Seedcorn Fund 2013-2014
Final Report

Final Report

CF0006 Title of Project: Bayesian statistical analysis to assess serological testing strategies for avian influenza surveillance in Europe

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1. Progress Against Project Milestones

Milestone from Project Proposal	Summary of Progress
1: Initial planning: Agreement concerning selection of a subset of all submitted sera for testing, plus review any useful existing data. *Due Month 2, Dec 2012.*	Telephone conference calls were completed in Dec 12 with all the other three Project Partners, namely CVI (NL), DTU (DK) and SVA (SE). These served to focus the aims of this CoVetLab Project onto testing galliforme sera (chickens and turkeys) for the comparison between the H5/H7 haemagglutination inhibition (HI) tests and the AI antibody detection ELISA. Galliformes were chosen and agreed as this project would complement earlier similar work which had been led-up by AHVLA Avian Virology in testing anseriforme sera (ducks and geese) as part of its EU AI Reference Laboratory remit. All partners agreed to this focus on galliforme sera and the use of the IDEXX "Multi Species" blocking ELISA for the CoVet Lab project to ensure a consistent approach. All partners provided assurance that galliforme sera are being collected in their countries as part of their AI national surveillance programmes. All also agreed that AHVLA (ex-CERA) will be doing the Bayesian modelling on the data collected from all four partners. This will prevent unnecessary duplication of statistical analyses at the other institutions, although AHVLA has offered to be transparent and is prepared to share information concerning Bayesian modelling with statisticians at the partner institutes if they so wish.
2: Completion of ELISA and HI serological testing within the project. *Due month 8, May 2013.*	Testing data has been supplied from the four Club 5 partner labs which is summarised as follows: **DK:** 428 sera have been tested from 40 chicken flocks. 36 of the 40 flocks were negative by both IDEXX ELISA and negative by H5/H7 HI testing. The other four chicken flocks were all H5/H7 HI negative, but included the following IDEXX ELISA results: Farm 6830431: One IDEXX 'borderline' serum out of 10. Farm 7136167: One IDEXX positive serum out of 10. Farm 8050531: 9/10 sera are IDEXX positive.

Farm 8151280: 9/10 sera are IDEXX positive.

Interestingly these four are all 'free range' / 'organic' egg production units.

Continuing work (summer 2013): Our Danish colleagues will continue to test more galliforme sera during the summer. These are highly likely to be exclusively chickens as there are very few turkeys in Denmark.

Autumn 2013: Verbal assurance was received at the annual EU AI National Reference Laboratories (NRL) Meeting (Helsinki, Finland, 24-26 September 2013) that the remaining Danish data, compiled during summer 2013, would be forthcoming. This will not include any H5/H7 seropositive flocks, but can contribute to firming-up the specificity aspects of the analysis. The member of staff who has been engaged at the centre of the serological testing left the institute during summer 2013, but the Danish colleagues have been sent a reminder concerning the completion of their contribution to this project.

SE:
The IDEXX ELISA has been used to test 2520 sera from chickens (2140 sera from 214 flocks) and turkeys (380 sera from 38 flocks). Two hundred and ten of the chicken flocks and all the turkey flocks were IDEXX ELISA negative. The four AI positive chicken flocks included:

Farm U120921-0206 (organic layer): 9/10 sera are IDEXX positive.

Farm U121205-0028 (organic layer): One IDEXX positive serum out of 10.

Farm U130222-0048 (broiler): 3/10 sera are IDEXX positive.

U120514-0126 (broiler breeder): One IDEXX positive serum out of 10.

The 14 IDEXX positive sera from the above four chicken flocks were negative by H5/H7 HI testing. However, it appears that none of the IDEXX negative flocks were tested by H5/H7 HI.

Continuing work (summer 2013): While it is appreciated that the Swedish colleagues have the smallest budget in this project, it would be informative if some of the IDEXX negative flocks were to be also tested by H5/H7 HI. This would help contribute to any specificity data which may emerge from the analysis.

Autumn 2013: A discussion ensued with the Swedish colleague at the EU AI NRL Meeting (Helsinki, Finland, 24-26 September 2013) and a reminder has been sent.

NL:
Our Dutch colleagues have provided data from the testing of 25 chicken and two turkey flocks which corresponds to testing of 735 and 58 sera respectively from these two species. All 27 flocks are IDEXX ELISA positive flocks. H5/H7 HI testing identified three H5 seropositive flocks (two chicken and one turkey) and six H7 seropositive flocks (chickens), but unfortunately the H5/H7 HI testing was done by a different protocol to that used by the other project partners. Therefore this potentially valuable data cannot be readily included with the data generated by the other three partners. Discussions at AHVLA considered whether this data may be utilised for other purposes, *eg* an evaluation of the efficacy of surveillance, but further correspondence with the NL lab revealed that the additional necessary information was not available.

	Continuing work (summer 2013): The NL partner has subsequently identified six recent AI seropositive flocks, five H7 positive and one H5 positive. Confirmation is pending that these are from chickens or turkeys in order to be relevant to this project. Four of the flocks have been tested by the IDEXX ELISA (105 sera in total), and the NL have agreed to retest the remaining two flocks with the same ELISA. Importantly, the NL partner has agreed to retest all available sera from these six flocks by the EU-recommended H5/H7 HI antigens. This would allow these six important notifiable AI-positive flocks to be included in the project's analysis together with the chicken / turkey testing that has been done by the other three partners. **Autumn 2013:** In October 2013, the Dutch partner provided serological data from four H5 and three H7 seropositive flocks that had been tested by the IDEXX ELISA and retested by the respective EU-recommended antigens. These seven flocks included a total of 190 sera, and these are in the process of being included into the model for statistical analysis by Dr Mark Arnold at AHVLA Sutton Bonnington. **UK:** Chickens: 245 sera were tested from 17 flocks: All are negative by IDEXX ELISA and by H5/H7 HI testing. Turkeys: 435 sera were tested from 35 flocks: 434 of these sera are negative by IDEXX ELISA and by H5/H7 HI testing. One serum was IDEXX ELISA positive but negative by H5/H7 HI testing. In addition, 47 sera collected from an H9N2 turkey outbreak (April 2013, Suffolk) have also been tested. None of these 47 turkey sera were positive by the H5/H7 HI testing, and this was unsurprising as the outbreak was due to a H9 virus. However, IDEXX ELISA testing revealed 12 positive and 6 'borderline' sera. Other than the event of an AI outbreak, it is unlikely that any more testing of UK chicken / turkey will be done within the lifetime of the project. This is because UK national AI poultry surveillance tends to collect sera from these species almost exclusively during the autumn. **Continuing work (summer 2013):** Ongoing activities at AHVLA have included internal discussion with ex-CERA colleagues concerning what may and may not be done with the accumulated data from all four partner labs in terms of statistical analysis. Liaison with the partners is ongoing in order to ensure that adequate data is generated. The major problem that has been identified is that the overwhelming majority of galliforme poultry sera tested in DK, SE and UK are AI-negative by the IDEXX ELISA, and absolutely none of these sera are H5/H7 HI positive. Therefore this data alone is inappropriate for a sensitivity comparison of IDEXX ELISA and H5/H7 HI. The NL partner is, however, an important source of H5/H7 HI and IDEXX ELISA positive sera. As noted above, discussions have continued with Dutch colleagues to encourage appropriate retesting of a recent tranche of six such seropositive flocks. It is hoped that this will be completed before the project's conclusion, and that this will be sufficient for some meaningful statistical analysis. **Autumn 2013:** As noted above, in October 2013 AHVLA (UK) received the crucial data from seven H5/H7 seropositive flocks from the NL partner. Analysis of the combined data from all the partner institutes is ongoing in order to assess both the sensitivity and specificity of the IDEXX ELISA and H5/H7 HI approaches to serosurveillance.
3. Change emphasis to complete the Bayesian analysis	**Spring 2014:** These three milestones summarise the work that was done in order to complete the project, which also necessitated resolution and / or correction of outstanding matters noted above. Key details for project completion are as follows:

of serological data 4. Conduct and complete any additional serological testing that may be indicated by Milestone 3 5. Concluding analyses, written outputs (below) and final report *Written for Final Report, 17 June 2014.*	1. *Chicken sera and testing:* A total of 1055 chicken sera from the four institutes (40 from SVA (SE), 245 from AHVLA (UK), 284 from CVI (NL) and 486 from DTU (DK)) were all tested by: *a.* HI, using the EU-recommended primary and secondary H5/H7 antigens (below) used to screen and confirm respectively for past infection with notifiable AI (NAI). *b.* The ELISA (IDEXX) which detects AI antibodies to any H-subtype. Results from these 1055 chicken sera featured in the Bayesian analysis (below). As noted above, AHVLA (UK) also tested 435 turkey sera, but these did not include any H5/H7 positive sera so were excluded from the analysis. None of the other three institutes tested any turkey sera in the project, therefore the aims were focused purely on chicken surveillance for NAI. 2. *Project aim:* The central question being addressed in the project was refined as follows: **What is the sensitivity and specificity of the two first-line serological screening methods in these European chicken populations?** A Bayesian statistical model was constructed to compare testing by (a) HI using the primary H5/H7 antigens and (b) the (IDEXX) ELISA testing. This addresses the question as to whether the ELISA may be acceptable as an alternative to primary H5/H7 HI as the first-line screening test. 3. *HI testing:* The classical serological surveillance algorithm for NAI is based purely around HI. The 1055 chicken sera were all tested by (i) initial screening with the primary HI antigens for H5 or H7. These flocks were then (ii) confirmed as being H5/H7 infected through testing with the respective H5/H7 secondary antigens. This was in accord with current EU guidelines (EC 2006) where all four partner institutes used the EU-recommended HI antigens to test for evidence of past infection with notifiable avian influenza (NAI). The antigens were provided by AHVLA in its role as the EU Reference Laboratory for AI, and were supplied as inactivated virus preparations: **Primary HI antigens (screening for NAI):** H5: A/teal/England/06 (H5N3 LPAI) H7: A/turkey/England/77 (H7N7 LPAI) **Secondary HI antigens (confirmation of NAI):** H5: A/chicken/Scotland/59 (H5N1 HPAI) H7: A/African starling/Q-England/79 (H7N1 LPAI) 4. *ELISA (IDEXX) testing:* A new surveillance algorithm under consideration in this project is based on initial first-line screening by the ELISA. Any ELISA reactors are then tested by H5/H7 HI as outlined above, whereby any primary H5/H7 HI positives are rested with the respective H5/H7 HI antigen to confirm evidence of past NAI infection. However, for this algorithm to be accepted, the performance characteristics of the ELISA (sensitivity and specificity) were compared to the primary H5/H7 antigens, i.e. see (2) above. 5. *Serology results:* A total of 21 AI (past infection due to **any** AI subtype) positive chicken flocks were identified in the project. These included six H5 flocks (four in the Netherlands, one in Denmark) and five H7 flocks (five in The Netherlands, one in Denmark) as evidence of past NAI infection. The 11 NAI seropositive flocks were all confirmed by HI testing with the H5 and H7 secondary antigens accordingly. In the case of one of the H7 seropositive flocks sampled in The Netherlands, an H7N7

LPAI virus was also successfully isolated as evidence of an active NAI infection in the chickens during the serological sampling. Ten other flocks were IDEXX ELISA positive but negative by primary H5/H7 HI, indicating past infection by non-NAI, i.e. two in The Netherlands, four in Denmark and four in Sweden. One of the non-NAI infected flocks in The Netherlands was shown to be positive for H6 antibodies in 18 of the 30 submitted sera. The results for the 423 sera from the 21 AI seropositive flocks (Table 1) were divided into the four possible results categories:

a. ELISA positive and primary H5/H7 HI positive
b. ELISA positive and primary H5/H7 HI negative
c. ELISA negative and primary H5/H7 HI positive
d. ELISA negative and primary H5/H7 HI negative

The numbers of sera in the four different results permutations represent important data which was fed into the Bayesian model. In addition to the flocks listed in Table 1, 36 chicken flocks from Denmark (387 sera) and 17 chicken flocks from the UK (245 sera) were all negative by both the ELISA and primary H5/H7 HI testing. This data was also included in the Bayesian model.

6. *The Bayesian model:* Key assumptions included:
   a. Non-informative Bayesian priors were the sensitivity and specificity of all the serological tests, as well as the within flock prevalence and flock prevalence.
   b. Infected flocks were assumed to have been exposed to only one strain of AI. This was confirmed by HI testing which showed that H5 seropositive flocks did not include any sera which were H7 positive, and vice versa.
   c. The sensitivity of primary HI testing with the H5 and H7 antigens was assumed to be equal. The primary H5 and H7 HI results were considered as one, i.e. positive or negative for NAI.
   d. The sensitivity and specificity of ELISA was assumed to be the same for detection of chicken antibodies produced in response to infections with all AI subtypes.

In addition, the bespoke model took into account the different specifications of the ELISA and primary H5/H7 HI tests, i.e. the former detects antibodies to all AI subtypes, while the latter detects antibodies only to H5/H7 (NAI) AI subtypes..

7. *Sensitivity and specificity of the serological tests (Bayesian):* These outcomes are shown in Table 2. The sensitivity and specificity of the IDEXX ELISA is high, and the same was observed for the specificity of the primary H5/H7 HI. However, the sensitivity of the primary H5/H7 HI was low at 53%. It is speculated that this may be due to the particular antibodies which are responsible for inhibiting haemagglutination being:

   a. Weaker or sub-optimal in recognising the receptor binding region on the haemagglutinin protein of the particular H5 or H7 AIV which was responsible for the infection in a given flock; or:

   b. A temporal effect, whereby an early humoral response (i.e. sera collected soon after infection) may be detected much more readily by the ELISA (which detects antibodies to the nucleoprotein which is conserved among all AIV subtypes), while the level of antibodies targeted to the haemagglutinin / the haemagglutinin region responsible for HI remained low or undetectable at this early time-point.

   c. Alternatively, in the case of sera collected at a later time after active infection had cleared, the temporal effect may be manifesting itself in faster decline in the titre of

antibodies which are detected by primary H5/H7 HI compared to those detected by the ELISA.

8. *Fit of the Bayesian model:* A comparison can be made between the Bayesian model and the observed results in the AI-infected chicken flocks. For example, in the case of the 11 AI seropositive chicken flocks from The Netherlands, the specificity of both tests is reflected in a very good fit between the numbers of observed and modelled sera which were negative by both tests (Fig 1). This is evident by comparing the 'ELISA negative / primary H5/H7 HI negative' results among the 11 flocks, where in most cases the numbers of sera predicted in the model are identical to the observed numbers of sera in this results category.

   The sensitivity of HI had been determined as 53% (Table 2) as an average value calculated across all the chicken sera in this study. However, when individual chicken flocks are considered, the sensitivity of HI was observed to be either greater or less than the 53% value in different flocks. This can be gauged visually by comparing the numbers of sera which are in the 'ELISA positive / primary H5/H7 HI positive' category (Fig 1). For example, in flock # 1 tested in The Netherlands, the number of sera predicted by the model in this results category are greater than those observed, therefore the primary H5/H7 HI sensitivity is <53%. In the case of flock # 6, the opposite is observed, therefore the primary H5/H7 HI sensitivity is >53%.

9. *Seroprevalence and estimates of sample size for chicken surveillance:* Bayesian modelling was also applied to calculate the distribution of within-flock prevalence of AI. This is shown in Fig 2, and shows that a high seroprevalence for AI was evident in the the majority of previously AI-infected chicken flocks. Additional mathematical calculations indicated that a sample size of 10 sera per chicken flock are sufficient for first-line screening with the ELISA.

10. *Conclusions and recommendations:* A Bayesian approach was used in this project in order to extract quantitative estimates from complex data. This enabled the sensitivity of two screening approaches for NAI surveillance in chickens, namely the use of primary H5/H7 HI and the IDEXX ELISA, to be compared without the need for one of these tests being accepted as a supposed 'gold-standard'. In summary, the results revealed:

    a. High specificity for both the primary H5/H7 HI and the ELISA.
    b. A higher sensitivity in the chicken flocks for the ELISA for use in screening, whereas the H5/H7 HI had a clearly lower sensitivity.
    c. A high AI seroprevalence was noted in the majority of previously AI-infected chicken flocks. Together with the high sensitivity of the ELISA, this indicated that a sample size of 10 sera is sufficient for screening for NAI by ELISA in chicken flocks.
    d. These outcomes show that the IDEXX ELISA is indeed acceptable for use in NAI surveillance in chickens as the first-line screening test.
    e. AHVLA (UK) is willing to share the Bayesian model which was used in this project with interested colleagues at the CoVetLab institutes. Details are included in an appendix supplied with this Final Report.

11. *Subsequent work / recommendations:*
    a. The Avian Virology Workgroup at AHVLA is the EU Reference Laboratory for AI, and will continue its liaisons with AI National Reference Laboratories (NRLs) of the EU Member States (MSs) as regards the acceptance of ELISA as a front-line screening test for NAI.
    b. Additional work may still be done, e.g. to analyse the results of secondary H5/H7 HI testing through the Bayesian model. The secondary H5/H7 HI

tests are important to confirm H5/H7 seropositive flocks (i.e. to confirm NAI) initially detected by primary H5/H7 HI testing. Secondary H5/H7 HI data was collected during the current project, and may be readily investigated as part of the EU AI Reference Laboratory activities at AHVLA.

c. The choice of H5 and H7 AI viruses as the primary and secondary HI antigens is another area which may merit investigation in a future project. Antigenic cartography would provide a rigorous scientific approach for selection of fit-for-purpose H5/H7 antigens. This would require testing of a large number of H5 and H7 AI viruses submitted from across Europe in recent years. While this was noted in the original project proposal to CoVetLab, in practical terms this was beyond the practical scope and budget of the current project.

12. *Reference:* EC (European Commission). 2006. Guidelines on the implementation of survey programmes for avian influenza in poultry and wild birds to be carried out in the Member States in 2007. Available at http://ec.europa.eu./food/animal/diseases/controlmeasures/avian/guidel_ai_surv_wb_poul_2007_en.pdf

2. Will you achieve the project plan within agreed timescales? No (possibly!)

3. Will you deliver the project on budget? Yes

4. If you answered no to either of the questions above please explain, including reasons for requesting an extension to time or extra budget allocation.

As explained above in the UK section of "Progress Against Project Milestones" under "Continuing work (summer 2013)", there is a continuing need for a sufficient number of H5/H7 HI seropositive flocks to be identified. This is in order to provide enough data for a statistical comparison of the selected IDEXX ELISA with the H5/H7 HI testing. It is important that the H5/H7 HI testing is done uniformly by all four partner labs by using the agreed EU-recommended H5/H7 HI antigens. The role of the Dutch partner is crucial as this is the only country which has identified such H5/H7 seropositive galliforme flocks.

However, much of the NL H5/H7 HI testing data from 27 galliforme flocks is based on their own choice of H5/H7 HI antigens, so this is inappropriate for the current project. Unfortunately these sera are no longer available for H5/H7 HI retesting with the agreed antigens. Following discussion, in late June the Dutch colleagues agreed to retesting six recent H5/H7 seropositive flocks by using the EU-recommended H5/H7 HI antigens. These were supplied from AHVLA in early July. At the time of writing I have contacted the NL Project Leader to check for progress, and hope to receive a reply by mid-August. In the event of the testing in the NL lab continuing into September, the project may require a time extension in order for the statistical analysis to be done at AHVLA.

Autumn 2013: As stated above, the necessary data from seven H5/H7 seropositive flocks has been received following retesting by using the H5/H7 EU-recommended antigens. The NL data also includes H5/H7 HI testing which employed locally-selected antigens, and consideration will be given to see whether the choice of particular H5/H7 antigens may influence the outcome of the test sensitivity / specificity analysis.

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June 2014 (Final Report): While the vast majority of the serology data had been submitted to AHVLA (UK) for analysis by December 2013, there remained several small but important questions which required resolving with the participating institutes in order to ensure that the Bayesian analysis would be completed accurately. The Bayesian model has been ready since late 2013, and was run as soon as the outstanding questions were answered during spring 2014. The project outputs, its conclusions and recommendations were presented at the CoVetLab meeting at CVI in The Netherlands on 12-13/6/14, and this Final Report completed accordingly.

5. Any other information.

Fig 1: Fit of the Bayesian model to observed data for each of 11 AI positive chicken flocks from The Netherlands tested with both ELISA and HI. Flock numbers correspond to those listed in Table 1, left to right, beginning in the top row and then continuing left to right in the second row etc…

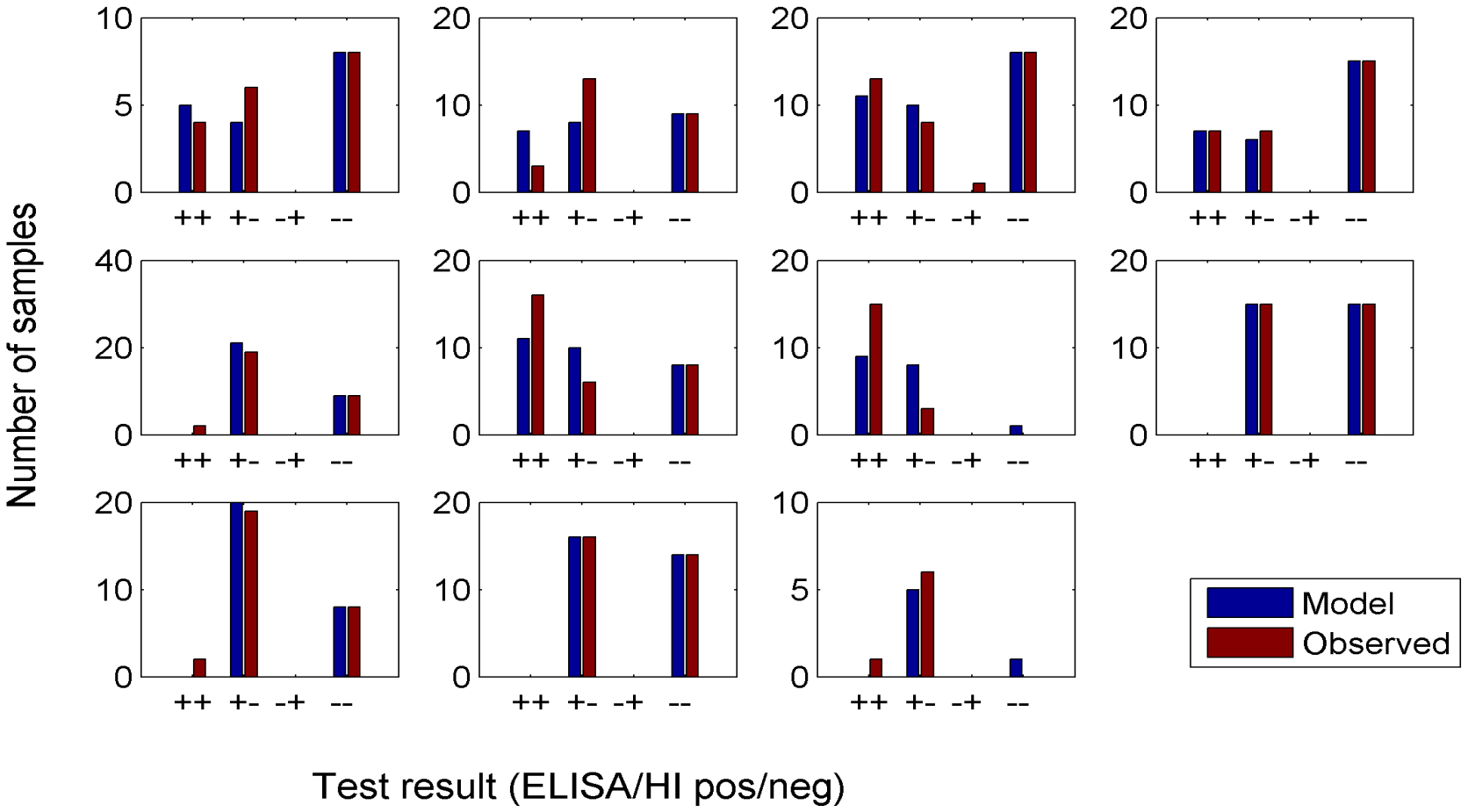

Fig 2: Distribution of the estimates of AI seroprevalence from a Bayesian model applied to chicken flocks sampled from the UK, Denmark, The Netherlands and Sweden* which were tested by primary

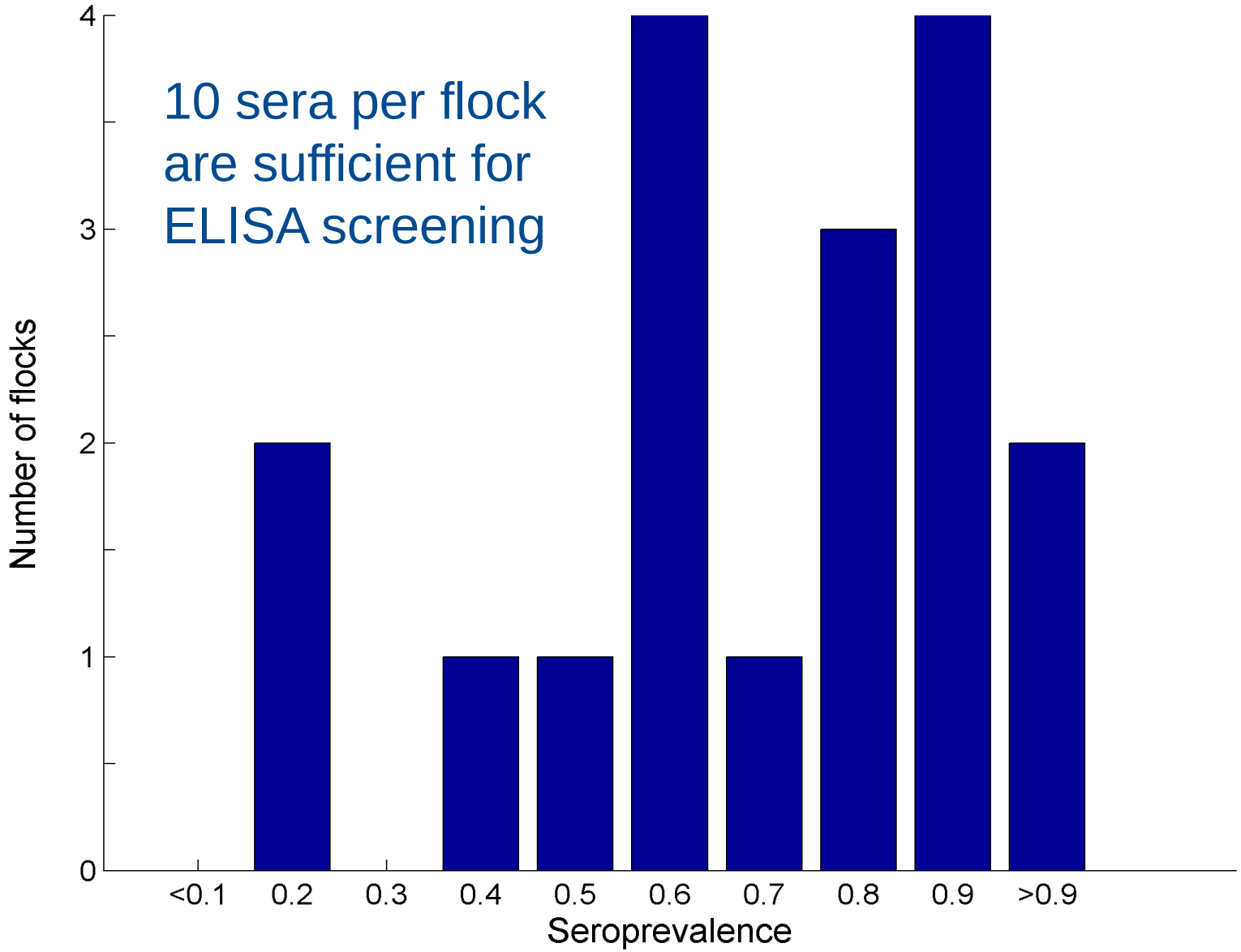

Table 1. AI seropositive chicken flocks. Numbers of sera are indicated for each the four possible permutations of ELISA and primary H5/H7 HI results. Red, blue and black type indicate H5, H7 and non-NAI seropositive flocks respectively.

Farm ID (numbers of sera tested)	Results: ELISA / HI			
	Pos/Pos	Pos/Neg	Neg/Pos	Neg/Neg
NL				
1 (18)	4	6	0	8
2 (25)	3	13	0	9
3 (38)	13	8	1	16
4 (29)	7	7	0	15
5 (30)	2	19	0	9
6 (28)	16	6	0	8
7 (18)	15	3	0	0
8 (30)	0	15	0	15
9 (29)	2	19	0	8
10 (30)	0	16	0	14
11 (7)	1	6	0	0
DK				
12 (10)	0	1	0	9
13 (10)	0	1	0	9
14 (10)	0	9	0	1
15 (10)	0	9	0	1
16 (39)	10	28	0	1
17 (20)	15	2	1	2
SE				
18 (10)	0	9	0	1
19 (10)	0	1	0	9
20 (10)	0	3	0	7
21 (10)	0	1	0	9

Table 2: Bayesian estimates of the sensitivity and specificity of HI and ELISA for NAI detection in chicken flocks

Parameter description	Bayesian estimates	
	Median	2.5 & 97.5 percentiles
Sensitivity of HI	0.53	(0.44, 0.62)
Specificity of HI	0.993	(0.985, 0.998)
Sensitivity of ELISA	0.96	(0.89, 0.99)
Specificity of ELISA	0.993	(0.985, 0.997)

AHVLA
Animal Health and Veterinary Laboratories Agency

Appendix to CoVetLab Project CF0006 Final Report:

The Bayesian model used to estimate the sensitivity and specificity of H5/H7 haemagglutination inhibition (HI) and ELISA for detection of notifiable avian influenza (NAI) antibodies in chicken flocks

Background

Additional background information and other details are presented in the main text of the Final Report:

A total of 1055 chicken sera were tested. All were tested by the (i) primary H5/H7 HI tests and (ii) the IDEXX ELISA. These are the two approaches for NAI screening which are being considered and compared, please refer to text in the Final Project Report.

It is assumed that the sensitivity of the primary H5 HI and primary H7 HI tests is the same. Chicken flocks were either:

(i) Uninfected with AI: All sera negative by all tests.
(ii) Infected with a non-H5/H7 AI subtype (i.e. infected with non-NAI): At least one serum in the flock was positive by the ELISA, but all sera were negative by the primary H5 & H7 HI tests.
(iii) Infected with NAI: At least one serum was positive by primary H5 or H7 HI testing, and follow-up by HI testing with the respective secondary H5 or H7 HI test confirmed NAI infection.

Statistical methods

Assumptions are indicated by yellow highlight:

Let the within flock prevalence of H5/H7 avian influenza (i.e. NAI) and non-H5/H7 avian influenza (i.e. non-NAI) be denoted by π_{H5} and π_{AI} respectively. For the data from the Netherlands, intensive efforts were made to establish the strain type, and there was no evidence of multiple strains infected any of the flocks. Therefore it was assumed that birds have been exposed to only one strain of avian influenza, i.e. we assume that birds will not have been infected by multiple H-subtypes of different AI viruses. Let the sensitivity and specificity of HI to detect H5/H7 avian influenza be denoted by Se_H, Sp_H , and the sensitivity and specificity of ELISA to detect avian influenza of any subtype be denoted Se_E, Sp_E. We assume that there is no difference in the sensitivity and specificity of ELISA according to sub-type, so that it will detect H5/H7 and non-H5/H7 with the same likelihood.

With these assumptions, the likelihood of a both HI and ELISA testing positive for a random bird within a flock is given by the sum of (i) the probability that the bird has antibodies to H5 AI and correctly identified by both tests, $\pi_{H5} Se_H Se_E$ (ii) the probability that the bird only has antibodies to non-H5 AI, correctly identified by

ELISA but incorrectly classified by H5/H7 HI, $(1-\pi_{H5})\pi_{AI}(1-Sp_H)Se_E$ and (iii) the probability that the bird is not infected with AI and incorrectly classified by both tests $(1-\pi_H)(1-\pi_{AI})(1-Sp_H)(1-Sp_E)$. Similar reasoning can be applied to derive the probabilities for the other test outcomes. Denoting by $p_{ij}, i, j = 0,1$ the likelihood that H5/H7 HI or ELISA were negative (i,j =0 respectively) or positive (i,j=1) respectively, the other test outcomes had the following probability:

$$p_{00} = (1-Se_H)(1-Se_E)\pi_{H5} + Sp_H(1-Se_E)(1-\pi_{H5})\pi_{AI} + Sp_H Sp_E(1-\pi_{H5})(1-\pi_{AI})$$
$$p_{01} = (1-Se_H)Se_E\pi_{H5} + Sp_H Se_E(1-\pi_{H5})\pi_{AI} + Sp_H(1-Sp_E)(1-\pi_{H5})(1-\pi_{AI})$$
$$p_{10} = Se_H(1-Se_E)\pi_{H5} + (1-Sp_H)(1-Se_E)(1-\pi_{H5})\pi_{AI} + (1-Sp_H)Sp_E(1-\pi_{H5})(1-\pi_{AI})$$

Since it is assumed that each flock has only been exposed to one strain of avian influenza, if it is positive for H5/H7 it is assumed negative for non-H5/H7, and vice-versa. The indicator function for whether a flock was H5/H7 positive was assumed to follow a Bernoulli distribution with a beta prior.

The outcome for each bird in each flock arises from a multinomial distribution with the probability of each of the 4 possible outcomes given by p_{ij}. The multinomial model was fitted to the data from each flock for each species using WinBUGS 3.1. 10,000 iterations with a burn-in of 5,000 were used to generate the final (posterior) estimates, and convergence assessed using the Gelman-Rubin statistic, as implemented in WinBUGS. Vague priors in the form of beta distributions with both parameters set equal to 1 were used (i.e. uniform in the range 0-1).

Results

A lot of this information is also summarised in the main text of the Final Report and its accompanying figures:

- Only 2 sera of 1055 tested were negative for ELISA but positive for H5/H7 HI out of all the 18 AI seropositive flocks (Table 1 in this appendix). This results in a very high estimate of sensitivity of ELISA (Table 2 in this appendix). This is because there is little evidence in the data of ELISA failing to detect seropositive birds.
- Far greater numbers of ELISA positive sera were observed compared to H5/H7 HI positive sera in H5/H7 HI positive flocks (205 ELISA positives compared to 90 HI positives). This leads to an estimate of H5/H7 HI sensitivity of 53% (Table 2 in this appendix).
- Model fit was good overall – but some evidence of variability of H5/H7 HI sensitivity between flocks. For example, compare flocks 2 and 7 in Table 2 in this appendix. In flock 2 only the minority of ELISA positives are detected by H5/H7 HI, but in flock 7 the majority of positive samples are positive for both H5/H7 HI and ELISA.
- Estimates of the flock seroprevalence were produced by the model – this indicated that the seroprevalence in AI seropositive flocks is high (Fig. 3 in this appendix). This has implications for sample sizes, see main text of Final Report.

- It may not be possible to draw conclusions concerning the randomness of the sampling of the flocks. It is accepted that the detailed testing was done at the four partner institutes on only a small proportion of chicken flocks which are submitted as part of the respective National Poultry Surveillance Programmes for AI. The Netherlands, for example, provided the majority of H5/H7 seropositive flocks, but no AI seronegative flocks which were however supplied in sufficient numbers by the UK and Denmark. If possible, it may be informative to note different prevalence of flocks with AI between Sweden, Denmark and UK.

Table 1
The results from positive farms from the testing of chickens for avian influenza with both Haemagglutination Inhibition (HI) and ELISA in The Netherlands, Denmark and Sweden

Farm ID	ELISA result/ HI result			
	Pos/Pos	Pos/Neg	Neg/Pos	Neg/Neg
The Netherlands				
1	4	6	0	8
2	3	13	0	9
3	13	8	1	16
4	7	7	0	15
5	2	19	0	9
6	16	6	0	8
7	15	3	0	0
8	0	15	0	15
9	2	19	0	8
10	0	16	0	14
11	1	6	0	0
Denmark and Sweden				
12	0	1	0	9
13	0	1	0	9
14	0	9	0	1
15	0	9	0	1
16	10	28	0	1
17	15	2	1	2
18 *	0	14	0	26

* Flock # 18 is an amalgamation of the four Swedish chicken flocks (ten sera submitted from each), see Table 1 in the Final Report which shows these as four discrete flocks.

Table 2
Bayesian Estimates of the sensitivity and specificity of HI and ELISA for detection of avian influenza in chicken flocks.

Parameter	Description	Bayesian estimates	
		Median	2.5 and 97.5 percentiles
SeH	Sensitivity of HI	0.53	(0.44, 0.62)
SpH	Specificity of HI	0.993	(0.985, 0.998)
SeE	Sensitivity of ELISA	0.96	(0.89, 0.99)
SpE	Specificity of ELISA	0.993	(0.985, 0.997)

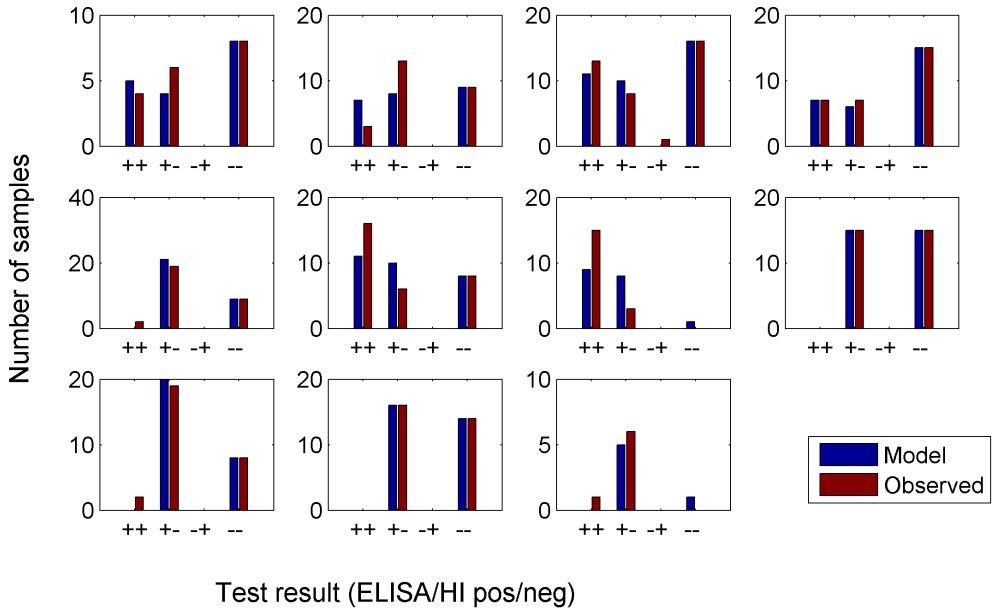

Figure 1. The fit of the Bayesian model (blue bars) to the observed data (brown bars) for each of 11 avian influenza positive flocks (Table 1 in this appendix) tested with both primary H5/H7 HI and ELISA in The Netherlands. The fit overall is good, but with some evidence for sensitivity of H5/H7 HI being variable between flocks, although not to the same extent as observed in a parallel project which similarly examined H5/H7 HI and ELISA testing of farmed anseriformes (i.e. domestic ducks and geese). In the current study, there are flocks that have a small number of H5/H7 HI positives, but lots of ELISA positives – the model is assuming that the birds have only been infected with one strain of AI, and thus low HI sensitivity is observed.

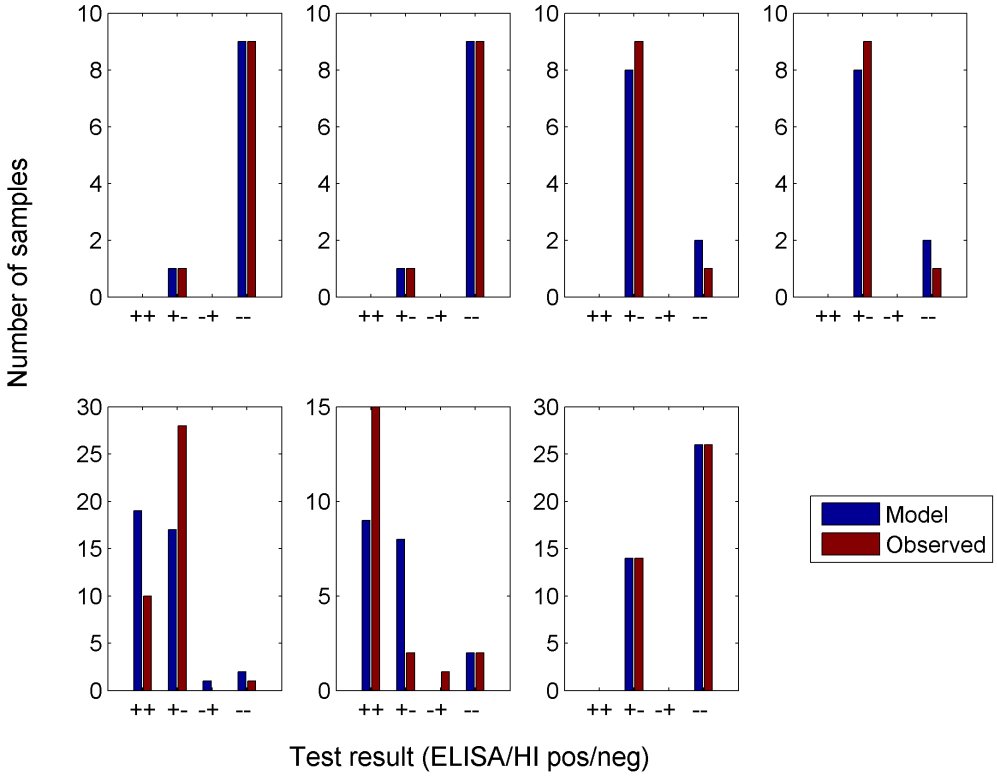

Figure 2. The fit of the Bayesian model (blue bars) to the observed data (brown bars) for each of 7 avian influenza positive flocks tested with both primary H5/H7 HI and ELISA in Denmark (flocks 12-17) and Sweden (flock 18, Table 1 in this appendix).

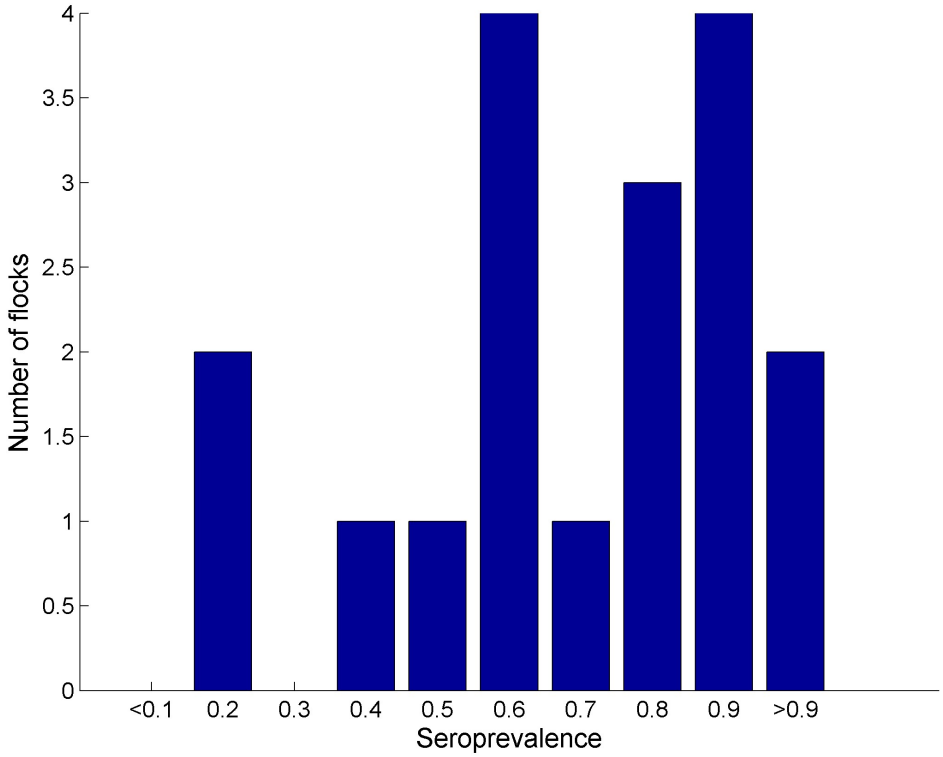

Figure 3. The distribution of the estimates of avian influenza seroprevalence from a Bayesian model applied to data on sampling chickens with HI and ELISA from The Netherlands, Denmark, Sweden and the UK.