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Using egg production data to quantify within-flock transmission of low pathogenic avian influenza virus in commercial layer chickens

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ABSTRACT

Even though low pathogenic avian influenza viruses (LPAIv) affect the poultry industry of several countries in the world, information about their transmission characteristics in poultry is sparse. Outbreak reports of LPAIv in layer chickens have described drops in egg production that appear to be correlated with the virus transmission dynamics. The objective of this study was to use egg production data from LPAIv infected layer flocks to quantify the within-flock transmission parameters of the virus. Egg production data from two commercial layer chicken flocks which were infected with an H7N3 LPAIv were used for this study. In addition, an isolate of the H7N3 LPAIv causing these outbreaks was used in a transmission experiment. The field and experimental estimates showed that this is a virus with high transmission characteristics. Furthermore, with the field method, the day of introduction of the virus into the flock was estimated. The method here presented uses compartmental models that assume homogeneous mixing. This method is, therefore, best suited to study transmission in commercial flocks with a litter (floor-reared) housing system. It would also perform better, when used to study transmission retrospectively, after the outbreak has finished and there is egg production data from recovered chickens. This method cannot be used when a flock was affected with a LPAIv with low transmission characteristics ($R_0 < 2$), since the drop in egg production would be low and likely to be confounded with the expected decrease in production due to aging of the flock. Because only two flocks were used for this analysis, this study is a preliminary basis for a proof of principle that transmission parameters of LPAIv infections in layer chicken flocks could be quantified using the egg production data from affected flocks.

1. Introduction

Low pathogenic avian influenza (LPAI) is a mild disease of various avian species, which is caused by Influenza A viruses belonging to one of 16 Hemagglutinin (H) and 9 Neuraminidase (N) subtypes (Fouchier et al., 2005; Alexander, 2007). LPAI virus (LPAIv) infections in poultry with H5 or H7 virus subtypes are of major importance due to their ability to mutate to a highly pathogenic avian influenza virus (HPAIv) (Alexander, 2007). In addition, H9 and H6 LPAIv subtypes in particular, have been affecting the poultry industry of different countries in Asia (Cheung et al., 2007; Xu et al., 2007; Hadipour, 2011; Park et al., 2011).

LPAIv surveillance programmes have been implemented in many countries (Gonzales et al., 2010). Although

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these programmes may be useful to determine whether the prevalence of infected birds is below a pre-set level, their usefulness in early detection is still unknown. To establish the latter (Graat et al., 2001; Fischer et al., 2005), quantitative knowledge of transmission of LPAIv is necessary.

The transmission characteristics of a pathogen can be determined in transmission experiments (van der Goot et al., 2003; Velthuis et al., 2007) or in field outbreaks (Stegeman et al., 1999; Bos et al., 2009). Transmission experiments allow for quantifying transmission parameters in a controlled environment, but, in case of LPAIv, there appears to be considerable variation in the transmission characteristics of different virus strains even within the same H subtype (van der Goot et al., 2003; Gonzales et al., 2011, 2012). To quantify the existing variability experimentally would be very costly. An alternative would be the quantification of transmission from field data. The latter would have the following benefits: (i) the quantified transmission parameters would be a direct indicator of the transmission characteristics of the virus in the field, (ii) transmission could be studied faster than with transmission experiments, and (iii) the use of indicators already available would be cheaper and more desirable from the perspective of animal welfare.

LPAIv infections in poultry are often subclinical or present unspecific clinical signs. However, drops in egg production have been often reported during outbreaks involving chicken layers flocks (Henzler et al., 2003; Zanella, 2003; de Wit et al., 2004); with sudden drops in production ranging from 10% (de Wit et al., 2004) to 40% (Zanella, 2003) in a couple of weeks followed by a slight increase (from the biggest drop level) some weeks later. Such drops in egg production have been also observed in experimentally infected layer chickens (Trampel et al., 2006; Gonzales et al., 2012). Consequently, it would be worthwhile to examine whether the drop in egg production can be used to estimate transmission parameters. This would be a cheap alternative to transmission experiments.

In 2003, a cross-sectional serological survey was performed in The Netherlands (de Wit et al., 2004) and a high prevalence of seropositive animals to H7N3 LPAIv was detected in a cluster of three farms: one turkey (10 seropositives out of 10 samples) and two free-range layer chicken farms (30 seropositives out of 30 samples). The H7N3 virus was later isolated from the turkey farm (de Wit et al., 2004; Velkers et al., 2006). The objective of this study was to estimate the transmission characteristics of this H7N3 LPAIv in a transmission experiment and from the egg production data of the two infected layer flocks.

2. Methods

2.1. Experimental estimation of within-flock transmission parameters

The chicken-to-chicken transmission characteristics of the H7N3 LPAIv (cleavage side: PEIPKGR*GLF (Velkers et al., 2006)) causing the outbreaks here analysed were first quantified in a transmission experiment. The experimental procedure and data analysis was similar to that described elsewhere (van der Goot et al., 2003; Gonzales et al., 2011). Briefly, two experimental trials were carried out. Each trial consisted of 10 specified pathogen free (spf) White Leghorn chickens (6 weeks old). Five chickens were inoculated and the remaining five were kept as contacts. Chickens were inoculated both intranasally and intratracheally with 0.1 ml/route of inoculum containing 10^6 EID_{50} (50% egg infectious dose)/ml. Virus transmission was monitored by regularly collecting cloaca and trachea swab samples, which were examined for the presence of virus (virus isolation in embryonated chicken eggs). Samples were taken daily from day post inoculation (d.p.i.) 1 to d.p.i. 10 and later at d.p.i 14, 17 and 21. The data from this experiment were used to estimate the transmission rate parameter β (day^{-1}), which is the expected number of contact infections caused by an infectious individual per day, the infectious period T, which is the average time (days) that an infected individual remains infectious, and the recovery rate γ (day^{-1}), which is the expected number of animals recovering from infection per day. β was estimated using a generalised lineal model (GLM) method, assuming a latent period $\leq$1 day. The mean length of T was estimated using a parametric survival model with a Weibull distribution (the distribution that best fitted the data) and γ was estimated as the inverse of T ($\gamma = 1/T$). The basic reproduction ratio R_0 was estimated as the product of β and T. Because the correlation between β and T was unknown, confidence intervals for R_0 were derived by Monte Carlo (MC) simulations assigning to β and T Lognormal and Weibull distributions, respectively (Table 2). All the analysis were performed using the statistical package "R" (R Development Core Team, 2005). The library Survival was used for the survival analysis.

The transmission experiment was approved by an ethical committee and complied with the Dutch law on Animal experiments. The experiment was carried out in the High Containment Unit at the Central Veterinary Institute part of Wageningen University and Research centre, in Lelystad, The Netherlands.

2.2. Estimation of within-flock transmission parameters from egg production data

Egg production data from the two infected free-range layer chicken flocks, here referred to as Farm-3 and Farm-4 as reported by de Wit et al. (2004), were used for the analysis. Egg production data consisted of weekly averages of daily egg production. For both flocks, we selected data from week 38 (calendar week) of 2002 – when production in both flocks appeared to be maximal and stable – to the last week (week 10 of 2003) that production was reported by de Wit et al. (2004). This period resulted in a total of 25 data points (Fig. 1). To analyse these data, we simulated the infection dynamics in these flocks constructing deterministic susceptible-infectious-recovered (SIR) and susceptible-exposed-infectious-recovered (SEIR) models, in which we assumed a homogeneous contact structure (Keeling and Rohani, 2008). The transmission term was formulated as $\beta S(t)I(t)/N(t)$, with $S(t)$, $I(t)$ and $N(t)$ denoting the number of susceptible S and infectious I chickens in the total population of size N at time t (days). This formulation implies that the transmission pressure is independent

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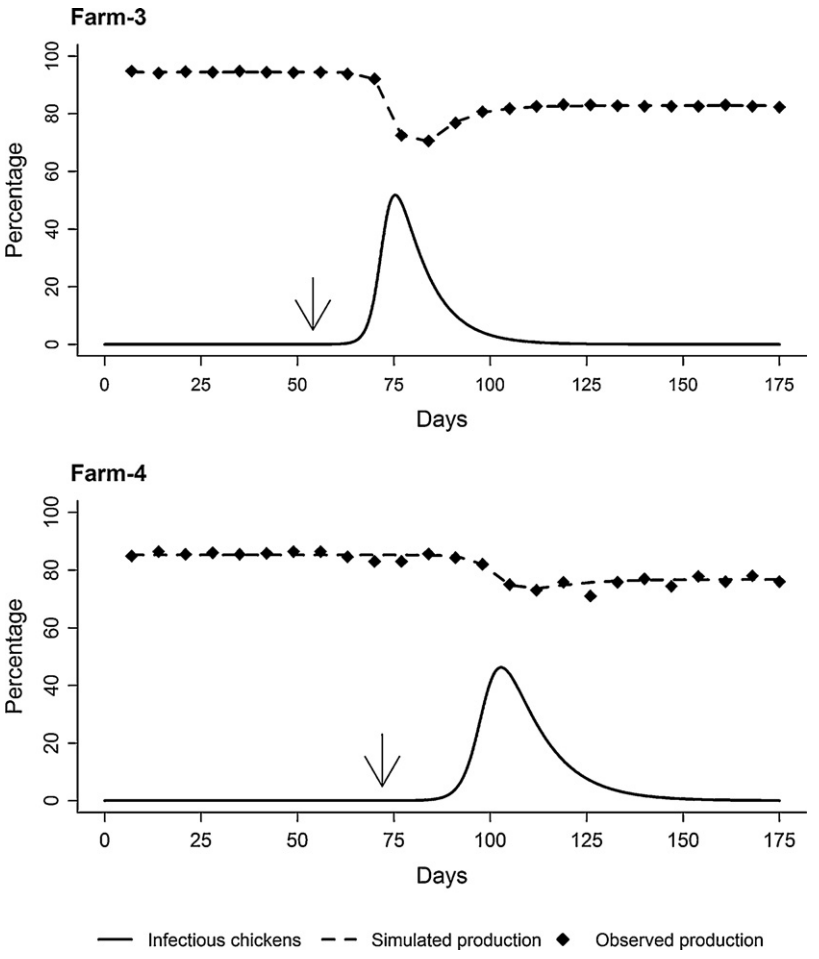

Fig. 1. Simulated LPAI outbreaks based on egg production data of affected flocks. Egg production data (diamonds), simulated susceptible-infectious-recovered (SIR) infection dynamics (solid line, showing only infectious chickens) and simulated egg production (dashed line) based on the fitted parameter values as presented in Table 3 for Farm-3 and Farm-4. The arrows indicate the estimated day of start of the outbreak. Day zero represents the first day of week 38 of the calendar year 2002.

of population size, which appears to be appropriate here (Bouma et al., 1995). By applying this model to both data sets, from the simulations and from the transmission experiment, the results could be compared.

Egg production in latently infected chickens was assumed to be equal to that of susceptible chickens, and is denoted as *ps*. Egg production (*pe*) was simulated as a function of the expected production (*ps*, *pi* and *pr*) of the susceptible chickens (*S*) plus that of the exposed (latently infected) (*E*), infectious (*I*) and recovered (*R*) chickens at time *t* in the epidemic.

$$pe(t) = [S(t) + E(t)]ps + I(t)pi + R(t)pr \quad (1)$$

For the simulations, the starting values (initial guesses) for the transmission parameters ($\beta = 0.49$ and $\gamma = 1/7.7$) were derived from Gonzales et al. (2011). The starting values for the production parameters *ps* and *pr* were derived from the production data by calculating the average egg production from the first 7 weeks (we assumed that infection was not yet introduced) and the last 6 weeks (production was stable and we assumed that both outbreaks were finished) of the study period (Farm-3: $ps = 0.944$, $pr = 0.827$ and Farm 4: $ps = 0.852$, $pr = 0.766$). The starting value for $pi = 0.56$ (for both farms) was derived from experimental estimates reported elsewhere (Gonzales et al., 2012).

The predicted egg production was averaged weekly and compared with the observed data. The squared deviation of the predicted production from the observed production was calculated and the total sum of squared deviations (SSQ) was used as a measure for goodness of fit. The set of parameter values with the lowest SSQ was selected as the best fit. The simulation models were specified by the transmission and production parameters described in Table 1.

The SIR/SEIR simulations and parameter optimization – using an in-built routine to minimize the SSQ – were carried out in Excel® using the add-in tool "PopTools" (Hood, 2010) and selecting the Marquardt method for the estimation of the covariance matrix (the Excel® file can be provided upon request). The time step (Δt) used in the simulations was 1/40 days (this small time step was optimised for duration and accuracy of the simulations). The fitting routine was iterated until the SSQ reached a constant value. The Marquardt fitting method finds local minima for the SSQ.

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Table 1
Parameters used for the susceptible-infectious-recovered (SIR) and susceptible-exposed-infectious-recovered (SEIR) models simulations intended to fit the dynamics in egg production.

Parameter	Description	Units
β	Transmission rate parameter	day^{-1}
γ	Recovery rate	day^{-1}
T	Infectious period, equal to $1/\gamma$	day
L	Latent period, this parameter was used in the SEIR model	day^{-1}
$I(0)$	Starting value for the Infectious (SIR model) or the Exposed (SEIR model) compartment. This parameter allows the model to identify the time the epidemic started in each flock.	day
ps[a]	Level of egg production of Susceptible and Exposed chickens. Since Exposed animals are latently infected, their production is expected to be the same as that of Susceptible chickens	
pi[a]	Level of egg production of Infectious chickens	
pr[a]	Level of egg production of Recovered chickens	

[a] The values of these parameters are included as proportions in the models.

It might well be that with different initial values guessed, different local minima, and thus different parameter values, are found. To test the robustness of the fitted results to the initial conditions, in particular the estimates of β and γ, we also initiated fitting iterations with the parameter values estimated in the transmission experiment in this study (Section 3.1). Both approaches yielded the same local minima. Comparison between SIR and SEIR model fits were done with the Akaike's Information Criterion (AIC).

3. Results

3.1. Experimental estimation of within-flock transmission parameters

All inoculated chickens in both trials (five per trial) became infected and transmitted virus to their contact chickens (Table 2). No apparent clinical signs were observed and all inoculated chickens were positive in virus isolation at d.p.i. 1, the latter indicating that the latent period might be less than one day. The mean length of the infectious period (T) was significantly different ($P < 0.05$) between inoculated and contact-infected chickens. The mean estimate of T in inoculated chickens was 13.32 days, while the mean T in contact chickens was 10.03 days. The latter gives a recovery rate $\gamma = 0.10\,day^{-1}$. The mean estimate of β was $0.91\,day^{-1}$. The MC result for R_0 using T of contact-infected chickens was 9.1. Table 2 provides an overview of all parameter estimates and their 95% confidence intervals (CI).

3.2. Estimation of within-flock transmission parameters from egg production data

Optimization of parameter estimates using the data from Farm-4 did not converge as easily as that using data from Farm-3. This was because egg production in Farm-4, after the peak of the outbreak (here referred to as recovery phase), was more variable (Fig. 1), and optimizations resulted in unexpected estimates for pi and γ (e.g. $pi = 10\%$ and $\gamma = 0.46\,day^{-1}$). To improve the optimisation procedure for this farm, we reduced the number of parameters to be optimised, by keeping the production parameters ps and pr fixed to the starting values. This resulted in robust estimates of the optimised parameters (Table 3).

Results of the parameter estimation using egg production data from Farm-3 and Farm-4 are summarised in Table 3. These estimates were robust and insensitive to different starting conditions. All simulations converged to the same minimum (Table 3, Fig. 1). Parameter estimates were similar when using either a SIR or a SEIR model. The mean estimates of the latent period (L), in the SEIR model, were close to zero. Based on the SSQ and AIC of these models, the SIR model showed a better fit than the SEIR model (Table 3). The estimated β was lower in Farm-4 than in Farm-3, while the estimates of γ were similar for both farms. The results of the simulations also show that Farm-3 was affected between 2 and 3 weeks before Farm-4. The day the first infectious chicken was present in the flock ($t(I = 1)$) in Farm-3 was around day 54 (counting from the start of the study period in week 38), and around day 72 in Farm-4 (Table 3).

4. Discussion

The transmission characteristics of the H7N3 LPAIv in chickens, evaluated using either egg production data of the affected flocks (here referred to as the field method) or the transmission experiment, showed that this virus – in relation to other LPAIv (van der Goot et al., 2003; Gonzales et al., 2011, 2012) – was highly transmissible in chickens. In addition, with the field method, we were able to estimate the period of introduction of the virus in each flock. There was a period of two to three weeks between the day of introduction of the virus in Farm-3 and Farm-4. This is in accordance with both the difference in the time at which the lowest mean egg production was observed in each flock and field reports that described these outbreaks (de Wit et al., 2004). Information about the transmission characteristics of a LPAIv and its introduction into a flock is relevant for the implementation, design or evaluation of control measures (backward and forward tracing, surveillance, etc.). Hence, whenever a seropositive flock is detected by surveillance, it would be advisable to obtain – retrospectively – daily egg production data from that flock.

The mean estimates of the transmission rate (β) and the reproduction ratio (R_0) obtained experimentally appear to be higher (because of low statistical power due to the low number of observations in this study, it is not interesting to statistically test this difference) than those obtained with the field method. A similar trend is also observed when comparing the outcomes of two separate studies on transmission of an H7N7 HPAIv: one experimental (van der Goot et al., 2005) and the other based on field data (Bos et al., 2009). Group transmission experiments appear to be better suited to compare treatments, e.g. effect on vaccination on transmission (De Jong and Kimman, 1994), than to provide precise estimates of transmission parameters

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Table 2
Experimental estimates of transmission parameters and basic reproduction ratio of the H7N3 LPAIv[a].

Latent period (days)	Infectious period T[b] (days) (95%CI[d])	Recovery rate γ[c] (day^{-1}) (95%CI)	Transmission rate β (day^{-1}) (95%CI)	Reproduction ratio R_0 (95% CI)[e]
≤1	Contacts: 10.03 (8.50–11.56)	0.10 (0.09–0.12)	0.91 (0.45–1.62)	9.1 (3.6–19.5)
	Inoculated: 13.32 (11.28–15.35)	0.07 (0.06–0.09)		

[a] Low pathogenic avian influenza virus.
[b] The estimated infectious period (T) of contact infected chickens was significantly different from the estimated T of inoculated infected animals.
[c] The recovery rate was estimated from $\gamma = 1/T$.
[d] CI = confidence interval.
[e] The limits of this interval are the 2.5% and 97.5% quantiles of the Monte Carlo procedure. $R_0 = \beta T$, where β was assigned a Lognormal distribution (mean = −0.094, standard deviation = 0.322), and T was assigned a Weibull distribution (shape = 4.616; scale = 10.977). The Weibull parameters are those estimated for T of contact-infected chickens.

(Velthuis et al., 2007). Hence, extrapolation of experimental results, where conditions (age, breed, management, etc.) are different from the field, should be carried out carefully. However, in the absence of field data, experimentally derived estimates offer a useful insight into the transmission of a pathogen. In this study, the experimental results showed that this LPAIv is highly transmissible in chickens, which is in agreement with the results obtained with the field method. In addition, the experimental estimates – from this and other studies (Gonzales et al., 2011, 2012) – provided information for the initial parameter values for the optimization process in the field method.

It has been shown that the level and duration of virus shedding is directly proportional to the inoculation/infectious dose (Stoyanov and Vladimirov, 2008; Chaves et al., 2011). This experiment used the same inoculation dose as that used in other transmission experiments using LPAIv in chickens (van der Goot et al., 2003; Gonzales et al., 2011). However, in contrast to those experiments, the length of the infectious period (T) in the inoculated-infected chickens was longer than that of the contact-infected chickens. We hypothesize that, for the H7N3 LPAIv, this difference is a consequence of a possible difference in the infectious doses received by the inoculated- and contact- infected animals, with the former receiving a higher dose, to a level that resulted in the observed difference in T. However, we expect that the inoculation dose had no significant effect on the infectiousness of the inoculated chickens, and therefore, the estimates of the transmission parameters (Spekreijse et al., 2011).

Both the LPAIv and the characteristics of the infected flock have influence in the transmission dynamics. Hence, variation in the within-flock transmission characteristics of a virus between flocks can be expected (Comin et al., 2011). This variation could be related to different management conditions, breed of the chicken, presence of concomitant diseases, age of production and others. In this study, the main difference between Farm-3 and Farm-4 was observed in the transmission rate (β), which was higher in Farm-3 than in Farm-4. This implies that the virus spread faster in

Table 3
Estimated (optimized) transmission parameters by fitting models to egg production data. Values between brackets are the 95% confidence intervals. If no brackets, then values are fixed.

Parameters[b]	Farm-3		Farm-4	
	SIR[a]	SEIR[a]	SIR	SEIR
β (day^{-1})	0.72 (0.68–0.77)	0.73 (0.69–0.77)	0.50 (0.45–0.55)	0.50 (0.42–0.59)
γ (day^{-1})	0.13 (0.09–0.17)	0.13 (0.10–0.16)	0.11 (0.05–0.16)	0.11 (0.04–0.17)
T (day)[c]	7.69 (5.88–11.11)	7.69 (6.25–10.00)	9.09 (6.25–20.00)	9.09 (5.88–25.00)
L (day)		0.02 (0.01–0.04)		0.03 (0.01–0.04)
pi (%)	47.4 (38.3–56.4)	47.1 (38.6–55.7)	66.4 (60.2–72.5)	66.3 (56.3–76.3)
pr (%)	82.8 (82.3–83.3)	82.8 (82.3–83.3)	76.6	76.6
ps (%)	94.4 (94.0–94.9)	94.4 (94.0–94.9)	85.2	85.2
$I(0)$	8.2×10^{-15} (5.7×10^{-16}–3.9×10^{-14})	7.5×10^{-15} (1.4×10^{-15}–2.5×10^{-14})	5.0×10^{-13} (2.9×10^{-13}–7.9×10^{-13})	4.4×10^{-13} (1.8×10^{-13}–9.2×10^{-13})
Sum of Squares	1.98	1.98	49.17	49.17
AIC[d]	29.13	31.10	105.38	107.38
Compound parameters[e]				
R_0	5.6 (4.3–7.7)	5.6 (4.4–7.8)	4.7 (3.0–8.6)	4.7 (2.2–11.0)
$t(I = 1)$ (day)[f]	54 (49–62)	54 (50–61)	72 (61–89)	72 (58–97)

[a] SIR = susceptible-infectious-recovered. SEIR = susceptible-exposed-infectious-recovered.
[b] Transmission rate β, recovery rate γ, infectious period T, latent period L, egg production of infectious pi, recovered pr and susceptible ps chickens, initial value of I when time is zero $I(0)$, Reproduction ratio R_0.
[c] The infectious period T was calculated as $T = 1/\gamma$.
[d] AIC = Akaike's information criterion.
[e] Confidence intervals for the compound parameters were estimated by Monte Carlo sampling. The limits of these intervals are the 2.5% and 97.5% quantiles.
[f] $t(I = 1)$ denotes the time $I = 1$ meaning the day that the first infectious animal was present in the flock. This was estimated by solving t from the formula describing the growth of an epidemic $I(t) = I(0)e^{(\beta-\gamma)t}$ (Keeling and Rohani, 2008), where $I(t) = 1$ and $I(0)$, β and γ are the above estimated values.

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Farm-3. Unfortunately, some characteristics of the flocks, such as breed, were not provided in the paper by de Wit et al. (2004). A possible explanation for the difference in β could be that Farm-3 also suffered from a nematode infection at the time of the outbreak (de Wit et al., 2004). The latter could have contributed to: (i) increase the susceptibility of the flock, which consequently enhanced transmission, and (ii) induce, together with the viral infection, a lower egg production of infectious chickens (*pi*).

Egg production data before the outbreak and from the last phase of the outbreak define the production parameters for susceptible (*ps*) and recovered (*pr*) chickens, respectively. These parameters are influential in the estimation process of the transmission parameters. For the outbreaks used for this study, only weekly averaged egg production data were available, which resulted in fewer data to be used to optimise *ps* and *pr*. As a result, in the case of Farm-4, for the reason already explained (Section 3.2), we found that better convergence and fit was obtained by estimating these parameters separately from the data and subsequently keeping them fixed. Having had daily data, the estimates would surely have been more robust than our results with weekly data points. We confirmed this with simulated data (results not shown). Therefore, it would be advisable to obtain daily egg production data from the flock, which will provide more information for the optimization process than weekly averages.

Because *pr* is influential for the optimization process, this method is best suited to be applied in situations where the outbreak has already died out. This is likely to be the case, when surveillance programmes are performed with a low frequency (e.g. once a year) (Gonzales et al., 2010). In these circumstances, this method would help to study transmission of LPAIv, that have been circulating in commercial layer flocks. In addition, the estimates of the day of introduction could be used to reconstruct an epidemic to investigate between farm spread. Occasionally, serological surveillance (Elbers et al., 2007) or early detection systems discover on-going outbreaks, e.g. in 2011, two LPAIv outbreaks in layer chickens in The Netherlands (OIE). Detection is most likely to happen at the time of the biggest drop in egg production. In such a situation, with no data available for the estimation of *pr*, the transmission parameters and the time of introduction could be still optimised, by setting an assumed value for *pr*. This assumed value could be obtained from previous outbreaks or reported studies such as this study.

The SIR models appeared to explain the egg production dynamics better than the SEIR models. The estimated latent (exposed) periods in the SEIR models were close to zero. This is in agreement with transmission experiments with other H5 or H7 LPAIv strains (van der Goot et al., 2003; Gonzales et al., 2011, 2012), which showed that all inoculated chickens were already positive for PCR or virus isolation one d.p.i. The results of this study suggest – considering the law of parsimony – that the use of a SIR model would be preferable above a SEIR model for simulating or analysing the within-flock transmission dynamics of LPAIv infections in chickens, since it requires fewer parameters. However, the latent period varies depending of the virus strain or inoculation dose (Spickler et al., 2008; Bouma et al., 2009; Spekreijse et al., 2011), with some studies reporting latent periods longer than one day (Spekreijse et al., 2011). Therefore, the decision to use a SIR or a SEIR model might depend on the virus to be analysed.

Some LPAIv spread slowly within a flock ($\beta < 0.22\ \text{day}^{-1}$; $R_0 < 1.5$) (van der Goot et al., 2003; Gonzales et al., 2012) and the prevalence of infectious chickens at any moment in time would be low (in the peak of the outbreak, the prevalence would be lower than 10%). Therefore, drops in egg production might be unnoticed or confounded with the expected decrease in production due to aging of the flock. By using simulated data, we saw that with $R_0 < 2$, the field method was not able to reproduce the original parameter values consistently (data not shown). Therefore, for low transmitting viruses, the method proposed here would not be applicable, and other methods to study transmission should be applied (Comin et al., 2011). Other than this, there are also other limitations to this method. First, this method performs better with consistent egg production data as is the case in commercial layer flocks unlike hobby/backyard flocks. Secondly, the assumption of homogeneous contact structure limits the application of this method to commercial flocks (free-range and indoors) with a litter (floor reared) housing system.

Outbreaks of LPAI have also been associated with increased mortality, with the peak of mortality around the time of the biggest drop in egg production (de Wit et al., 2004; Beltrán-Alcrudo et al., 2009). We did not include mortality in the model because: (i) the increased number of parameters to be estimated could lead to convergence problems; and (ii) mortality was very low (the peak mortality was below 0.5% per week and mortality before and after these peak was below 0.25% per week) (de Wit et al., 2004), and was therefore assumed not to have a big influence on the population dynamics of the infection and on the estimated transmission parameters.

It should be noted that data from only two flocks were used for analysis, and therefore, this study is a preliminary basis for a proof of principle that transmission parameters of LPAIv infections in layer chicken flocks could be quantified using the egg production data from affected flocks. This opens the opportunity to study the transmission characteristics of different LPAIv affecting chickens with a practical and inexpensive method, provided that these viruses do induce a drop in egg production in the affected commercial flock. This information will be of importance to develop appropriate control measures against LPAI epidemics.

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