Role of Backyard Flocks in Transmission Dynamics of Highly Pathogenic Avian Influenza A(H5N8) Clade 2.3.4.4, France, 2016–2017

Marie Souvestre, Claire Guinat, Eric Niqueux, Luc Robertet, Guillaume Croville, Mathilde Paul, Audrey Schmitz, Anne Bronner, Nicolas Eterradossi, Jean-Luc Guérin

Highly pathogenic avian influenza A(H5N8) clade 2.3.4.4 spread in France during 2016–2017. We assessed the biosecurity and avian influenza virus infection status of 70 backyard flocks near H5N8-infected commercial farms. One flock was seropositive for clade 2.3.4.4. Backyard flocks linked to commercial farms had elevated risk for H5 infection.

In the past 2 years, major outbreaks of highly pathogenic avian influenza (HPAI) occurred in Europe, resulting in severe socioeconomic effects on the poultry industry (*1*,*2*). During November 28, 2016–March 23, 2017, a total 484 HPAI poultry outbreaks associated with influenza A(H5N8) clade 2.3.4.4 viruses of Eurasia A/goose/Guangdong/1/1996 lineage were reported in France (*2*). Virus introduction into the index farm probably was associated with wild birds; however, other transmission pathways for virus spread between farms have been considered, including trade-related movements and spatial proximity (*2*). Although most outbreaks occurred in commercial flocks (n = 464), outbreaks in ≈20 backyard flocks also were reported (*2*). Backyard flocks are generally assumed to be at risk for avian influenza virus (AIV) introduction from wildlife and from nearby commercial poultry flocks during influenza outbreaks (*3*,*4*). Because little is known about the prevalence of AIV in backyard flocks contiguous to commercial farms, we aimed to quantify the seroprevalence of AIV and H5 subtype and to identify risk factors for infection in backyard flocks near commercial farms affected by HPAI H5N8 during the 2016–2017 epidemic.

Author affiliations: Ecole Nationale Vétérinaire, Institut National de la Recherche Agronomique, Université de Toulouse, Toulouse, France (M. Souvestre, C. Guinat, L. Robertet, G. Croville, M. Paul, J.-L. Guérin); Agence Nationale de Sécurité Sanitaire, Ploufragan, France (E. Niqueux, A. Schmitz, N. Eterradossi); Direction Générale de l'Alimentation, Paris, France (A. Bronner)

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The Study

We conducted our study in Gers Department (1 of the 101 administrative units in France). Gers accounted for 19.8% (96/484) of the HPAI H5N8 outbreaks reported during the epidemic; 55.2% (53/96) of the Gers outbreaks were spatiotemporally clustered during December 11, 2016–January 4, 2017 (*2*). Our study targeted backyard flocks that were located within a 1-km radius from HPAI H5N8 outbreaks reported on commercial farms in Gers (n = 169) (Figure). At the time of our study, no backyard flock in Gers had been reported as HPAI infected.

Using a 28-question form, we conducted face-to-face interviews with each backyard flock owner during March 31–May 10, 2017. The 28 closed or semiclosed questions concerned the species of poultry, biosecurity practices, contacts with other flocks, and health status of the birds. We explained the purpose and methods of the study to all participants, who gave their consent to participate.

We sampled all backyard flocks up to a limit of 10 birds >6 months of age, which ensured that all sampled birds had been exposed to the HPAI outbreaks. Because flock size was as high as 60 birds (median 14 birds), detection thresholds ranged from 20% to 30% with a 95% CI. Not all flock owners consented to or were available for the study; in all, we were able to include 70 of the 169 backyard holdings.

We collected blood samples, tracheal swabs, and cloacal swabs. Blood was stored at 4°C after shipment, then serum was extracted and stored at –20°C. Tracheal and cloacal swabs were stored at –80°C until analysis. We performed serologic testing for AIV by using ELISA (IDVet ID Screen Influenza A Antibody Competition Multi-Species kit, http://www.id-vet.com). We considered a backyard flock as seropositive if ≥1 bird was found to be positive. We then tested AIV-seropositive backyard flocks for H5 antibodies by using the same IDVet ELISA kit, and we used hemagglutination inhibition tests to detect clade 2.3.4.4 H5 or other H5 Eurasian viruses (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/25/3/18-1040-App1.pdf). Finally, we individually tested all birds from seropositive backyard flocks for AIV gene M and subtype H5 by using reverse transcription PCR (*5*,*6*). We performed descriptive statistics to assess how seroprevalences

of AIV and H5 subtype were affected by flock owners' practices (Appendix).

Estimated overall flock-level seroprevalence was 25.7% (95% CI 16.9%–37.0%) for AIV and 11.4% (95% CI 5.9%–21.0%) for H5 (Table 1). Estimated overall bird-level seroprevalence was 5.9% (95% CI 4.3%–8.1%) for AIV and 3.3% (95% CI 2.1%–5.0%) for H5. All birds tested were PCR-negative for gene M and H5.

Among H5 ELISA-seropositive birds, only 3 belonging to the same flock showed positive hemagglutination inhibition titers against a clade 2.3.4.4 HPAI H5N8 antigen, and we could not confirm detection of clade 2.3.4.4–specific H5 antibodies with a second clade 2.3.4.4 H5N5 antigen in 1 of these birds. This backyard flock included chickens and ducks and was not adjacent to a commercial farm, and the owner reported working in a poultry meat processing plant.

Other H5 ELISA-positive birds were mainly seropositive for a couple of antigens from other H5 Eurasia lineages instead of clade 2.3.4.4 H5 HPAI virus. We could not distinguish between antibodies targeting low-pathogenicity or HPAI H5Nx viruses that spread in the region during 2015–2016 (*1*) (Appendix Table 1). This finding suggests that backyard flocks might have played a limited role in HPAI H5N8 transmission between farms during the 2016–2017 epidemic. Seroprevalence was higher in ducks than in chickens for AIV (13.1% [95% CI 8.2%–20.2%] vs. 4.1% [95% CI 2.7%–6.3%]) and H5 (9.0% [95% CI 5.1%–15.4%] vs. 1.9% [95% CI 1.0%–3.5%).

Backyard flocks that included ducks were more likely to be AIV-positive (odds ratio [OR] 2.3, 95% CI 1.1–5.1) and H5-positive (OR 5.7, 95% CI 1.6–30.6) than those having only chickens. These results are consistent with several studies emphasizing the role of ducks on AIV shedding and transmission (*1*). Specific attention was paid to flocks having ducks in the sampling design in the field because duck species could be considered as an additional risk factor (*1*). Thus, our study might overestimate the overall seroprevalence at the backyard flock and bird levels. Backyard flocks that had no fencing outdoors or had no covered food distribution area could be considered at higher risk for exposure to wild birds. However, these risk factors were not statistically associated with increased AIV or H5 seroprevalence (Appendix Table 2).

Backyard flocks located on or in close proximity to a commercial poultry farm were significantly more likely to be AIV-positive (OR 6.0, 95% CI 1.5–24.5) and H5-positive (OR 20.5, 95% CI 3.2–215.8). To date, proximity of commercial units to backyard flocks has not been considered as a risk factor, despite airborne transmission being suspected to spread disease (*7*,*8*). On the basis of the influenza A(H7N7) epidemic in the Netherlands, researchers constructed a model that assumed that infected backyard flocks were an example of spillover from commercial farms and that backyard flocks played no part in transmission (*9*). Our results highlight the importance of considering the impact of human activities in both the commercial and backyard flock settings. For commercial flocks, human activities have been described as a main source of secondary spread (*10*), with contacts through persons or shared equipment increasing the risk for AIV transmission (*11*). Consequently, a lack of biosecurity practices for backyard flocks belonging to commercial poultry farmers might have contributed to an increased risk for AIV infection of backyard poultry (Table 2).

Conclusions

We detected high flock- and bird-level seroprevalence of AIV in the backyard flocks we sampled after the 2016–2017 H5N8 epidemic in France. However, we observed very limited circulation of the H5N8 subtype, which indicates the minor role of backyard flocks in the transmission dynamics of H5N8. Backyard flocks belonging to commercial poultry farmers showed a significantly higher risk for infection with other H5 AIVs than backyard flocks having no links with commercial farms. These findings suggest that, from a risk-based perspective, surveillance of AIV circulation in backyard flocks should be focused on those flocks that have ducks and those connected to commercial poultry farms. On that basis, transmission of other more persistent pathogens of interest, such as mycoplasma or herpesviruses, should be further investigated at the backyard–commercial poultry interface (*12*).

Table 1. Results of serologic assays for 70 backyard flocks and 608 birds, by bird species comprising the flock, Gers Department, France, 2016–2017

	Avian influenza virus			Influenza A virus subtype H5		
Species comprising flock	Positive	Total	Seroprevalence, % (95% CI)	Positive	Total	Seroprevalence, % (95% CI)
All backyard holdings	18	70	25.7 (16.9–37.0)	8	70	11.4 (5.9–21.0)
Backyard holdings with only chickens	9	48	18.8 (10.2–31.9)	2	48	4.2 (1.2–14.0)
Backyard flocks with ducks	9	22	40.9 (23.3–61.3)	6	22	27.3 (13.2–48.2)
All birds	36	608	5.9 (4.3–8.1)	20	608	3.3 (2.1–5.0)
Chickens	20	486	4.1 (2.7–6.3)	9	486	1.9 (1.0–3.5)
Ducks	16	122	13.1 (8.2–20.2)	11	122	9.0 (5.1–15.4)

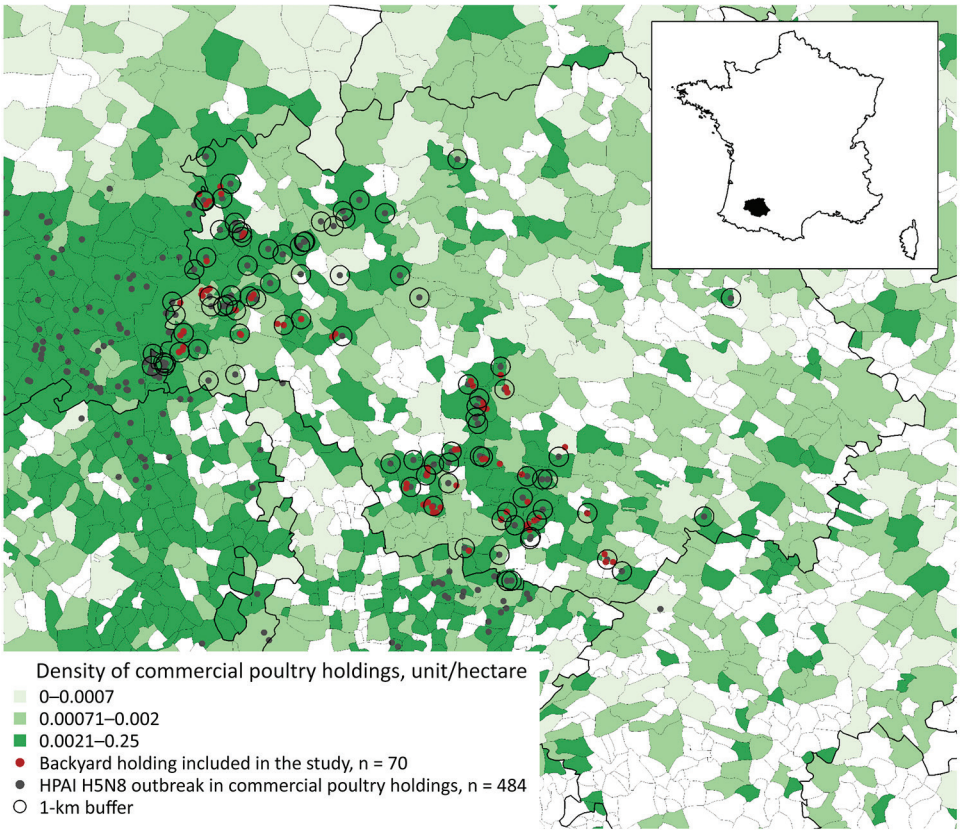

Figure. Locations of 484 commercial poultry holdings with reported outbreaks of HPAI H5N8 and the 70 backyard poultry holdings included in our study, Gers, Department, France, 2016–2017. HPAI H5N8, highly pathogenic avian influenza A virus subtype H5N8.

Table 2. Variables included in the final multivariable logistic regression with avian influenza virus and influenza A virus subtype H5 seroprevalences as outcome variables, Gers Department, France, 2016–2017

Outcome and variable	Odds ratio (95% CI)	p value
Avian influenza virus		
Species included*	2.3 (1.1–5.1)	0.036
Link with poultry industry†	5.8 (1.5–24.5)	0.011
Influenza A virus subtype H5		
Species included*	5.7 (1.6–30.6)	0.019
Link with poultry industry†	20.5 (3.2–215.8)	0.003

*Backyard flocks having ducks (yes vs. no).
†Professional activity of the backyard owner or member of the family home in connection with poultry industry (yes vs. no).

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About the Author

Dr. Souvestre is a veterinarian and a PhD candidate in poultry medicine at the Ecole Nationale Vétérinaire, Institut National de la Recherche Agronomique, Université de Toulouse, Toulouse, France. Her research interests focus on poultry health, the role of backyard poultry in the epidemiology of avian influenza, and the interface between backyard and commercial poultry.

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Address for correspondence: Jean-Luc Guerin, UMR IHAP 1225, École Nationale Vétérinaire de Toulouse, 23, chemin des capelles 31076 Toulouse CEDEX 3, France; email: jl.guerin@envt.fr

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Appendix

Methods

Multivariable logistic regression analysis was conducted to investigate risk factors statistically associated with the infection status (AIV and H5). First, associations were tested between each outcome and explanatory variable using a chi-squared test or Fisher's test (Appendix Table 1). Explanatory variables with a p-value below 0.2 were selected to be included in multivariable logistic regressions. Pairwise collinearity was tested between all selected explanatory variables by computing Cohen's kappa coefficient and considered significant if the absolute value of the coefficient exceeded 0.7. For the multivariable regressions, stepwise backward elimination was performed and explanatory variables retained if statistically significant ($p<0.05$). All analyses were performed using R statistical software (version 3.4.1).

HIT (hemagglutination inhibition tests) were performed following international standards, using different couple of antigens specific for clade 2.3.4.4 H5 viruses, H5N8 A/decoyduck/France/161105a/2016 and H5N5 A/muteswan/ Croatia/102/2016, or for viruses belonging to other H5 Eurasian lineages, H5N3 A/muscovy duck/France/070090b/2007 and H5N2 A/chicken/France/03426a/2003 (http://www.oie.int/en/standard-setting/terrestrial-code/access-online). These pairs of H5 antigens, with different neuraminidase subtypes, were tested to exclude cross-reactivity driven by neuraminidase-specific antibodies.

Antigens Used for HIT

H5N2 A/chicken/France/03426a/2003 and H5N3 A/muscovy duck/France/070090b/2007: sera displaying HI titers higher than 16 with both antigens are H5-positive in HIT. The antigens used have a broad cross-reactivity against H5 antibodies induced by infection or immunization with Eurasian lineage H5Nx low pathogenic or HPAI, except H5Nx A/goose/Guandong/1/1996-lineage clade 2.3.4.4 HPAI.

H5N5 A/muteswan/ Croatia/102/2016 (clade 2.3.4.4 A/goose/Guandong/1/1996 lineage) and H5N8 A/decoyduck/France/161105a/2016 (clade 2.3.4.4 A/goose/Guandong/1/1996 lineage): sera displaying HI titers higher than 16 with both antigens are H5-positive in HIT. The antigens used have a narrow cross-reactivity against H5 antibodies induced by infection or immunization with H5Nx A/goose/Guandong/1/1996-lineage clade 2.3.4.4 HPAI.

Appendix Table 1. ELISA, HIT and PCR analyses performed on AIV seropositive backyard poultry.

Positive ELISA AIV backyards	Bird ID	Species	ELISA H5	HIT H5N2	HIT H5N3	HIT H5N5	HIT H5N8	PCR M	PCR H5
V5	V5–02	Poultry	–	ND	ND	ND	ND	–	–
V9	V9–01	Duck	+	<4	<2	16	16	–	–
	V9–02	Poultry	+	ND	<2	ND	32	–	–
	V9–03	Duck	±	<4	<2	8	16	–	–
	V9–09	Duck	–	<4	<2	8	8	–	–
	V9–13	Duck	–	<4	<2	<4	<2	–	–
V12	V12–04	Duck	–	ND	ND	ND	ND	–	–
V18	V18–09	Poultry	–	ND	ND	ND	ND	–	–
V21	V21–04	Duck	±	<4	<2	<4	<2	–	–
	V21–06	Poultry	+	ND	ND	ND	ND	–	–
V22	V22–10	Duck	±	<4	<2	<4	<2	–	–
V24	V24–04	Poultry	–	ND	ND	ND	ND	–	–
V32	V32–02	Poultry	–	ND	ND	ND	ND	–	–
V33	V33–02	Poultry	–	ND	ND	ND	ND	–	–
	V33–08	Poultry	–	ND	ND	ND	ND	–	–
V37	V37–06	Poultry	+	16	32	<4	<2	–	–
	V37–07	Poultry	+	16	16	<4	<2	–	–
V50	V50–03	Poultry	+	<4	8	<4	<2	–	–
V59	V59–01	Goose	+	32	32	<4	<2	–	–
	V59–02	Goose	+	16	16	<4	<2	–	–
	V59–03	Duck	+	32	16	<4	<2	–	–
	V59–05	Duck	+	<4	<2	<4	<2	–	–
	V59–07	Poultry	+	ND	<2	ND	<2	–	–
	V59–08	Poultry	±	<4	<2	<4	<2	–	–
	V59–09	Poultry	+	<8	16	<4	<2	–	–
	V59–10	Poultry	+	4	8	ND	<2	–	–
V62	V62–03	Goose	–	ND	ND	ND	ND	–	–
V69	V69–01	Duck	+	32	8	<4	<2	–	–
	V69–02	Duck	+	<4	<2	<4	<2	–	–
V78	V78–02	Poultry	–	ND	ND	ND	ND	–	–
	V78–06	Poultry	–	ND	ND	ND	ND	–	–
	V78–09	Poultry	–	ND	ND	ND	ND	–	–
V81	V81–08	Duck	–	<4	<2	<4	<2	–	–
	V81–09	Duck	+	<4	<2	<4	<2	–	–
V87	V87–02	Poultry	–	ND	ND	ND	ND	–	–
V85	V85–03	Poultry	–	ND	ND	ND	ND	–	–

*HIT, hemagglutination inhibition test; ND, Not Done; +, positive results; –, negative results; ±, uncertain results.

Appendix Table 2. Binary variables examined for association with AIV and H5 seropositive holdings and results given by the univariate analysis with $p < 0.05$ * and $p < 0.01$ **.

Variable	Description	Backyard holdings, % (proportion)	p value chi-squared or Fischer test AIV	p value chi-squared or Fischer test H5
Species	Backyard flocks with ducks*	31 (22/70)	0.02*	0.03*
Fenced outdoor	Access to a defined free-range area	76 (53/70)	0.99	0.99
Covered backyard holding	Covered chicken house and/or free-range	19 (13/70)	0.19	0.19
Covered food	Water and food distribution in a covered area	57 (40/70)	0.28	0.46
Change practice	Modification of practices following the first H5 cases in South-Western France	37 (26/70)	0.38	0.70
Clothes	No specific clothing for backyard care	97 (68/70)	0.99	0.99
Shoes	No specific shoes for backyard care	83 (58/70)	0.43	0.71
Handwashing	No handwashing before or after visiting the backyard	33 (23/70)	0.98	0.85
Animal introduction	Bird introduction during the last year	73 (51/70)	0.92	0.70
Backyard holdings' production	Selling or giving eggs to family or neighbors	74 (52/70)	0.51	0.51
Backyard holdings' age	Owners having backyards holdings for more than 30 y	59 (41/70)	0.15	0.33
Bird exhibition visit	Visit of a bird exhibition during the last three months	4 (3/70)	0.99	0.31
Link with poultry industry	Professional activity of the backyard owner or member of the family home in connection with poultry industry **	17 (12/70)	0.01**	0.01**
Farmer's assistance	Owner giving assistance to a poultry farmer	7 (5/70)	0.99	0.99
Hunting	Owner or member of family home being a hunter	31 (22/70)	0.93	0.42
Backyard holding proximity	According to the owner, close distance to another backyard holding (1km or less)	74 (52/70)	0.99	0.99
Mortality	Abnormal mortality during the three last months	4 (3/70)	0.99	0.99
Ducks' clinical signs	Clinical signs on ducks during last three months	0 (0/70)	–	–
Poultry clinical signs	Clinical signs on poultry during last three months	6 (4/70)	0.99	0.99
Veterinary consultation	Veterinary visit or consultation during the three last months	1 (1/70)	0.26	0.11