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Presence of avian influenza risk birds in and around poultry free-range areas in relation to range vegetation and openness of surrounding landscape

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Abstract Free-range areas contribute to the welfare of poultry. Chickens are most likely to use these areas if there is sufficient cover by trees. However, wild birds in free-range areas may infect the chickens with avian influenza (AI). This study aimed to investigate the relation between the presence of AI risk birds and woody vegetation within the range areas as well as in the landscape surrounding the range areas. During two seasons all wild birds were counted in the free-range areas of 11 poultry farms and their immediate surroundings. More high-risk birds were observed in free-range areas with less than 5% woody cover, compared to free-range areas with more woody cover. Furthermore, more high-risk birds were observed in the surroundings of free-range areas in open landscapes, compared to half-open landscapes. As for low-risk birds, no relation was found between woody cover or openness of the landscape and the presence of these birds in free-range areas or surroundings. However, interpretation of the results was hampered by the incomplete factorial design, which did not allow to differentiate between the effect of woody cover within the range area and openness of the surrounding landscape. The results of this pilot study need to be confirmed with further experimental research on the relation between the presence of AI risk birds and woody vegetation in and around poultry free-range areas.

Keywords Free-range poultry · Avian influenza · Agroforestry

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Introduction

Free-range areas contribute to the welfare of laying hens. Feather-pecking, a generally recognized indicator of chicken welfare (Rodenburg et al. 2013), is less prevalent if more hens of a flock go outside (Bestman and Wagenaar 2003; Green et al. 2000). The number of chickens going outside has been found to depend on the degree of cover provided by trees or artificial structures in the range area (Bestman and Wagenaar 2003; Zeltner and Hirt 2003). These findings were corroborated by Bright et al. (2016), who found less feather-pecking damage in chickens that had more trees in their free-range area. Furthermore, range use also depends on the relative number of cockerels, the age of the hens on arrival at the laying farm (Bestman and Wagenaar 2003), and flock size (Bubier and Bradshaw 1998; Hirt et al. 2000; Appleby and Hughes 1991). Besides reduced feather-pecking, an additional benefit of (tree) cover is a more even distribution of chickens across the range area, which may reduce the risk of parasitic contamination (Bray and Lancaster 1992) and local accumulation of nitrogen and

phosphate (Dekker et al. 2012). Thus, outdoor areas with (tree) cover contribute not only to the welfare, but also to the health of free-range chickens, and may also have environmental benefits.

Chickens ranging outside can come into contact with wild birds and their feces. If these wild birds are infected with the avian influenza (AI) virus, the virus may be transmitted to the chickens. Among wild birds, migratory water birds are being regarded as the most probable transmitters of the AI virus to poultry, while birds of prey are considered low-risk with regard to AI transmission (Verhagen et al. 2015; van der Goot et al. 2015). However, it is not certain whether wild birds transmit the virus to poultry directly, or whether intermediate hosts, such as pigeons or rats, (also) play a role (EFSA 2006).

Our first research question was whether there is a relation between the degree of tree cover in free-range areas ('woody cover' hereafter) and the number of AI high-risk and low-risk birds observed within these areas. Another question was whether there is a relation between the openness of the landscape surrounding the poultry farm and the number of AI high-risk and low-risk birds in the immediate surroundings of the free-range areas.

Methods

Farm selection

At the start of the project we selected ten poultry farms from our network, based on the varying degree of woody cover in their free-range areas. The woody cover on these farms consisted of fruit trees, biomass willows, or Miscanthus (*Miscanthus giganteus*). Although Miscanthus is a grass, we regarded it as 'woody cover' since it grows up to 4 meters high and has a density comparable to biomass willows. The degree of woody cover on the selected farms ranged from 0 to 90% (Table 1). The landscape surrounding the ranges was classified either as 'open' or 'half-open', with the open landscapes mainly consisting of grassland and only very few trees or bushes, and the half-open landscapes containing woodland strips or tree plots within 500 ms from the border of the free-range area. After analyzing the results of the first season, we added an eleventh farm to our study. This was a farm with a high degree of woody cover in the

Table 1 Characteristics of the study farms

	No of hens, rounded to 1000	Size of free-range area in hectares	Woody cover in % of free-range area	Cover category*	Type of vegetation in free-range area	Vegetation of surrounding landscape	Openness of surrounding landscape
1	24,000	12	0	1	Grass	Grassland	Open
2	18,000	8	35	3	Grass, fruit, Miscanthus	Agriculture**, woodland strips, forest	Half-open
3	30,000	17	8	2	Grass, trees, bushes	Agriculture, woodland strips	Half-open
4	15,000	6	75	4	Miscanthus, grass	Agriculture, woodland strips, forest	Half-open
5	12,000	5	90	4	Fruit, grass	Agriculture	Half-open
6	17,000	8	0	1	Grass	Grassland	Open
7	16,000	6	35	3	Grass, fruit	Agriculture, woodland strips	Half-open
8	15,000	8	50	3	Fruit, biomass willows, grass	Agriculture, woodland strips	Half-open
9	15,000	7	10	2	Grass, fruit	Agriculture, woodland strips, forest	Half-open
10	24,000	10	10	2	Grass, fruit	Agriculture, woodland strips, forest	Half-open
11	6000	2	90	4	Fruit, diverse bushes	Grassland	Open

* See "Methods"

** 'Agriculture': maize or wheat (=arable crops related to livestock farms)

range area and which was located in an open landscape, a combination of characteristics that was missing from our initial farm sample. Table 1 shows the characteristics of the 11 farms. The farms varied in the type of vegetation and were unevenly distributed across open and half-open landscapes. The degree of woody cover (% of free-range area) was estimated after walking around and through the entire range area during an initial visit.

Bird observations

The first observation period (hereafter called 'spring') ran from February 4 to April 23, 2014. The second observation period (hereafter called 'autumn/winter') ran from October 10, 2014 to February 2, 2015. Bird observations were carried out on 10 farms in spring, and on 11 farms in autumn/winter. In each season, we visited each farm four times.

At the first visit of each farm, maps of the free-range area, farm buildings, farm yard, and direct surroundings up to a distance of 500 m were made. For bird counts in the surroundings, we selected two plots bordering (or close to) the range area, which could be observed from a car from the public road. On each farm, bird observations started with observing the surrounding plots from the car for 30 min, after which the observer continued on foot to visit the range area and farm yard. The range area was observed while walking around or standing at predefined spots. A range area of 5–10 ha took approximately 60 min, a range area of 10–15 ha 75 min and a range area of 15–20 ha 90 min. The mean observation time for a range area, farm yard and surrounding plots together was 90 min.

On most farms, pop-holes opened at 11:00 am and chickens would be outside until dark. Since we expected less disturbance and better visibility of wild birds if no chickens were present in the outdoor ranges, we started our observations at 9:00 am, when the chickens were still inside. Per day, one farm was visited. In the seldom case of 'extreme' weather, such as heavy rain or fog, the farm visit was postponed for an hour or moved to another day. Birds were considered present in the free-range area if they touched the ground or the vegetation within the free-range area. Birds were considered present in the surroundings if they flew over the free-range area, the surrounding plots or the farmyard, or if they were sitting in the surroundings or the farmyard. Although we could not do observations inside dense vegetation such as Miscanthus, we did not expect this to be a problem, since the most important species of our study (water fowl and birds of prey) were not expected to be present there.

Risk categories

Wild birds were classified into three categories (high-risk, low-risk, and no/unknown-risk species), based on large-scale wild bird monitoring for prevalence of AI (Breed et al. 2011), a categorization made by Veen et al. (2007), and expert judgement (personal communication, Roy Slaterus, Sovon, Dutch center for field ornithology). High-risk species, i.e. species with a high prevalence of infection with the AI virus, are water birds and (long-legged) wading birds, such as geese, ducks, swans, gulls, oystercatchers and lapwings. Low-risk birds are not as vulnerable to influenza infection as the high-risk birds, but can still carry the virus after contact with infected birds. This category includes all birds of prey and carrion-feeding birds as corvids; for example, hawks, buzzards, crows and ravens. The no/unknown-risk birds include all other birds (mainly songbirds). Birds in this category are rarely or never found to be infected with AI, and are no longer monitored in the European AI monitoring programme (Breed et al. 2011).

Data analysis

For data analysis, the counted wild birds were categorized into three risk classes, as described above. Furthermore, depending on where the birds were observed, their location was classified as either "free-range area" or "surroundings". Free-range areas were categorized according to their degree of woody cover: cover category 1: <5%; cover category 2: 5–20%; cover category 3: 20–50%; and cover category 4: >50%. The surrounding landscape was classified either as open or half-open (see Farm selection, above). Per farm, the total number of birds observed per season (summed over 4 observations per season) were used for the analysis: (1) all high-risk birds in the free-range area, (2) all low-risk birds in the free-range area, (3) all high-risk birds in surroundings and (4) all low-risk birds in surroundings. The no/unknown-risk birds were not included in the statistical analysis. Hence, for each combination of bird category and

location (range area versus surroundings), a total of 21 observations were available for analysis (10 farms in spring, 11 farms in autumn/winter). Data were natural-log transformed to normalize distributions, and analyzed using the General Linear Models procedure in Genstat, using the following model:

Ln (total number of birds observed per farm and per season) = season + woody cover + openness landscape

where Season = block (spring or autumn/winter); woody cover = woody vegetation within range area (four levels); openness landscape = absence or presence of woody vegetation in the immediate surroundings of the range area (two levels: open or half-open).

Results

In total, 24,103 wild birds were counted during this study, of which 5706 were either high-risk or low-risk birds (Table 2). The complete list can be obtained from the main author.

High-risk birds in free-range areas

If either woody cover or openness of the landscape was used in the model, this resulted in models with significant factors (Table 3). Due to the incomplete factorial design it was not possible to run models with both factors together. Hence it was not possible to differentiate the effect of woody cover within the range area from the effect of openness of the landscape surrounding the range area.

Table 2 Results used for the statistical analysis, categorized as either high-risk or low-risk and observed either within the free-range areas or the surrounding landscape

Order	Free-range area	Surroundings	Total
High-risk birds			
Ducks	28	308	
Geese	108	1443	
Charadriiformes	85	1435	
Other	47	186	
Subtotal	268	3372	3640
Low-risk birds			
Bird of prey	24	194	
Corvid	403	1445	
Subtotal	427	1639	2066
Total	695	5011	5706

Regression analysis with woody cover as fixed factor (Table 3, model 1) resulted in the following model ($p = 0.026$; $R^2 = 35$; SE = 15.8):

Ln (total number of high-risk birds in free-range area) = 2.55 + (2.47 * Season Spring) + (−5.10 * Cover Category 2/ − 5.12 * Cover Category 3/ − 4.62 * Cover Category 4).

In this model, the number of high-risk birds in free-range areas was weakly related to season ($p = 0.052$) and significantly related to woody cover ($p = 0.042$). More high-risk birds were observed in spring than in autumn/winter, and more high-risk birds were observed if woody cover was low. The number of high-risk birds in the woody cover category <5% differed significantly ($p < 0.02$) from the other cover categories (5–20, 20–50 and >50% woody cover).

Using openness of the landscape as a fixed factor (Table 3, model 2) resulted in the following model ($p = 0.029$; $R^2 = 25$; SE = 19.3):

Ln (total number of high-risk birds in free-range area) = −2.38 + (2.7 * Season Spring) + (3.37 * Open Landscape).

In this model, the number of high-risk birds was weakly related to season ($p = 0.066$) and significantly related to openness of the landscape ($p = 0.040$), with more high-risk birds observed in free-range areas surrounded by open landscapes, compared to free-range areas surrounded by half-open landscapes.

Low-risk birds in free-range areas

The number of birds of prey and corvids in free-range areas was not significantly related to woody cover within the range area (Table 3, model 3: $p = 0.613$; SE = 2.5) nor to the openness of the surrounding landscape (model 4: $p = 0.701$; SE = 2.48). Furthermore, no effect of season was found.

High-risk birds in surroundings of free-range areas

If either openness of the landscape or woody cover in the range area was used in the model, this resulted in

Table 3 Results from the general linear model analysis

Model	Response variable	Fixed factor	Model R^2	SE	P	Variables (P)		
						Season	Cover	Openness
1	Ln (total HRB in FR)	Woody cover	35	15.8	0.026	NS (0.052)	0.042	Excluded
2	Ln (total HRB in FR)	Openness landscape	25	19.3	0.042	NS (0.066)	Excluded	0.040
3	Ln (total LRB in FR)	Woody cover	–	2.5	0.613	NS	NS	Excluded
4	Ln (total LRB in FR)	Openness landscape	–	2.5	0.701	NS	Excluded	NS
5	Ln (total HRB in SUR)	Openness landscape	39	1.3	0.005	0.013	0.016	Excluded
6	Ln (total HRB in SUR)	Woody cover	52	1.3	0.016	0.007	Excluded	0.010
7	Ln (total LRB in SUR)	Openness landscape	–	1.3	0.580	NS	NS	Excluded
8	Ln (total LRB in SUR)	Woody cover	–	1.3	0.924	NS	Excluded	NS

Ln natural logarithm, *HRB* high-risk birds, *FR* free-range area, *LRB* low-risk birds, *SUR* surroundings, *NS* not significant

models with significant factors (Table 3). Due to the incomplete factorial design it was not possible to run models with both factors together. Hence it was not possible to differentiate the effect of openness of the surrounding landscape from the effect of woody cover within the range.

Regression analysis with openness of the landscape as a fixed factor (Table 3, model 5) resulted in the following model ($p = 0.005$; $R^2 = 39$; $SE = 1.3$):

$$\begin{aligned}\text{Ln(total number of high-risk birds in surroundings)} &= 2.10 + (-0.37 * \text{Season Spring}) \\ &\quad + (0.456 * \text{Open Landscape})\end{aligned}$$

In this model, the number of high-risk birds in the surroundings of free-range areas was significantly related to season ($p = 0.013$) and openness of the landscape ($p = 0.016$). More high-risk species were observed in the surroundings of free-range areas if these surroundings were open landscapes, and more high-risk birds were observed in autumn/winter than in spring.

Regression analysis with woody cover as a fixed factor (Table 3, model 6) resulted in the following model ($p = 0.003$; $R^2 = 52.1$; $SE = 1.34$):

$$\begin{aligned}\text{Ln (total number of high-risk birds in surroundings)} &= 2.55 + (-0.40 * \text{Season Spring}) \\ &\quad + (-0.72 * \text{Cover Category 2}/ \\ &\quad - 0.24\,\text{Cover Category 3}/ - 0.27 \\ &\quad * \text{Cover Category 4})\end{aligned}$$

In this model, the number of high-risk birds in the surroundings of free-range areas was significantly related to season ($p = 0.007$) and woody cover within the range area ($p = 0.01$). More high-risk species were observed in the surroundings of free-range areas with low woody cover, and more birds were observed in autumn/winter than in spring. The difference between Cover Category 1 and 2 was significant, as was the differences between Cover Category 2 and Cover Categories 3 and 4. The lowest numbers of high-risk birds were observed in the surroundings of the free-range areas, if these had a woody cover of 5–20%.

Low-risk birds in surroundings of free-range areas

Neither openness of the landscape (Table 3, model 7: $p = 0.58$; $SE = 1.3$) nor woody cover as fixed factor (model 8: $p = 0.924$; $SE = 1.34$) were significantly related to the numbers of birds of prey and corvids observed in the surroundings of free-range areas. Furthermore, no effect of season was found.

Discussion

High-risk birds in free-range areas

The largest numbers of high-risk birds were observed in free-range areas with a minimum (<5%) of woody cover. As soon as woody cover was more than 5%, numbers of high-risk birds were significantly lower. A likely explanation for this result is that geese and ducks (the main groups of high-risk birds) prefer areas with short grass and without bushes, where they can

spot their predators in time (Loonen and Bos 2003). Moreover, these species move around in large groups, for which they need space (Stahl, personal information; Sovon, Dutch center for field ornithology). However, interpretation of our results is hampered by the incomplete design of this pilot study: in our farm sample, the two farms with 0% woody cover were both located in an open landscape. Hence, it is not possible to say whether the larger number of high-risk birds on these farms was related to the absence of woody cover in their free-range areas or to the openness of the surrounding landscape (or both). In these cases, the larger number of water birds present in range areas with minimal woody cover may be partly due to the larger 'reservoir' of water birds in the open landscape surrounding the range areas in question.

Low-risk birds in free-range areas

No relation was found between the number of birds of prey or corvids in free-range areas and woody cover within these areas, nor between the number of these birds and the openness of the surrounding landscape. In general, the presence of birds of prey was low compared to other birds. If more observations had been done on a larger number of farms, a relation with woody cover or openness of the landscape might have emerged, but it is also possible that other factors played a stronger role in attracting (or keeping off) birds of prey. In another study (Bestman, unpublished results) a buzzard nest was found in a tree a few hundred meters from an organic poultry house, where the entire buzzard family was observed hunting in the free-range area. This observation indicates that a relation with woody vegetation (trees for nesting) within and outside the range area is conceivable. While the number of corvids was much larger than the number of birds of prey, no relation between corvid abundance and woody cover or openness of the landscape was found, either. This group of birds may have been attracted to carrion in the free-range areas or feed spills in the farmyard.

High-risk birds in the surroundings of free-range areas

More high-risk birds were observed in the surroundings of free-range areas if these surroundings were an open landscape, rather than a half-open landscape. However, interpretation of the results was hampered by the incomplete design of our pilot study: of the three farms in an open landscape, two had 0% woody cover and one had 90% woody cover in the range area. The largest numbers of high-risk birds were observed in the surroundings of the farms with 0% woody cover, but it is not possible to say whether this was due to the absence of woody cover in the free-range area or to the openness of the surrounding landscape (or both). In general, the presence of geese and ducks in open landscapes can be explained by the fact that these species prefer large open spaces, where they can spot their predators in time (Loonen and Bos 2003) and where they can move around in large groups (Stahl, personal communication).

Low-risk birds in the surroundings of free-range areas

No relation was found between the numbers of birds of prey and corvids in the surroundings of free-range areas and openness of the landscape or degree of woody cover within the free-range area. As argued above, the numbers of birds of prey were low and relationships between their abundance and woody vegetation might have emerged if more observations had been made on a larger number of farms. As for the corvids, it is possible that they feel at home in both open and half-open landscapes.

Limitations of the study

Ideally, this pilot study would have covered two sets of poultry farms with a varying degree of woody cover, one complete set located in open landscapes and one complete set in half-open landscapes. However, our choice of farms was limited, in particular of farms with substantial woody cover within the range area. While poultry farmers show a growing interest in planting trees in free-range areas, the number of poultry farms with substantial woody vegetation is still limited. This is due, most importantly, to the high costs of planting trees, and to the legal restrictions on cutting trees once they are grown. This limits their uses and makes agricultural land with trees less valuable. In addition, when interviewed about agroforestry, farmers tended to emphasize the general complexity of working with trees and difficulties with mechanization (Graves et al.

2009). Although we did find farms with differing degrees of woody cover, the vegetation varied from fruit trees and biomass willows to Miscanthus. Furthermore, our farm sample size and number of observations was limited by time; we had only one observer available and only one farm could be visited per day because of farm-hygienic reasons. An additional limitation was that, for practical reasons, we did our observations only during the daytime. However, some high-risk birds such as mallards are known to be nocturnal (Kleyheeg et al. 2015): during the day they are observed (resting) in other places than during the night (foraging). Hence, birds visiting poultry free-range areas at night are not covered by our study.

Implications of findings for further research

The results of this pilot study need to be confirmed with further research to investigate the effect of planting woody vegetation in poultry free-range areas as a measure to reduce the presence of AI high-risk birds. Such follow-up studies could be based on an experimental approach, where the number of high-risk and low-risk birds is followed in free-range areas that previously had no woody cover but where trees are planted as part of the experiment. Such "before-and after" experiments should be carried out in both open and half-open landscapes. Similarly, experiments could be set up by planting vegetation in the surroundings of free-range areas and observing the effects on the presence of the various bird risk categories within and outside the range areas. Additionally, further studies could compare bird presence in planted ranges versus open ranges within the same season, as season and year may also affect the number of birds present in the range areas. As soon as more is known about what animal species serve as intermediate AI hosts, these should be included in the study with appropriate (if necessary) nocturnal observations.

Conclusions

- In poultry free-range areas with a higher degree of woody cover, fewer AI high-risk birds were observed than in free-range areas without or with very limited (<5%) woody cover. However, due to the incomplete design of this pilot study, the effect of woody cover within the range areas could not be differentiated from the effect of the surrounding landscape: most of the investigated free-range areas with higher degrees of woody cover were located in half-open landscapes, where the presence of woody vegetation was statistically related to lower abundances of high-risk birds within the free-range areas.
- In open landscapes, more high-risk birds were observed in the surroundings of free-range areas than in half-open landscapes. However, due to the incomplete design of this study, the effect of openness of the landscape could not be differentiated from the effect of woody cover within the range areas, which was statistically related to lower abundances of high-risk birds in the surroundings.
- No relation was found between the numbers of low-risk birds in free-range areas or their surroundings and the degree of woody cover or openness of the landscape.
- Our results need to be confirmed with further studies to investigate the relations between woody cover in and around poultry free-range areas and the presence of AI high-risk birds.

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