Epidemiol. Infect. (2010), **138**, 813–824. © Cambridge University Press 2009
doi:10.1017/S0950268809991038

Evaluation of interventions and vaccination strategies for low pathogenicity avian influenza: spatial and space–time analyses and quantification of the spread of infection

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(*Accepted 23 September 2009; first published online 22 October 2009*)

SUMMARY

In recent years the control of low pathogenicity avian influenza (LPAI) viruses of the H5 and H7 subtypes has increasingly become a concern. We evaluated the measures (stamping out, controlled marketing, emergency and preventive vaccination, farm density reduction and restocking in homogenous areas) implemented to control the LPAI epidemics that occurred in Italy between 2000 and 2005, using a combination of spatial and space–time analyses and estimates of the basic reproduction ratio (R_0). Clustering of infected farms decreased over the years, indicating the effectiveness of the control strategies implemented. Controlled marketing [relative risk (RR) 0·46, 95% confidence interval (CI) 0·27–0·80], emergency (RR 0·47, 95% CI 0·39–0·57) and preventive vaccination (RR 0·19, 95% CI 0·09–0·41) were the most effective measures, yet $R_0 < 1$ was only for preventive vaccination. Our results are useful for identifying the most effective measures for reducing the risk of the spread of LPAI and optimizing the allocation of resources.

Key words: Avian flu, epidemics, infectious disease control, mathematical modelling, veterinary epidemiology.

INTRODUCTION

Strategies for the control of avian influenza (AI) have primarily focused on the eradication of highly pathogenic AI (HPAI) in poultry populations [1]. However, HPAI viruses may emerge by mutation from low pathogenicity AI (LPAI) viruses of the H5 and H7 subtypes circulating in domestic poultry [2, 3], and both LPAI and HPAI viruses can infect humans [4–6], which stresses the need for the monitoring and control of LPAI viruses in poultry.

Experiences in the control and eradication of LPAI infection in poultry have demonstrated the importance of implementing a combination of measures, potentially including vaccination, especially in areas with high densities of turkey farms, given that turkeys are a particularly susceptible species [7–9]. Since there is no single, universal approach to AI control, different prevention and control policies could be adopted depending on the specific eco-epidemiological situation and the characteristics of the poultry industry at risk [10]. However, limited epidemiological data exist on how to define LPAI control strategies or on what set

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of measures need to be implemented when confronting a LPAI epidemic. The identification of a set of measures that are sustainable in the long term is of great importance, especially when the risk of introduction and spread of AI viruses persists.

To evaluate the effectiveness of measures for controlling HPAI epidemics, both spatial analyses and estimates of the basic reproduction ratio (R_0) have been adopted [1, 11, 12]. Spatial analyses have been used to generate hypotheses regarding AI risk factors and to identify areas at higher risk of infection, through the detection of spatial and space–time clusters. The R_0 estimate relies on a threshold that allows determination of whether an infection will be cleared from a population ($R_0 < 1$) or will generate an outbreak ($R_0 > 1$), and it has been used to identify the most effective control measures and extent of their application [13].

In Italy, between 2000 and 2005, four epidemics of LPAI due to H5 or H7 subytpes occurred on poultry farms in a densely populated poultry area (DPPA). To control these outbreaks, a series of measures were implemented, including emergency and preventive vaccination [8, 14]. The objective of the current study was to evaluate the effectiveness of these measures using a combination of spatial and space analyses and R_0 estimates.

METHODS

Study area and population at risk

The study area included the municipalities in which an outbreak had occurred and the neighbouring municipalities. All four epidemics occurred in the adjoining Regions of Veneto and Lombardy (north-eastern Italy) and involved mainly meat turkey farms. The beginning and the end of each epidemic were defined, respectively, by the date of detection of the first and the last infected farm (IF). The population at risk for each epidemic was defined as all of the industrial poultry farms located in the study area with an ongoing production cycle during the epidemic. Backyard flocks were not included because the available information was not reliable.

Data sources

For all of the poultry farms included in the study, information on spatial coordinates, size (number of birds per production cycle), species raised and type of production was obtained from the regional databases of the industrial poultry farms of the Veneto and Lombardy regions. The results of monitoring activities in vaccinated and unvaccinated poultry farms and confirmation of AI infection in each IF were obtained from the Italian Reference Laboratory for AI.

Control measures

For all four epidemics, the following interventions were carried out by the Regional Veterinary Services in the areas involved in the epidemics: monitoring of flocks at risk of infection, stamping out or controlled marketing of all birds on IFs, ban or controlled restocking and movement restrictions for live birds, vehicles and staff [15, 16].

Other interventions were specific to each epidemic. In particular, once the second epidemic in 2002–2003 had ended, in order to reduce poultry density, the Veneto Region placed two consecutive temporary bans on the restocking of a number of meat turkey farms in the DPPAs involved. The first ban involved 137 farms and lasted from October 2003 until April 2004; the second ban involved 45 farms and lasted from April 2004 to October 2004.

In the same DPPAs, for the meat turkey farms that had remained fully operational, starting in October 2003 restocking was managed by applying 'homogenous areas', which were generated according to farm density and geographical criteria [17]. In each of these areas, restocking and slaughtering were performed by applying the 'all-in-all-out' system at the area level, and the 'all-in-all-out' was obtained by performing restocking and slaughtering at all the farms in that area within a limited time-frame (20 days). To date, controlled restocking is still in place and involves 384 meat turkey farms in 88 homogeneous areas.

Emergency vaccination was performed in the first and second epidemics only, in particular, from November 2000 to September 2001 for the first epidemic and from December 2002 to October 2004 for the second epidemic. Preventive vaccination was implemented from October 2004 to December 2006. Both emergency and preventive vaccination were carried out using inactivated vaccines and only for long-living poultry species and production types (mainly meat turkeys and layers), which were considered to be at higher risk of LPAI infection. Species-specific vaccination protocols were applied, and the

vaccine strains were selected based on the circulating AI strain and the DIVA strategy (Differentiating Infected from Vaccinated Animals) [8, 18]. The emergency vaccination was based on the use of a monovalent H7 heterologous inactivate strain, whereas the preventive vaccination involved the use of a bivalent H5/H7 vaccine.

Spatial and space–time analyses

For each of the epidemics, the spatial and space–time clustering of IFs were determined in relation to the control measures implemented. A homogeneous Poisson point process was assumed, considering a constant risk throughout each study area. For each epidemic, a complete dataset which included cases and population at risk was produced, and the analysis was repeated for each dataset.

K function [19, 20] was used to test both the clustering of farms in general and the clustering of IFs. This function measures the number of events that occur within increasing radiuses. A simple formula for estimating the *K* function is: $K(d)$ = (average number of events within distance d of a randomly chosen event)/(average number of events per unit area).

In evaluating clustering, the maximum search radius was considered to be equal to half of the maximum Euclidean distance between farms. The estimated distributions of $K(d)$ were then compared to the expected distributions, previously linearized to $L(d)$, which, under the null hypothesis, is equal to d [21]. Clustering was defined if the observed $L(d)$ exceeded the expected $L(d)$. The excess of clustering for IFs in respect of the farms at risk was calculated using the formula:

$$\Delta L(d) = L(d)_{\text{out}} - L(d)_{\text{pop}},$$

where $L(d)_{\text{out}}$ and $L(d)_{\text{pop}}$ are, respectively, the linearized K for the IFs and the at-risk farms.

The local clustering of IFs was analysed using the spatial scan statistic [22]. The statistical significance of the clusters was established using Monte Carlo hypothesis testing [23], assuming that the IFs were randomly distributed across the at-risk farms. Purely spatial analysis was performed using the Poisson probability model [22]. The size of the scanning window in the spatial scan statistic was set to include up to 10% of the total population.

The local space–time cluster analysis was performed using a space–time permutation scan statistic [24]. Only IFs were considered, adjusting for any purely spatial or purely temporal clusters and looking for clusters due to the interaction of space and time. The spatial window of search was set as described above, whereas the maximum temporal window was set at up to 15 days.

Univariate and multivariate analyses

Data were fitted into a SID (Susceptible, Infectious, Depopulated) format, and a database was created containing the total number of farms in the study area in each class (described below) per week (week record) [25, 26]. The farms were assigned to a specific class using the following criteria: farms that were still operational and negative to AI testing were defined as 'susceptible' (S); once the LPAI virus entered a farm, the farm was considered to be infected (but not yet infectious) and defined as a 'case' (C) and subsequently as 'infectious' (I). Farms became either 'removed' (R), if the end of the production cycle was reached without infection being diagnosed, or 'depopulated' (D), if LPAI infection was diagnosed. Based on the frequency of monitoring, a farm was considered to be a case (C) starting 3 weeks before a positive test result, and it was considered to be infectious (I) starting 2 weeks before a positive result and until being depopulated (D). The response variable C was assumed to have a Poisson distribution, with $\log[S(t)^*I(t)/N(t)]$ ($N = S + I + C$) as offset variable and a log-link function. All farms were assumed to be equally susceptible; infectivity was assumed to be homogeneous in infectious farms and over time, and all infectious farms were assumed to constitute an independent risk to susceptible farms.

Each control measure was represented by a dummy variable that was assigned a value of 1 if it was in force for $\geqslant 4$ days of the week and 0 if in force for < 4 days or not implemented at all. The control measures were coded as: stamping out = 1; controlled marketing = 2; vaccination = 3 (3a = emergency; 3b = preventive); reduction of density through ban on restocking = 4; and homogenous areas = 5. Because movement restrictions and the ban on restocking were implemented for all four epidemics, it was not possible to comparatively estimate their effect on the spread of AI.

The LPAI epidemics were divided into periods of time based on the dates that the control measures started and ended. Univariate and multivariate analyses were performed to evaluate the development of the

Table 1. *R_0 estimates in the univariate model by periods and combinations of control measures for each epidemic*

Epidemic	Period	Period code (duration in weeks)*	Control measures†	Duration of control measure enforcement (in weeks)	Infectious farms	*P* value	β	R_0
1 (H7N1)	14 Aug. 2000–20 Mar. 2001							
	24 July 2000–31 Aug. 2000	1 (7)	—	5	32	Ref.‡	0·4	2·15
	1 Sep. 2000–14 Nov. 2000	2 (10)	1, 2	10	16	<0·0001	0·1	0·53
	15 Nov. 2000–12 Feb. 2001	3 (13)	1, 2, 3a	13	21	0·002	0·2	0·9
	13 Feb. 2001–26 Mar. 2001	4 (6)	1, 3a	6	2	0·46	0·3	1·25
2 (phase A, H7N3)	20 June 2002–12 Aug. 2002							
	30 May 2002–12 Aug. 2002	5 (11)	—	4	1	0·18	0·1	0·56
2 (phase B, H7N3)	10 Oct. 2002–29 Sep. 2003							
	19 Sep. 2002–16 Oct. 2002	6 (4)	—	3	9	0·91	0·4	2·06
	17 Oct. 2002–25 Oct. 2002	7 (1)	1	1	8	0·45	0·6	2·9
	26 Oct. 2002–9 Dec. 2002	8 (7)	1, 2	7	159	0·4	0·4	1·83
	10 Dec. 2002–29 Sep. 2003	9 (42)	1, 2, 3a	42	188	<0·0001	0·1	0·67
3 (H7N3)	15 Sep. 2004–10 Dec. 2004							
	25 Aug. 2004–11 Oct. 2004	10 (7)	3b, 4, 5	6	19	0·03	0·2	1·14
	12 Oct. 2004–26 Oct. 2004	11 (2)	2, 3b, 4, 5	2	0	1	0	0
	27 Oct. 2004–09 Dec. 2004	12 (6)	2, 3b, 5	6	2	0·005	0·1	0·28
4 (H5N2)	11 Apr. 2005–11 May 2005							
	21 Mar. 2005–25 Apr. 2005	13 (5)	3b	4	5	0·06	0·2	0·88
	26 Apr. 2005–15 May 2005	14 (3)	1, 3b	3	0	1	0	0

* Period code refers to the categorical variable included in the model.
† See description in Materials and Methods for references on the control measures.
‡ Ref. defines the reference period for the analysis.

epidemics over time and to quantify the effect of specific control measures. The relative risk (RR) and the R_0 were calculated, respectively, for the different control measures and periods. Because there were no control measures implemented in period 1 (Table 1), this was considered as the reference period for the univariate analysis.

Since control measures were rarely implemented individually, a multivariate model was used to analyse the effect of a specific measure, taking into account other simultaneous measures. The transmission rate parameter β was estimated from the intercept and the estimates for the covariables (as $e^{\text{intercept}+\text{estimate}}$), and the R_0 followed from the product of β and the estimated mean infectious period. The mean infectious period was the difference in days between diagnosis and depopulation, plus 2 weeks. This 2-week extension was based upon the average time between two consecutive tests, which was 4 weeks, and the hypothesis that the farm became infected in the middle of this period. The effect of a single measure is expressed as the RR, considering implementation *vs.* non-implementation, with the RR estimated as e^{estimate}.

RESULTS

2000–2001 H7N1 LPAI epidemic

The epidemic occurred between 14 August 2000 and 20 March 2001 and involved 73 meat turkey farms, one layer farm and six quail farms.The overall spatial distribution showed an excess clustering of cases compared to the expected value, with peak clustering within a 0–16 km radius. A second peak was observed in the 32–38 km radius, although it was not significant (Fig. 1*a*).

At the beginning of the epidemic (period 1), the R_0 was 2·15. After the implementation of control interventions, R_0 decreased to <1 ($R_0=0{\cdot}53$ in period 2 and 0·9 in period 3). In period 4, the infection involved the provinces of Padua and Vicenza, which were located outside the vaccination area, and $R_0=1{\cdot}25$.

The local spatial statistic revealed two clusters with $P<0{\cdot}005$ (Fig. 2*a*), one in the southern part of Verona Province (radius 5170 m, 37 cases out of a total of 76 farms) and the other in Padua Province (radius 2145 m; 13 cases out of a total of 18 farms). The space–time cluster analysis identified three

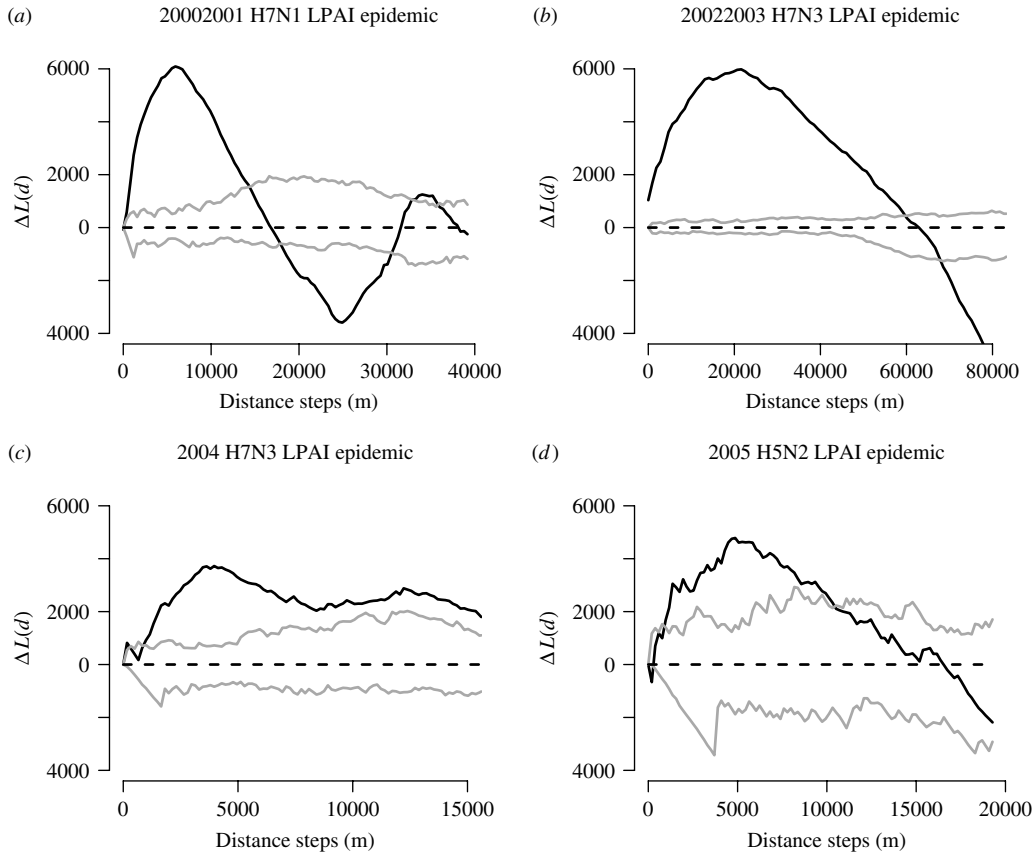

Fig. 1. Global spatial analysis: excess of clustering [$\Delta L(d)$] of infected farms compared to the poultry population at risk. (*a*) 2000–2001 H7N1 LPAI epidemic; (*b*) 2002–2003 H7N3 LPAI epidemic; (*c*) 2004 H7N3 LPAI epidemic; (*d*) 2005 H5N2 LPAI epidemic. ———, $\Delta L(d)$; - - -, Expected $\Delta L(d)$; ——, 95% confidence interval.

clusters: the first occurred from 14 to 23 August 2000 and included five IFs within a radius of 705 m ($P=0{\cdot}01$); the second occurred from 8 to 22 October 2000 and included five IFs within a radius of 4593 m ($P=0{\cdot}005$); the third cluster occurred from 6 to 20 January 2001 and included seven IFs, within a radius of 2634 m ($P=0{\cdot}002$) (Fig. 3*a*). The second and third space–time clusters partially overlapped the two purely spatial clusters.

2002–2003 H7N3 LPAI epidemic

From 20 June 2002 to 12 August 2002, five IFs were identified in Lombardy. From 12 August 2002 to 9 October 2002, no cases occurred. Then, from 10 October 2002 to 29 September 2003, another 388 IFs (86% meat turkey farms, 88 of which were vaccinated) were identified in the same area and in an adjoining area in Veneto. Because of the interval in the occurrence of infections, this epidemic was divided into two phases (referred to as 'epidemic 2A' and 'epidemic 2B', Table 1). Emergency AI vaccination was begun in December 2002; a total of 83 million doses were delivered, and beginning in March 2003 ~90% of the meat turkey farms and 78% of the layer farms were vaccinated.

The IFs showed greater clustering than the poultry farms in general, with an excess in the 0–60 km radius and a peak at about 20 km. Over 60 km, the IFs were over-dispersed compared to the distribution of the population at risk (Fig. 1*b*).

In epidemic 2B, R_0 was >1 when only stamping out and controlled marketing were in force (Table 1, epidemic 2B, periods 6, 7, and 8, $R_0=2{\cdot}06$, $2{\cdot}90$ and $1{\cdot}83$, respectively), whereas the R_0 decreased to <1 following emergency vaccination (Table 1, epidemic 2B, period 9, $R_0=0{\cdot}67$).

The local spatial statistic detected three clusters; the first had a radius of 4505 m and included 23 IFs out of a total of 35 poultry farms; the second had a radius of 7419 m and included 69 IFs out of a total of 130 farms; and the third had a radius of 10 756 m and included 84 IFs out of a total of 194 farms (Fig. 2*b*). The local space–time analysis identified nine clusters

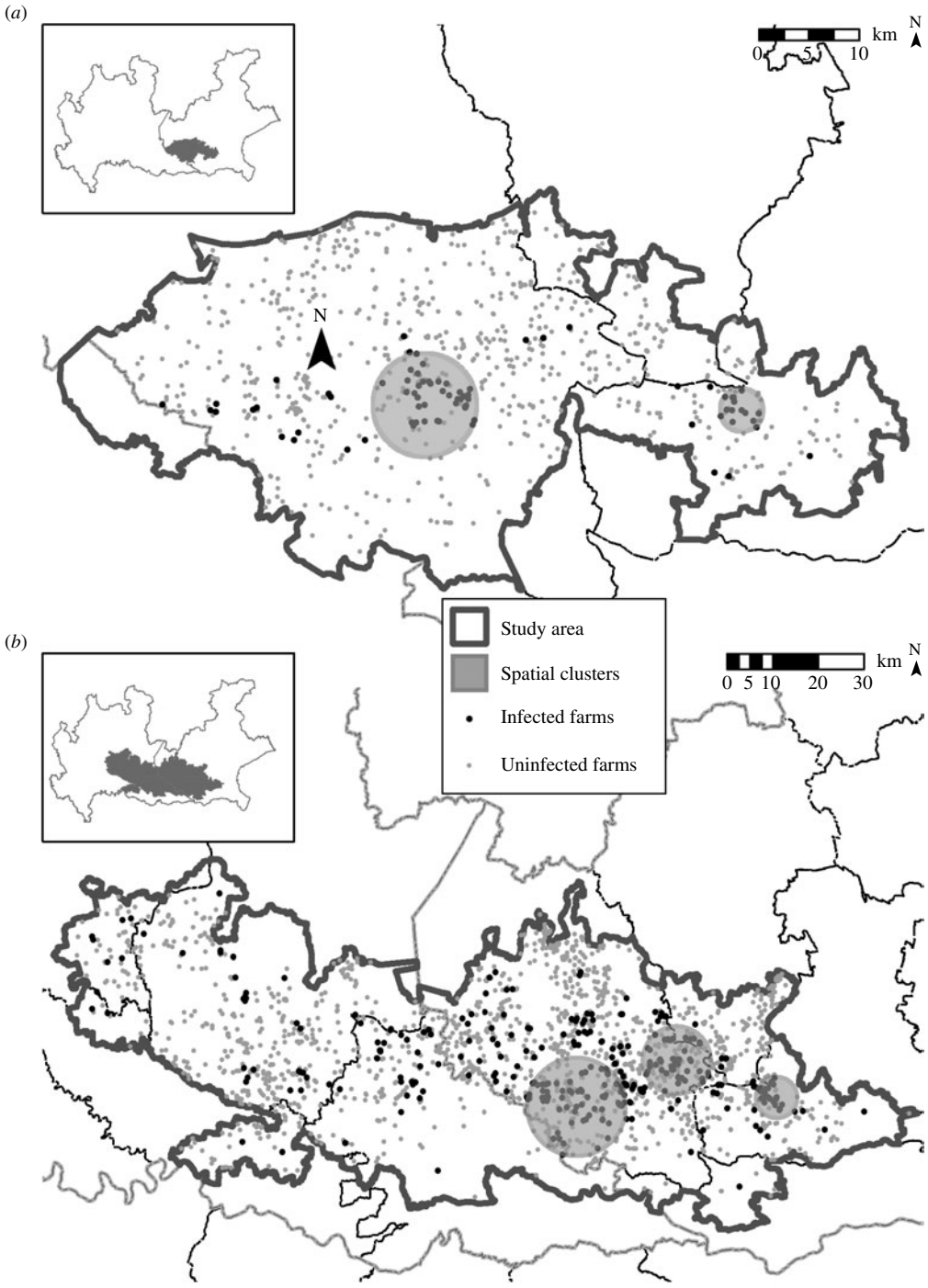

Fig. 2. Local level purely spatial clusters of infected farms in the study area. (*a*) 2000–2001 H7N1 LPAI epidemic (76 infected farms). (*b*) 2002–2003 H7N3 LPAI epidemic (375 infected farms).

between 1290 m and 12 339 m (Fig. 3*b*, Table 2), some of which overlapped the purely spatial clusters.

2004 H7N3 LPAI epidemic

On 16 September 2004, the LPAI H7N3 virus re-emerged in meat turkey flocks in Verona Province (Veneto Region). The epidemic lasted until 10 December 2004 and involved 28 farms (27 meat turkey farms and one quail farm).

The epidemic occurred in a vaccinated poultry subpopulation, given that the 2002–2003 emergency vaccination campaign was in force until October 2004 and was continued with a preventive vaccination plan

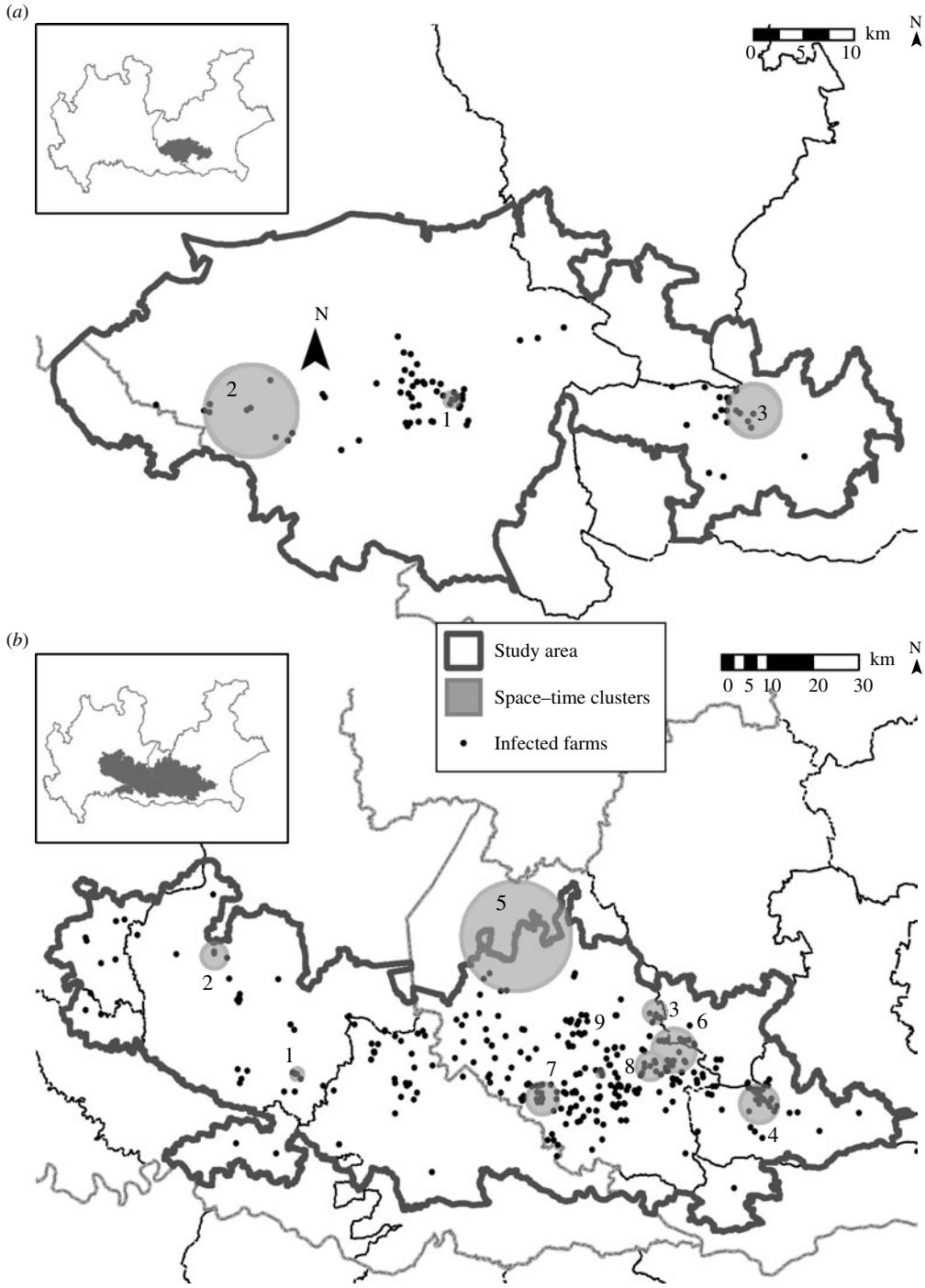

Fig. 3. Space–time clusters of infected farms in the study area. (*a*) 2000–2001 H7N1 LPAI epidemic (76 infected farms). (*b*) 2002–2003 H7N3 LPAI epidemic (375 infected farms).

[27]. Restocking by homogenous areas was also in place.

An excess of clustering of IFs compared to the total poultry-farm population was observed in the 0–15 km radius, with a peak at about 4 km (Fig. 1*c*). When the epidemic started, R_0 was slightly above 1 ($R_0 = 1{\cdot}14$); after the implementation of controlled marketing and booster vaccination (period 12, Table 1), R_0 decreased to 0·28.

At the local level, the spatial scan statistic identified two clusters ($P < 0{\cdot}01$). The first cluster had a radius of 5079 m and included 14 IFs, out of a total of 28 farms. The second cluster had a radius of 3594 m and included 10 IFs, out a total of 20 farms. Only four IFs

Table 2. *Significant space-time clusters ($P<0{\cdot}05$) detected in the 2002–2003 H7N3 LPAI epidemic*

Cluster	Radius (m)	Involved farms	First case	Last case	*P* value
1	1290	3	30 July 2002	13 Aug. 2002	0·015
2	2781	4	28 Sep. 2002	12 Oct. 2002	0·001
3	2412	7	12 Nov. 2002	26 Nov. 2002	0·002
4	4143	16	27 Dec. 2002	10 Jan. 2003	0·001
5	12 010	3	27 Mar. 2003	10 Apr. 2003	0·008
6	4750	18	26 May 2003	9 June 2003	0·001
7	3419	12	26 May 2003	9 June 2003	0·001
8	3008	7	10 June 2003	24 June 2004	0·001
9	700	3	24 Aug. 2003	7 Sep. 2003	0·009

were excluded from the clusters. No evidence of space–time interaction was detected at the local level.

2005 H5N2 LPAI epidemic

On 15 April 2005, an LPAI virus of the H5N2 subtype was detected in four meat turkey farms in Lombardy. Up to 15 May 2005, a total of 15 IFs were detected. Of these, 13 had been preventively vaccinated, whereas the other two were located outside the vaccination area. The only measures in place at the onset of the epidemic were preventive vaccination and monitoring. Additional measures were promptly enforced to eradicate the infection: restricted restocking and movement of live poultry, rapid depopulation of affected premises, booster vaccination and reduction of turkey densities in Veneto (an area bordering the affected area).

IFs were clustered within a radius of 14 km and the clustering peaked at about 5 km (Fig. 1*d*). During the entire epidemic R_0 remained below 1 ($R_0=0{\cdot}88$) and the infection died out quickly (Table 1, periods 13 and 14). The only cluster detected by spatial scan statistic ($P<0{\cdot}02$) had a radius of 3184 m and included eight IFs, out of a total of 20 farms. No space–time aggregation was identified during the epidemic.

Univariate and multivariate models

The results of the univariate models are shown in Table 3. Those measures that significantly reduced the risk of the spread of AI were: stamping out ($P=0{\cdot}05$), controlled marketing ($P<0{\cdot}0001$) and vaccination ($P<0{\cdot}0001$). The greatest reduction in the disease spread was obtained with vaccination (RR 0·45 for emergency vaccination and 0·25 for preventive vaccination). Preventive vaccination led to the smallest R_0 (0·39).

Controlled marketing and vaccination significantly contributed to the model ($P<0{\cdot}003$) (Table 4). When corrected for the other measures applied at the same time, controlled marketing and preventive vaccination reduced R_0 to below 0·9, with an estimated RR of 0·46 for controlled marketing, 0·47 for emergency vaccination, and 0·19 for preventive vaccination.

DISCUSSION

In the current study, the effects of different LPAI control measures and vaccination strategies were evaluated using spatial and space–time analyses and by estimating R_0. The performance of one of these methods alone would not have been as informative, particularly for understanding the local dynamics of the spread of infection and estimating the effectiveness of the diverse control strategies.

Regarding the overall spatial analyses, the excess of clustering of IFs, compared to the farms at risk, within relatively short distances suggests that the risk of infection may be related to the distance between farms. In the Italian and Dutch HPAI epidemics of 1999–2000 and 2003, respectively, local transmission played an important role [1, 11, 28]. However, our results on the extent of clustering in the 2002–2003 LPAI epidemic (within 60 km, with a peak at 20 km) could be a consequence of a medium and long-distance spread of the infection due to the transmission of AI viruses through the contact structure of the poultry production sector (i.e. direct or indirect contact with infected birds via live poultry, staff, vehicles, equipment or contaminated materials) [1, 29].

Table 3. *Relative risks and R_0 estimates for each control measure using the univariate models*

Parameter	Estimate*	*P* value	RR (95% CI)	β	R_0
Intercept	−1·44	<0·0001			
Stamping out	−0·26	0·05	0·77 (0·60–1·00)	0·18	0·92
No stamping out	Ref.			0·24	1·19
Intercept	−1·16	<0·0001			
Marketing	−0·57	<0·0001	0·57 (0·44–0·72)	0·18	0·88
No marketing	Ref.			0·31	1·56
Intercept	−1·14	<0·0001			
Vaccination	−0·84	<0·0001	0·43 (0·36–0·52)	0·14	0·69
No vaccination	Ref.			0·32	1·60
Intercept	−1·14	<0·0001			
Emergency vaccination	−0·80	<0·0001	0·45 (0·37–0·54)	0·14	0·71
Preventive vaccination	−1·40	<0·0001	0·25 (0·13–0·45)	0·08	0·39
No vaccination	Ref.			0·32	1·60
Intercept	−1·64	<0·0001			
Homogenous areas	−0·34	0·18	0·71 (0·46–1·10)	0·14	0·69
No homogenous areas†	Ref.			0·19	0·97
Intercept	−1·65	<0·0001			
Density reduction	−0·16	0·50	0·86 (0·54–1·35)	0·16	0·82
No density reduction	Ref.			0·19	0·96

RR, Relative risk; CI, confidence interval.
* Ref. defines the reference category in each model.
† Not in epidemic 4; not implemented in that area.

Table 4. *Relative risks and R_0 estimates for each control measure using the multivariate model*

Parameter	Estimate*	*P* value	RR (95% CI)	β	R_0
Intercept	−0·86	<0·0001			
Stamping out	0·40	0·20	1·49 (0·81–2·76)	0·63	3·17
No stamping out	Ref.				
Marketing	−0·77	0·006	0·46 (0·27–0·80)	0·20	0·98
No marketing	Ref.				
Emergency vaccination	−0·75	<0·0001	0·47 (0·39–0·57)	0·20	1·00
Preventive vaccination	−1·66	<0·0001	0·19 (0·09–0·41)	0·08	0·40
No vaccination	Ref.				
Density reduction	−0·03	0·97	0·97 (0·19–4·85)	0·41	2·05
No density reduction	Ref.				
Homogenous areas†	0·39	0·65	1·47 (0·28–7·76)	0·63	3·13
No homogenous areas	Ref.				

RR, Relative risk; CI, confidence interval.
* Ref. defines the reference category in each model.
† Not in epidemic 4; not implemented in that area.

The spatial and space–time analyses at the local level revealed the role of local factors in the evolution of the four epidemics. Clustering of IFs differed in the epidemics; in particular, space–time clustering was only observed for the first two epidemics (2000–2001 and 2002–2003), and the small clusters tended to disappear in 2004 and 2005. This reduction in the local spread of infection probably reflects the positive effect of the control measures. However, the assumptions made about the parameters used for the cluster analysis (i.e. length of the temporal scan windows and the date of virus introduction) could have affected the temporal clustering of IFs and led to these clusters being inaccurately identified. However, the testing of

a scan window up to 30 days did not produce significant differences in the clustering of IFs. Since the true dates of virus introduction were unknown and any estimate of the date of infection would have been to some extent speculative, no further testing was carried out.

The local spatial analysis showed that areas in the southern part of the Province of Verona were more frequently involved in the epidemics. These areas are characterized by a high density of farms that raise turkeys, which have been shown to be more susceptible to LPAI infection than other poultry species [30].

Of the control measures evaluated, controlled marketing and vaccination were the most effective in reducing the risk of infection. In areas with a high poultry density, the prompt depopulation of IFs and the consequent elimination of a huge number of recently infected birds could have contributed to the dispersion of infectious materials via different routes (e.g. organic debris, including feathers, dust, and faecal material) and the consequent transmission of the virus to neighbouring farms [15, 16]. However, the likelihood of the dispersion of infected materials would have been reduced by controlled marketing, which was based on strict quarantine and intensive monitoring of the evolution of infection at the flock level, to determine whether poultry from IFs could be moved to the slaughterhouse after the sharp reduction in the level of virus excretion, which occurs in meat turkeys 3–4 weeks after infection [31].

In Italy, emergency and preventive vaccinations have proven to be effective in controlling LPAI infection [7, 10]. In our study, the reduction in R_0 and RR was greater following preventive vaccination, compared to emergency vaccination, which was probably due to the high overall vaccination coverage at the beginning of the epidemic. In fact, emergency vaccination in 2000–2001 and 2002–2003 was implemented when the epidemics were still in progress and most infections had already occurred. Nonetheless, when combined with other control measures, emergency vaccination was able to reduce the R_0 to <1 and may have contributed to eradicating the disease.

The reduction in poultry density and the restocking of farms in homogenous areas, when considered alone, did not have significant effects on infection control (Table 4). However, when combined with other control measures such as vaccination, the effectiveness increased (Table 1, epidemic 3, periods 10–12). In fact, these two measures are not designed to directly lower the risk of infection at the farm level or in the area where the epidemic was still in progress; instead, they are meant to reduce contacts between farms in areas where the virus has been introduced, decreasing the risk of the massive spread of infection.

Since some of the control measures specified in EU legislation were enforced for all four epidemics (i.e. ban on restocking and movement restrictions) [27], it was not possible to analyse their impact on infection control. Furthermore, such measures might have interacted with those included in the models, contributing to lowering the risk of spread.

At certain points during the epidemics, the R_0 and the results of the space–time analyses seemed to be contradictory. In particular, during the 2002–2003 epidemic, newly infected IFs showed tight spatial clustering (Fig. 3), whereas the total number of IFs was decreasing ($R_0 < 1$). Neighbourhood spread of infection was probably one of the factors that contributed to the maintenance of infection in the vaccination area, allowing the epidemic to last for about 1 year. Although LPAI infection was never observed in vaccinated layers, the detection of LPAI outbreaks in vaccinated meat turkey farms could be related to several factors. In particular, the occurrence of immunosuppressive infections (e.g. haemorrhagic enteritis) and the adoption of certain management practices (e.g. inappropriate vaccine administration) may have contributed to impairing the immune response in vaccinated turkeys [32]. Vaccination cannot be considered as a long-term control measure because of the required resources and financial burden. Thus other measures for controlling LPAI epidemics in DPPAs, which could also be combined with vaccination, need to be identified.

A limitation of the study could be considered to be the exclusion of backyard flocks from the analyses. However, their role in the HPAI epidemics of 1999–2000 in Italy and 2003 in The Netherlands was considered almost negligible. Indeed, the limited number of outbreaks that occurred in backyards in these epidemics strongly suggested a clear separation between them and the industrial poultry farms, that were heavily affected [33, 34]. Reliable data on AI in backyard flocks in the EU have only been available from 2007, showing a very low prevalence of H5/H7 infection ($<0{\cdot}5\%$) [35]. On the other hand, backyard farms could be of greater concern in developing countries, where the control of AI in poultry raised with low biosecurity and poor disease prevention

measures is a difficult issue, increasing the risk of human exposure to AI viruses.

The results of the current study could help decision makers to develop strategies tailored to the specific field situation, allowing for the better use of available resources. Such strategies would have to be coupled with continuous monitoring, high biosecurity, movement control and emergency preparedness, to provide effective protection from the risk of the spread of AI.

ACKNOWLEDGEMENTS

This work was supported by grant FP6-513737 (Development of new integrated strategies for prevention, control and monitoring of epizootic poultry diseases – healthy poultry) from the EU Commission and by a grant from the Italian Ministry of Health.

DECLARATION OF INTEREST

None.

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