment Processing Protocol

Verify submission paperwork, hold samples at -80˚C, defrost overnight

Matrices

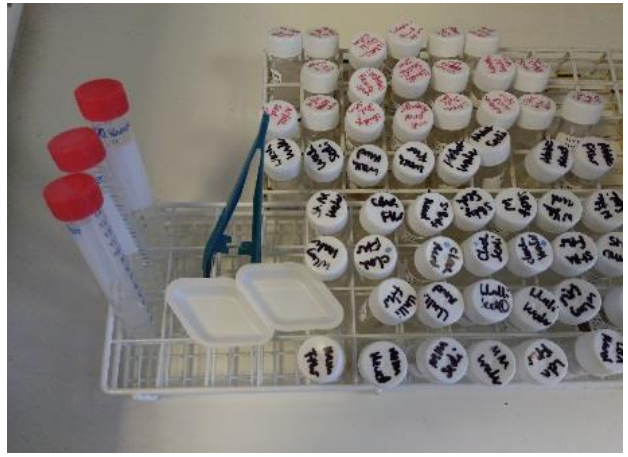
- Water, mud–faeces and sediment samples
  - Prepare a 10% suspension of each sample in 0.1 M PBS pH 7.2 with antibiotics
- Feather
  - Rough chop, add to 0.1M PBS for 10% suspension

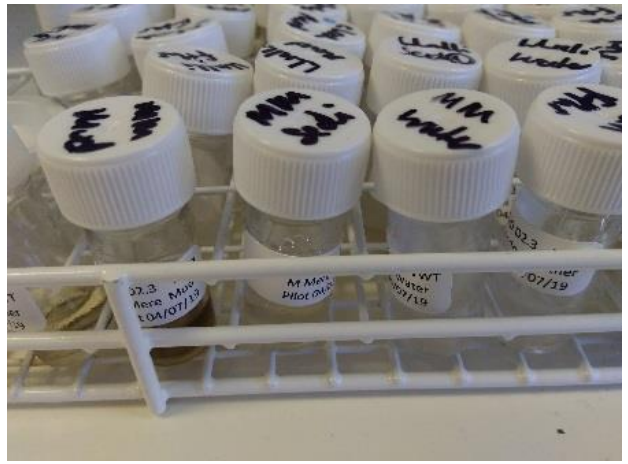
Store overnight at +4°C

Standard robotic extraction of RNA

- AVL-Ethanol protocol (Qiagen Viral RNA Extraction Kit)

RRT-PCR tested by M gene Nagy





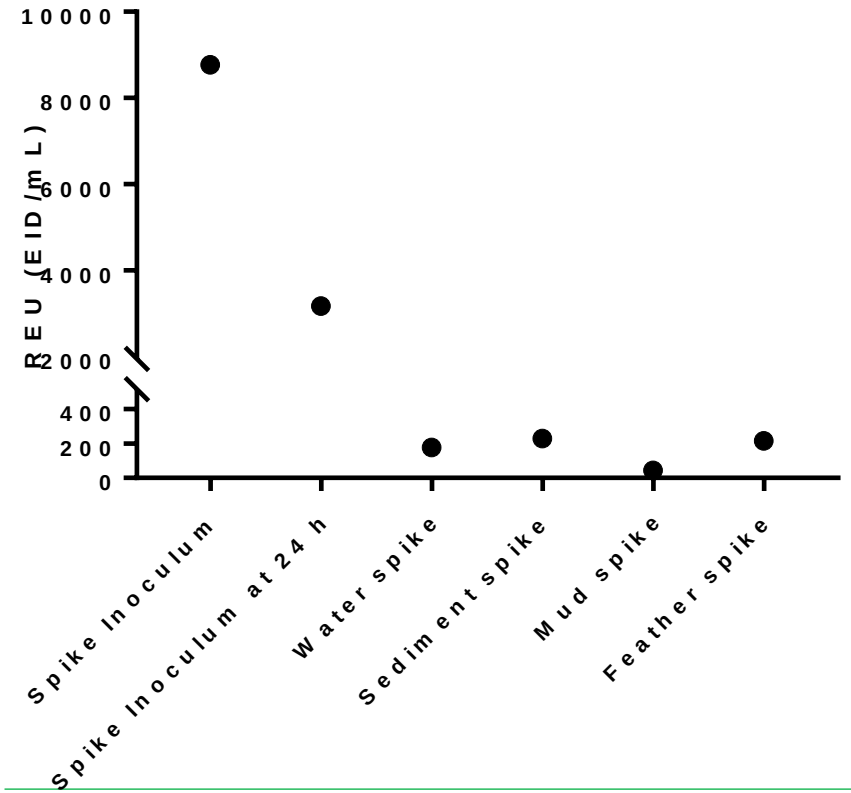
Summary

All 10 sites tested negative for AIV by RRT-PCR M gene Nagy

Testing Protocols with Spiked Matrices

Method

- Spiked negative samples collected from UK wetland with inactivated H5N6-2017 (1x 10^4 EID_{50}/ml).
- Processed samples as described.
- Assessed recovery by RRT-PCR.



Sample	Relative Concentration (EID50/mL)	Spiked Recovery (%)	Estimated Limit of Detection (EID50/mL)
Spike Inoculum	8.773E+03	100.00	
Spike Inoculum24 h	3.177E+03	36.22	
Water spike	1.765E+02	2.01	9.343E+02
Sediment spike	2.290E+02	2.61	7.201E+02
Mud spike	4.410E+01	0.50	3.739E+03
Feather spike	2.145E+02	2.45	7.686E+02

Summary

Reduced RNA recovery from mud compared to other matrices

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Conclusions

- AIV surveillance in wild birds is risk-based and involves laboratory testing of clinical samples from carcasses

- Collection of environmental samples during both outbreaks and experimental infections enables us to gain further information for disease investigations and identify potential pathways for introduction

- By actively testing environmental samples collected from wetlands outside of outbreaks may help to supplement the information gained from wild bird samples and provide insights into the AIVs that are present in the wild bird environment

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