ΔELTA-FLU

DYNAMICS OF AVIAN INFLUENZA
IN A CHANGING WORLD

Report on database of AIV contamination determinants, sediments and wild bird samples

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SUMMARY

Multiple epizootics of avian influenza virus (AIV) in recent years have resulted in AIVs circulating in wild birds, as well as causing poultry outbreaks, however the methods of surveillance of AIVs in wild birds vary between countries and institutions, making comparisons difficult. In Europe, the primary method of surveillance is sampling selected wild bird carcasses, however this is limited by difficulties in the acquisition and processing of such carcasses. Since AIVs are mainly excreted from birds in faeces, analyzing environmental samples containing avian faecal matter, such as sediment, from areas where it represents a risk to poultry and other susceptible species, could act a as an additional means to characterise the AIVs circulating in wild birds.

For WP2 Deliverable 2.3 (D2.3 Report on database of AIV contamination determinants, sediments and wild bird samples) a database has been produced for members of the DELTA-FLU consortium to record the different wild bird, sediment and environmental samples that have been collected, as well as any associated testing to allow comparisons between different methods of AIV surveillance. It is also hoped that the database will inform future studies regarding the type of environmental samples that can be taken and analysed in the future.

INTRODUCTION

DELTA-FLU WP2 Task 2.1 *"Epidemiological investigation on the environmental drivers for the incursion of AIV from wild birds to poultry"* sub-task 2.1.2 *"Genomic analysis of wetland sediments as a potential tool for AIV surveillance"* deliverable 2.3 *"D2.3 Report on database of AIV contamination determinants, sediments and wild bird samples"* features in this report.

Over recent years there have been multiple epizootics of AIV globally, resulting in AIVs circulating in wild birds, as well as causing poultry outbreaks. The methods and sample types used for surveillance of AIVs circulating in the wild bird population vary between countries and institutions making consolidation and comparison of data difficult. In the United Kingdom and Europe, clinical samples are taken from selected found dead wild bird carcasses, which are then screened for AIV by real-time PCR, followed by virus isolation and sequencing of non-negative samples. However, this approach is limited by difficulties in the acquisition and processing of such carcasses.

AIVs are mainly excreted from birds in faeces. Analysing environmental samples containing avian faecal matter, such as sediment, from areas where it represents a risk to poultry and other susceptible species, from areas where wild birds are known to congregate or surrounding poultry holdings, could potentially act as an additional means to characterise the AIVs circulating in wild bird. Such work has been performed in North America with success.

The aim of this deliverable was for a database to be produced by members of the DELTA-FLU consortium to record the different wild bird, sediment and environmental samples that have been collected, as well as details regarding the testing of such samples, and will continue to be added throughout the lifetime of the project. This will enable comparisons between wild bird and sediment samples across geographically diverse areas, as well as the testing and AIV subtypes identified, to be performed. It is also hoped that the database will stimulate ideas for future research questions that can be asked using such samples, as well as the type of samples that can be taken for future experiments during the lifetime of DELTA-FLU.

METHODS

A database was established based on archive data available at P3-DEFRA-APHA from various AIV outbreaks, as well as wild bird samples, experimental *in vivo* infection studies.

The database consists of four separate sheets:

1. **Poultry Outbreak Field Samples** – any environmental samples that were taken in relation to an outbreak or a disease investigation event.
2. **Wetland and Sediment Samples** – any environmental samples that are collected from the field, that are not related to an outbreak or disease investigation event.
3. **Wild Bird Samples** – any wild bird samples or carcasses that have been collected and tested for the presence of AIV.
4. **Laboratory Samples** – any 'environmental' samples that were collected from experimental *in vivo* AIV studies.

Within each sheet, there are then multiple fields for entering data regarding the sample. These fields can be broadly split into two groups: location data and testing data. The location data includes fields regarding the collection of the samples:

- A reference number for each set of samples and an identifier for each sample type collected from a single location/event.
- The agency who collected the samples.
- The year and date of collection.
- The location from which the samples were taken - country, Nomenclature of Territorial Units for Statistics (NUTS) code, region, latitude and longitude.
- The type of location from which they were collected (farm, backyard flock or wetlands).
- The species from which the sample was collected, or were present at the site from which the samples were collected.
- The sample matrix - faeces, feathers, litter, pond water, drinking water, sediment or mud.

The fields for location data are the same for sheets 1-3, however for sheet 4 (Laboratory Samples) (Fig. 1-4), the location data has been simplified to only include a reference, identifier, the agency, year and date of samples, the species and matrix. This was reduced as it was assumed that the majority of laboratory samples would be collected from the same site at any institute and that the location data would therefore not change.

Reference	Identifier	Agency	Year	Sampling Date	Country	NUTS Code	Region	Latitude	Longitude	Site	Species	Matrix
1	1	APHA	2007	01/11/2007	UK	UKH17	Norfolk			Farm	Turkey	Faeces
1	2	APHA	2007	01/11/2007	UK	UKH17	Norfolk			arm	Turkey	Feathers
2	1	APHA	2014	01/11/2014	UK	UKE12	East Riding of Yorkshire			arm	Duck	Feathers
3	1	APHA	2015	01/07/2017	UK	UKD45	Lancashire			arm	Chicken	Faeces
3	2	APHA	2015	01/07/2017	UK	UKD45	Lancashire			arm	Chicken	Litter
3	3	APHA	2015	01/07/2017	UK	UKD45	Lancashire			arm	Chicken	Feathers
3	4	APHA	2015	01/07/2017	UK	UKD45	Lancashire			Farm	Chicken	Feathers
4	1	FLI	2015	29/07/2015	DEU	DE949	Emsland, Lower Saxony			Farm	Wild ducks	Faeces
4	2	FLI	2015	29/07/2015	DEU	DE949	Emsland, Lower Saxony			Farm	Chicken	Pen inside swab
5	1	APHA	2016	01/01/2016	UK	UKM22	Fife			Farm	Duck	Litter
6	1	APHA	2016	01/12/2016	UK	UKE13	Lincolnshire			Farm	Turkey	Pond water
7	1	FLI	2016	11/11/2016	DEU	DEF03	Lübeck, Schleswig-Holstein			Farm	Dove	Feet swabs
7	2	FLI	2016	11/11/2016	DEU	DEF03	Lübeck, Schleswig-Holstein			Farm	Dove	Swab
7	3	FLI	2016	11/11/2016	DEU	DEF03	Lübeck, Schleswig-Holstein			Farm	Wild ducks	Feeding trough
7	4	FLI	2016	11/11/2016	DEU	DEF03	Lübeck, Schleswig-Holstein			Farm	Wild ducks	Faeces
8	1	APHA	2017	01/02/2017	UK	UKH17	Norfolk			Farm	Chicken	Feathers
9	1	FLI	2017	02/02/2017	DEU	DE80H	Vorpommern-Rügen, Mecklenburg-Westpommerania			Farm	Wild birds	Faeces
10	1	FLI	2017	09/02/2017	DEU	DEE04	Altmark, Saxony-Anhalt			Farm	Wild birds	Faeces

> **Latitude**
> The digital latitude for the location where the samples were collected.

Figure 1: Image of location data fields from sheet 1 – Poultry Outbreak Field Samples.

Reference	Identifier	Agency	Year	Sampling Date	Country	NUTS Code	Region	Latitude	Longitude	Site	Species	Matrix	
1	1	APHA	2019	26/02/2019	UK	UKH16	Norfolk			Wetland	Mallards	River water	
1	2	APHA	2019	26/02/2019	UK	UKH16	Norfolk			Wetland	Mallards	Sediment	
1	3	APHA	2019	26/02/2019	UK	UKH16	Norfolk			Wetland	Mallards	Mud	
2	1	APHA	2019	01/04/2019	UK	UKK13	Gloucestershire			Wetland	Mute swan,	Pond Water	
2	2	APHA	2019	01/04/2019	UK	UKK13	Gloucestershire			Wetland	Mute swan,	Sediment	
2	3	APHA	2019	01/04/2019	UK	UKK13	Gloucestershire			Wetland	Mute swan,	Mud/Faeces	
2	4	APHA	2019	01/04/2019	UK	UKK13	Gloucestershire			Wetland	Mute swan,	Feathers	
3	1	APHA	2019	01/04/2019	UK	UKK23	Somerset			Wetland	Waders	Water	
3	2	APHA	2019	01/04/2019	UK	UKK23	Somerset			Wetland	Waders	Sediment	
3	3	APHA	2019	01/04/2019	UK	UKK23	Somerset			Wetland	Waders	Mud/Faeces	
3	4	APHA	2019	01/04/2019	UK	UKK23	Somerset			Wetland	Waders	Feathers	
4	1	APHA	2019	01/04/2019	UK	UKD44	Lancashire			Wetland	N/A	Pond Water	
4	2	APHA	2019	01/04/2019	UK	UKD44	Lancashire			Wetland	N/A	Sediment	
4	3	APHA	2019	01/04/2019	UK	UKD44	Lancashire			Wetland	N/A	Mud/Faeces	
4	4	APHA	2019	01/04/2019	UK	UKD44	Lancashire			Wetland	N/A	Feathers	
5	1	APHA	2019	10/04/2019	UK	UKI75	London			Wetland	Coot, Moorh	Lake Water	
5	2	APHA	2019	10/04/2019	UK	UKI75	London			Wetland	Coot, Moorh	Sediment	
5	3	APHA	2019	10/04/2019	UK	UKI75	London			Wetland	Coot, Moorh	Mud	

Figure 2: Image of location data fields from sheet 2 – Wetland and Sediment Samples.

Reference	Identifier	Agency	Year	Sampling Date	Country	NUTS Code	Region	Latitude	Longitude	Site	Species	Matrix
1	1	APHA	2016	01/01/2016	UK	UKL14	Carmarthenshire			Wild bird	Wigeon	Carcass
2	1	FLI	2016	29/04/2015	DEU	DE80H	Vorpommern-Rügen, Mecklenburg-Westpommerania			Wild bird	Mallard	Swab
3	1	FLI	2016	12/04/2016	DEU	DE80H	Vorpommern-Rügen, Mecklenburg-Westpommerania			Wild bird	Mallard	Swab
3	2	FLI	2016	01/09/2016	DEU	DE80H	Vorpommern-Rügen, Mecklenburg-Westpommerania			Wild bird	Mallard	Swab
4	1	APHA	2017	01/12/2017	UK	UKK22	Dorset			Wetlands	Mute Swan	
5	1	FLI	2017	23/08/2017	DEU	DE80H	Vorpommern-Rügen, Mecklenburg-Westpommerania			Wild bird	Mallard	Swab
5	2	FLI	2017	23/10/2017	DEU	DE80H	Vorpommern-Rügen, Mecklenburg-Westpommerania			Wild bird	Mallard	Swab
5	3	FLI	2017	22/11/2017	DEU	DE80H	Vorpommern-Rügen, Mecklenburg-Westpommerania			Wild bird	Mallard	Swab
6	1	FLI	2017		DEU					Wild bird	Mallard	Swab
7	1	FLI	2017		DEU					Wild bird	Mallard	Swab
8	1	FLI	2017		DEU					Wild bird	Mallard	Swab
9	1	FLI	2017		DEU					Wild bird	Mallard	Swab
10	1	FLI	2017		DEU					Wild bird	Mallard	Swab
10	2	FLI	2017		DEU					Wild bird	Mallard	Swab
10	3	FLI	2017		DEU					Wild bird	Mallard	Swab
10	4	FLI	2017		DEU					Wild bird	Mallard	Swab
10	5	FLI	2017		DEU					Wild bird	Mallard	Swab
10	6	FLI	2017		DEU					Wild bird	Mallard	Swab

Figure 3: Image of location data fields from sheet 3 – Wild Bird Samples.

Reference	Identifier	Agency	Year	Sampling Date	Species	Matrix
1	1	APHA	2000	01/01/2000	Chicken	Drinking water (spiked)
1	2	APHA	2000	01/01/2000	Chicken	Faeces (spiked)
1	3	APHA	2000	01/01/2000	Chicken	Litter (spiked)
1	4	APHA	2000	01/01/2000	Chicken	Feathers
1	5	APHA	2000	01/01/2000	Turkey	Drinking water (spiked)
1	6	APHA	2000	01/01/2000	Turkey	Faeces (spiked)
1	7	APHA	2000	01/01/2000	Turkey	Litter (spiked)
1	8	APHA	2000	01/01/2000	Turkey	Feathers
2	1	APHA	2005	01/01/2005	Chicken	Drinking water (spiked)
2	2	APHA	2005	01/01/2005	Chicken	Faeces (spiked)
2	3	APHA	2005	01/01/2005	Chicken	Litter (spiked)
2	4	APHA	2005	01/01/2005	Chicken	Feathers
2	5	APHA	2005	01/01/2005	Turkey	Drinking water (spiked)
2	6	APHA	2005	01/01/2005	Turkey	Faeces (spiked)
2	7	APHA	2005	01/01/2005	Turkey	Litter (spiked)
2	8	APHA	2005	01/01/2005	Turkey	Feathers
3	1	APHA	2013	01/01/2013	Turkey	Feathers
4	1	APHA	2014	01/01/2014	Duck	Pond water

Figure 4: Image of location data fields from sheet 4 – Laboratory Samples.

The testing fields were then developed to capture the maximum amount of information regarding what tests have been performed on the samples and whether the result indicated the samples were positive or negative for AIV. It was decided that the exact values that were determined from each test were not required as the information regarding whether the samples were positive or negative for AIV was sufficient for the purposes of this database. The testing fields include:

- M-gene tested and result.
- H5/H7 tested and result.
- N-type tested and result.

- Whether the samples were sequenced and if so, whether the whole genome or only a partial genome was obtained.
- If virus isolation was attempted from the sample and the result.
- If serology was performed and the result.
- If the H-type, N-type, subtype and pathotype were determined and the virus name if known.

The testing fields are the same for sheets 1-4 (Fig. 5).

M-gene Tested?	M-gene Result	H5 Tested?	H5 Result	H7 Tested?	H7 Result	N-type Tested?	N-type Result	Sample Sequenced?	Whole Genome Sequenced
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
1	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
1	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0

Partial Genome Sequenced	VI Tested?	VI Result	Serology Tested?	Serology Result	H-Type	N-Type	Virus Subtype	Pathotype	Virus Name
0	1	1	1	1	Not Determined	Not Determined	H7N1	HP	A/Ostrich/Italy/984/2000
0	1	1	1	1	Not Determined	Not Determined	H7N1	HP	A/Ostrich/Italy/984/2000
0	1	1	1	1	Not Determined	Not Determined	H7N1	HP	A/Ostrich/Italy/984/2000
0	0	0	1	1	Not Determined	Not Determined	H7N1	HP	A/Ostrich/Italy/984/2000
0	1	1	1	1	Not Determined	Not Determined	H7N1	HP	A/Ostrich/Italy/984/2000
0	1	1	1	1	Not Determined	Not Determined	H7N1	HP	A/Ostrich/Italy/984/2000
0	1	1	1	1	Not Determined	Not Determined	H7N1	HP	A/Ostrich/Italy/984/2000
0	0	0	1	1	Not Determined	Not Determined	H7N1	HP	A/Ostrich/Italy/984/2000
0	1	1	1	1	Not Determined	Not Determined	H5N1	HP	A/turkey/Turkey/1/2005

Figure 5: Image of testing fields from sheet 4 – Laboratory Samples as an example, but the fields are the same for sheets 1-4.

The database has been created in such a way that the data within can be sorted, searched and analysed enabling the data to be used to generate outputs, such as maps, with minimal editing.

KEY FINDINGS

The database provides a centralised location for partners of the DELTA-FLU consortium to input data regarding their environmental and wild bird sampling and any associated testing. The database allows for information regarding the location, species, premises type, sample type and testing results to be collated from different institutes. This will allow further determination of any factors which may contribute to the identification of AIV in environmental and wild bird samples. It is hoped that this database will also encourage further scientific questions to be asked of the data that could be addressed in further studies exploring the wild bird/poultry interface by the DELTA-FLU consortium.

The structure of the database was discussed at the WP2 Virtual Meeting in March 2019, after which the database was circulated to all WP2 partners to input their first tranche of data. The current database will be presented at the DELTA-FLU Annual Meeting in September 2019, after which the database will be updated twice a year for review at further annual meetings.

LOCATION OF PRIMARY DATA

It is proposed that the database will be made available via the DELTA-FLU website (https://delta-flu.fli.de/de/login/) but kept private with access only to consortium partners before public release, at a date agreed by the consortium members. This will be relating to a publication output that is to be planned.

DELIVERABLE 2.3 DATABASE

Whilst the creation of the database fulfils the deliverable (D2.3 - Report on database of AIV contamination determinants, sediments and wild bird samples), it is an active deliverable – data generated from the collection of environmental and wild bird samples throughout the lifetime of the DELTA-FLU project by all consortium members can be added to the database as an ongoing process.

OTHER CONTRIBUTIONS TOWARDS DELIVERABLE 2.3

As well as contributions directly towards the database, the consortium partners involved in WP2 have also performed other work that contributes to this deliverable.

Partner 2 – EMC:

In November and December of 2014, the HPAI H5N8 GsGd virus (clade 2.3.4.4, group A, Buanlike) was detected in wild birds and poultry in various countries of Asia, Europe, and—for the first time—North America. The global spread of HPAI H5N8 virus in 2014/2015 raised the question whether this H5 virus was better adapted to wild birds. To answer this question, experimental infections were performed by P2-EMC, on four key waterbird species: Eurasian wigeon (*Anas penelope*), common teal (*Anas crecca*), mallard (*Anas platyrhynchos*), and common pochard (*Aythya ferina*). For each waterbird species, eight birds were inoculated with 1×10^4 TCID^{50} virus, 1.5 ml intratracheally and 1.5 ml intraesophageally. The results showed that subclinical infection occurred in all four waterbird species, and that excretion was highest in Eurasian wigeons. In conclusion, the evidence was strongest for Eurasian wigeons as long-distance vectors for HPAI H5N8 virus from 2014. The implications are that, while any of the tested wild bird species may potentially be subclinically infected and contamination of the environment around poultry farms (water/food/litter/turf/fomites) with this HPAI H5N8 virus, Eurasian wigeons may pose an increased risk. The results of this study were published recently in *Emerging Microbes & Infections* (*van den Brand et al., 2018),* and is available as an open access article, so that methodological data are freely available to other researchers.

Partner 3 – DEFRA-APHA:

Using collaborations with other UK organisations, including the Wildfowl and Wetland Trust (WWT), and Abbotsbury Swannery, P3-DEFRA-APHA is conducting a pilot study to collect and test sediment and environmental samples for the presence of AIVs.

For this pilot study, 10 locations have been selected based on the location of high pathogenicity AIV-positive wild bird samples, as shown in Fig. 6. These locations are well distributed throughout the country to ensure adequate sampling.
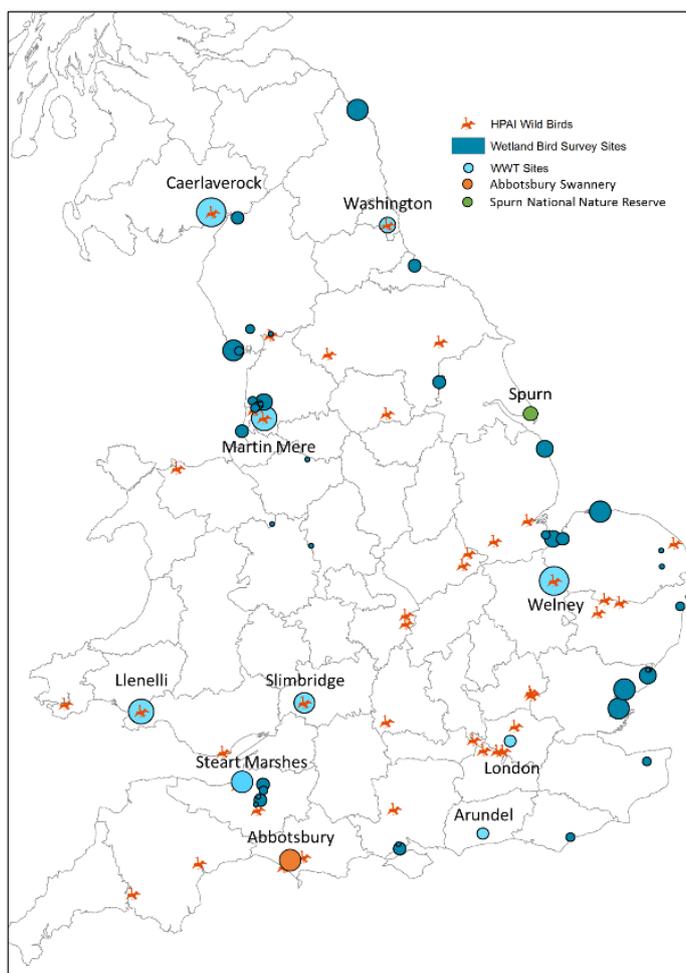
Figure 6: Map of Great Britain depicting the occurence of high pathogenicity AIV wild bird samples since 2014, and the location of available wetland sites. Selected sampling sites are labelled.

From these locations, the following samples will be collected from areas containing a water source, where wild birds are known to congregate:

- Water – from ponds, lakes, rivers etc.
- Sediment – taken up to 1 metre into the water from the water's edge.
- Mud/Faeces – taken from within 1 metre of the water's edge.
- Feathers – if present at the site.

To enable the collection of these samples a collection kit and associated guidance documents have been developed by P3-DEFRA-APHA to ensure that the samples can be returned to P3-DEFRA-APHA for testing. The collection kit includes a form to collect appropriate information that will be used to assist in later analysis of the samples.

Samples have been returned from seven of the sites and are awaiting processing. The details regarding the location have been entered into the database. The results of this pilot study will be presented at the Annual DELTA-FLU Meeting in September 2019, as well as part of a joint submission, between P3-DEFRA-APHA and the University of British Colombia to the EPIZONE Annual Meeting in August 2019. This study is being used as a logistical pilot to assess the feasibility of distributing sampling kits for third party volunteers to collect and return samples for testing. The study is planned to be expanded to more sites at a latter point in 2019 when AIVs are likely to be more prevalent in the wild bird population.

Partner 10 – CFIA:

P10-CFIA is engaged in an environmental collaboration with the British Columbia Centre for Disease Control (BC-CDC). The role of P10-CFIA has been to conduct isolation and sequencing of AI viruses from swabs collected from ducks around the ponds/lakes from where sediments were collected. P10-CFIA finished this part of the study and forwarded the results to BC-CDC who is the lead on the project. A detailed report is anticipated at some time during 2019, and P10-CFIA have indicated a willingness to share the key outcomes with the DELTA-FLU consortium at the Annual DELTA-FLU Meeting in September 2019. It is hoped that some of this data will be included in the D2.3 database, resources permitting.