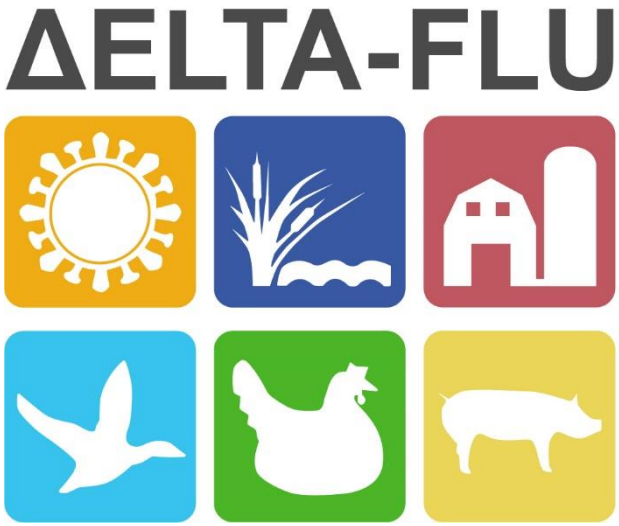

DYNAMICS OF AVIAN INFLUENZA IN A CHANGING WORLD

Report on standardization of experimental infection protocols

Version 1, 2018-08-01

Deliverable:	D1.1
Due date:	M12
Submission Date:	2018-08-07
Delivered by:	ERASMUS

Dissemination level

☒ Public

☐ Confidential, only for members of the consortium (including the Commission Services)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 727922.

TABLE OF CONTENTS

Table of Contents **1**

Background **3**

Goal **3**

Comparison of past protocols **3**

Standardization of methods 5

References **5**

BACKGROUND

The research on AIV in wild birds within Delta-Flu has two components. One (Task 1.1) is field research on the level of connectivity between Asia and Europe via migratory waterfowl, for example through their breeding grounds in the tundra and taiga of Russia. The other research component on AIV in wild birds (Task 1.2) is laboratory research on the dynamics of HPAIV infection in wild waterfowl compared to poultry. To understand the dynamics of HPAIV H5N8 and other AIV in wild waterfowl, key wild waterfowl species from Europe and North America (e.g., common teal, Eurasian wigeon, mallard, American wigeon, northern pintail, Canada goose) will be inoculated experimentally with different wild-type and genetically engineered HPAIV (e.g., H5N1, H5N2, H5N8, H7N7) in high containment laboratories of different consortium partners (EMC, FLI, DEFRA-APHA, SEPRL, CFIA) using standardized methods. Comparative experiments will be performed in relevant poultry species (chicken, turkey, domestic duck), and with typical wild-bird LPAIV and early H5N1 strains. Swabs and sera collected during the course of infection, and autopsy specimens collected after euthanasia, will be analyzed by virological, immunological, and pathological methods. In addition, NGS will be included to analyze whole genomes, virus populations, and virus quasispecies. Through these studies, it will be possible to determine whether an HPAIV could be maintained in wild waterfowl populations independent of its circulation in poultry.

GOAL

Part of this task, and the subject of this deliverable, is to standardize methods of virus inoculation (inoculum volume, virus dose, route of inoculation) and experimental design (group categories, group size) to mirror natural AIV infections and to allow measurement of the key determinants for endemic infection. Not only will this standardization of procedures allow comparison of results of experiments between consortium partners and generate directly comparable data sets for robust statistical analysis and transmission modelling, but this will also form a blueprint for a more general standardization of such experimental infections in the AIV research community.

COMPARISON OF PAST PROTOCOLS

Selected recent publications on experimental HPAIV infection in wild and domestic birds by Delta-Flu partners and others were reviewed (Table) for key methodological parameters of virus inoculation and experimental design.

Reference	Route of inoculation	Virus dose	Inoculum volume (ml)	Group categories	Group size
(van den Brand *et al.*, 2018)	trachea and oesophagus	1 x 10^4 $TCID_{50}$	3	Pathogenesis, excretion, negative control	4
(Ducatez *et al.*, 2017)	nose via nares, eye, and mouth	1 x 10^4 EID_{50}	1	Donor, direct contact, airborne contact	4
(Spackman *et al.*, 2017)	nose via choanae	1 x 10^2 to 1 x 10^6 EID_{50}	0.1	Pathogenesis	4 or 5
(Pantin-Jackwood *et al.*, 2017)	nose via choanae	1 x 10^2 to 1 x 10^6 EID_{50}	0.1	Donor, direct contact	5 or 8 (donor), 3 (direct contact)
(Berhane *et al.*, 2016)	nose via nares, mouth, and cloaca	1 x 10^4 PFU	n.r.	Donor, direct contact	15 (donor), 5 (direct contact)
(Nemeth *et al.*, 2013)	nose via choanae	1 x 10^4 EID_{50}	0.1	Pathogenesis	3 or 4

TCID50, median tissue culture infectious dose.
EID50, median egg infectious dose.
PFU, plaque-forming units.
n.r., not reported.

Birds may become infected with AIV by different routes. To emulate the natural route of infection, birds have been inoculated with AIV via different routes: eye, nasal cavity (either via nares or choanae), oral cavity, crop, oesophagus, stomach, trachea, and cloaca, via ingestion of infected meat or contaminated feathers, or a combination of above routes (reviewed in (Pantin-Jackwood et al., 2013). In some cases, the route of inoculation can affect excretion dynamics or virus-associated lesions. For example, inoculation of LPAIV via the cloaca can result in higher replication from the cloaca that inoculation via other routes (Franca *et al.*, 2012); and inoculation of LPAIV in the trachea can lead to pneumonia, while inoculation in the oesophagus does not (Kuiken, 2013)

Virus dose typically ranges between 1 x 10^2 to 1 x 10^6 EID_{50} or comparable unit of measure (Table). At the lower end of this range, some inoculated birds may not develop a productive infection. At the higher end of this range, a productive infection of more than 50% of the inoculated birds is highly likely, and any clinical signs and lesions are usually more severe.

Volume of inoculum depends in part on site of inoculation, and in part whether one or multiple sites are used per bird (Table). Inoculation into the nasal cavity requires a small inoculum to avoid spillage. In sites where this is less of an issue, e.g. trachea or cloaca, a larger inoculum enhances local spread of the virus.

The use of different group categories depends on the experimental set-up (Table). In the simplest experimental set-up, a group of inoculated birds is sampled over time to estimate virus excretion dynamics, and any birds that develop severe disease are euthanized to characterize virus-associated lesions. The disadvantage of this setup is birds often become ill at different time points after inoculation, so that there is substantial variation in the age of the lesions. Another set-up is to use one group to estimate virus excretion dynamics, and to euthanize another group at a specified time at an early time point of infection (typically between 2 and 4 dpi) to characterize virus-associated lesions. Additional groups may be used to determine rate of transmission to birds in direct or indirect contact. For bird species for which there are no historic negative controls for histological evaluation of tissues, a negative control group may be required.

Group size varies substantially, depending on the expected level of difference in variable of interest, and on availability of birds (Table). For some wild bird species, only small numbers are available for experimental infection.

The above comparison shows that the outcome of infection experiments depends in part on the chosen methods of virus inoculation and experimental design. Therefore, it would be desirable to standardize these procedures in order to allow comparison of results of experiments between consortium partners and generate directly comparable data sets.

STANDARDIZATION OF METHODS

After review and discussion among Delta-Flu partners, we have come to the following preferred values for key variables in infection experiments. The use of these values will make it easier to compare the results of different experimental AIV infections in wild and domestic birds.

1. Route of inoculation: nose via the choanae
2. Virus dose: 1 x 10^6 EID_{50} (or comparable unit of measure)
3. Passage history of virus stock: 4th egg passage or less
4. Inoculum volume: 0.1 to 0.2 ml, depending on the size of the bird
5. Group categories: One group for virus dynamics, one group for evaluation of virus-associated lesions.
6. Group size: Minimum 4 to 6 birds (dependent on expected level of difference in variable of interest).

REFERENCES

Berhane, Y., Kobasa, D., Embury-Hyatt, C., Pickering, B., Babiuk, S., Joseph, T., Bowes, V., Suderman, M., Leung, A., Cottam-Birt, C., Hisanaga, T. and Pasick, J. (2016). Pathobiological Characterization of a Novel Reassortant Highly Pathogenic H5N1 Virus Isolated in British Columbia, Canada, 2015. *Sci Rep,* **6,** 23380.

Ducatez, M., Sonnberg, S., Crumpton, J. C., Rubrum, A., Phommachanh, P., Douangngeun, B., Peiris, M., Guan, Y., Webster, R. and Webby, R. (2017). Highly pathogenic avian influenza H5N1 clade 2.3.2.1 and clade 2.3.4 viruses do not induce a clade-specific phenotype in mallard ducks. *J Gen Virol,* **98,** 1232-1244.

Franca, M., Poulson, R., Brown, J., Howerth, E. W., Berghaus, R. D., Carter, D. and Stallknecht, D. E. (2012). Effect of different routes of inoculation on infectivity and viral shedding of LPAI viruses in mallards. *Avian Dis,* **56,** 981-985.

Kuiken, T. (2013). Is low pathogenic avian influenza virus virulent for wild waterbirds? *Proc Biol Sci,* **280,** 20130990.

Nemeth, N. M., Brown, J. D., Stallknecht, D. E., Howerth, E. W., Newman, S. H. and Swayne, D. E. (2013). Experimental infection of bar-headed geese (Anser indicus) and ruddy shelducks (Tadorna ferruginea) with a clade 2.3.2 H5N1 highly pathogenic avian influenza virus. *Vet Pathol,* **50,** 961-970.

Pantin-Jackwood, M., Swayne, D. E., Smith, D. and Shepherd, E. (2013). Effect of species, breed and route of virus inoculation on the pathogenicity of H5N1 highly pathogenic influenza (HPAI) viruses in domestic ducks. *Vet Res,* **44,** 62.

Pantin-Jackwood, M. J., Stephens, C. B., Bertran, K., Swayne, D. E. and Spackman, E. (2017). The pathogenesis of H7N8 low and highly pathogenic avian influenza viruses from the United States 2016 outbreak in chickens, turkeys and mallards. *PLoS One,* **12,** e0177265.

Spackman, E., Prosser, D. J., Pantin-Jackwood, M. J., Berlin, A. M. and Stephens, C. B. (2017). The Pathogenesis of Clade 2.3.4.4 H5 Highly Pathogenic Avian Influenza Viruses in Ruddy Duck (Oxyura Jamaicensis) and Lesser Scaup (Aythya Affinis). *J Wildl Dis,* **53,** 832-842.

van den Brand, J. M. A., Verhagen, J. H., Veldhuis Kroeze, E. J. B., van de Bildt, M. W. G., Bodewes, R., Herfst, S., Richard, M., Lexmond, P., Bestebroer, T. M., Fouchier, R. A. M. and Kuiken, T. (2018). Wild ducks excrete highly pathogenic avian influenza virus H5N8 (2014-2015) without clinical or pathological evidence of disease. *Emerg Microbes Infect,* **7,** 67.