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Flock management and histomoniasis in free-range turkeys in France: description and search for potential risk factors

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SUMMARY

The relationship between flock management and histomoniasis, a re-emergent infection in poultry, was investigated by statistical techniques used in veterinary epidemiology to deal with various problems including: multicollinearity, confounding, interaction or sample size. Associations between the variables describing flock management were examined by multivariate descriptive analysis to reduce the number of independent variables, prior to investigating associations with the disease. No homogenous groups of farms were found in the 44 free-range turkey flocks sampled in France. *Histomonas meleagridis* was identified in 26/38 flocks and histomoniasis was confirmed in 19 flocks. Cleanliness of the building, wet litter and diarrhoea were linked with *H. meleagridis* and severity of histomoniasis. Sharing outdoor fields simultaneously with chickens was related to serious macroscopic lesions determined by post-mortem examinations. Contrary to general belief, acidification of drinking water with organic acid had consistent association with the presence of *H. meleagridis* in turkey caeca. These results confirm previous findings and provide several new hypotheses on the effects of hygiene and water management on *H. meleagridis* and histomoniasis.

Key words: *Histomonas meleagridis*, histomoniasis, infectious disease epidemiology, poultry, risk assessment.

INTRODUCTION

Histomoniasis or blackhead disease, caused by *Histomonas meleagridis*, a flagellated protozoon, is re-emerging in poultry after effective chemotherapeutics were banned in the USA and Europe [1, 2]. Parallel to recent research carried out on alternative drugs [3–7], it is necessary to develop or to validate non-drug methods in order to control this disease based on an improved knowledge of its epidemiology in the field.

Some outbreaks have been described in standard turkey production [2, 8–10], but in free-range farms very little precise information is available although birds are highly exposed to *H. meleagridis* by field contamination [1]. Here, we investigated traditional turkey production in the Bresse region with specific

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conditions under the framework of a European Protected Designation of Origin (PDO), where histomoniasis is commonly identified.

This study was designed to investigate the possible associations between management methods and the presence of histomoniasis at flock level, and as a first step towards the identification of risk factors. Data analysis was performed in three steps to deal with multicollinearity, confounding, interaction and small sample size usually reported in health and production studies in livestock: (i) describing the farming system in the Bresse region and searching if flocks could be classified in typology according to their common traits, with the view that histomoniasis could be associated with a particular farming system, (ii) describing the presence of *H. meleagridis* and the severity of histomoniasis, and (iii) searching for associations between histomoniasis and flock management practices that may represent potential risk factors.

METHODS

Study area and specific rearing conditions

We recorded information concerning flock management and the presence/absence of *H. meleagridis* and histomoniasis from farmers on a voluntary basis in September 2002 and 2003 on free-range turkey farms in the Bresse area benefiting from a PDO. Data consisted of completing a questionnaire and performing field investigations (e.g. pH measures in drinking troughs, capture of three turkeys for necropsy at the laboratory) for each flock. The French area of Bresse consists of a limited surface area of 3500 km^2 to the north of Lyon. Only 30 000 turkeys are reared each year by about 40 producers (43 producers in 2002 and 36 in 2003). The turkey strain is the Betina strain GB 191, a lightweight black strain. In accordance with the PDO guarantee, birds are reared in one flock per year, over a period of 7 months. The growth period usually starts at the end of June or the beginning of July on grassy pasture and lasts for at least 15 weeks. The feed consists only of cereals (maize and wheat) and milk products. Birds find complementary food in pastures. Drugs and feed additives are forbidden, except for herbal products and nifursol that was allowed up to April 2003.

Collection of management data

In order to describe and classify the farms into types according to their common characteristics, we used 44 qualitative variables (2–4 modalities) elicited from the questionnaires from all the investigated farms. We distinguished three sets of variables that characterized different levels of the farming system (Table 1). Each set was used to (i) search for a typology based on homogenous groups of farms, and (ii) to explore some hypotheses concerning *H. meleagridis* and histomoniasis.

The first set of variables, 'Farming', included the general husbandry methods (housing, feeding, etc.). We expected that large and small flocks would be reared in different conditions and that the disease could be associated with one of the farm types.

In the second set, 'Preventive measures', we considered variables describing the measures that are used in farms to prevent histomoniasis (disinfection, sanitary vacuum, etc.). This set included a general note on hygiene, estimated by the same external observer, based on litter aspect, odour and general cleanliness of the building. We expected that a high level of prevention would be associated with a low presence of histomoniasis.

In the third set, 'Sanitary situation', we included variables related to the current presence of pathogens or diseases. The variable 'stress' described all events that could disturb birds during the rearing period, e.g. unusually heavy rainfall or coolness in summer. The hypotheses were that (i) the presence of diseases or pathogens could be a marker of the presence of *H. meleagridis*, and (ii) some pathogens (e.g. *Heterakis* or *Eimeria*) could favour the presence of *H. meleagridis* or increase the severity of the lesions related to histomoniasis.

We included a variable 'year' in each dataset, after observing some differences between 2002 and 2003 for several variables. We thus aimed to distinguish the effect of other variables from the year-to-year variability.

Detection of *H. meleagridis* and histomoniasis

Four variables quantified the presence of *H. meleagridis* and the severity of histomoniasis. Two of them were recorded at the laboratory from the three turkeys provided at the end of the rearing period: Direct Examination (DE) and Lesion Index (LI). The two other variables were provided by the farmers and concerned the whole rearing period, i.e. 'Morbidity' and 'Mortality' that were believed to be due to histomoniasis.

Table 1. *Variables and modalities describing flock management in free-range turkeys in France, divided in three sets: (a) Farming, (b) Preventive measures and (c) Sanitary situation (n is the number of flocks for each modality)*

Name	Variable	Modalities	*n*
(*a*) Farming			
Year	Year	2002/2003	14/30
Nb-turkey	Number of turkeys in farm	< 500/500–1000/ > 1000	15/17/12
Period	Beginning of the flock	April/May	21/23
Time-farming	Time since the farm was created	< 5 years/ ⩾ 5 years	5/39
Chickens	Presence of chickens in the farm	Presence/absence	35/9
Guinea	Presence of guinea fowl in the farm	Presence/absence	21/23
Nb-build	Number of buildings used for turkeys	1 building/2 buildings/3 buildings or more	21/13/10
Time-build	Time since the building were built	< 5 years/ ⩾ 5 years	8/36
Origin-build	Use of the building since construction	Always turkeys/other poultry/other species	13/19/12
Litter	Litter used	Straw/other material	38/6
Freq-litter	Frequency of litter change	> Once per week/once per week to once per month/ < once per month	22/15/16
Perches	Presence of perches	Presence/absence	25/19
Field surface	Surface per turkey in the field	⩽ 20 m^2 per turkey/20 to 40 m^2 per turkey/ > 40 m^2 per turkey	7/21/16
Sharing	Is the field shared by other species	Sharing simultaneously/sharing successively/ no sharing	9/10/25
Soil-field	Soil of the field	Clay/sand	35/9
Water-field	Presence of wet areas in the field	Dry/wet	26/18
Topo	Topography of the field	Flat/slope on the field	14/30
Cover	Tree cover on the field	No tree cover/Tree cover	30/14
Origin-water	Origin of the drinking water distributed	Municipal network only/well	31/13
Int-water	Number of indoor drinking troughs per 200 turkeys	< 1 per 200/ ⩾ 1 per 200	17/27
Ext-water	Presence of drinking troughs on the field	Presence/absence	21/23
Puddles	Presence of puddles	Presence/absence	28/16
(*b*) Preventive measures			
Year	Year	2002/2003	14/30
Vacuum	Sanitary vacuum done	Disinfection during sanitary vacuum/ no disinfection or no vacuum	37/7
Hygiene	Overall hygiene note (see text)	Good/intermediate/poor/n.a.	17/13/10/4
Disinfection	Disinfection of the field between two lots	Yes/no	27/17
Acidification	Acidification of water	Permanent/non-permanent/absent	28/8/8
pH	pH of indoor drinking troughs	Acid/neutral/n.a.	16/19/9
Investigations	Complementary investigations if diarrhoea	By the vet/by the farmer/no investigation	12/8/24
Treatment	Is there any treatment against histomoniasis	Yes/no	23/21
Prophylaxis	Is there any prophylaxis against histomoniasis	Yes with nifursol/no/yes with other product	5/1/38
(*c*) Sanitary situation			
Year	Year	2002/2003	14/30
Diarrhoea	Presence of diarrhoea	Yes/no	37/7
Date	Date of the diarrhoea	June–July/Aug./Sept.–Oct./n.a. or no diarrhoea	19/15/4/6
Before	Any case of histomoniasis before this lot	Yes/no	39/5
Coccidiosis	Does the farmer think to have coccidiosis	Yes/no	8/36
Eimeria	Presence of *Eimeria* at coproscopy	Presence + /presence + + /absence/n.a.	16/10/12/6
Capillaria	Presence of *Capillaria* at coproscopy	Presence/absence/n.a.	25/13/6
Heterakis	Presence of *Heterakis* at coproscopy	Presence/absence/n.a.	5/33/6
Mycotoxin	Presence of at least one mycotoxin	Presence/absence	7/37
Stress	Disturbing events while rearing (see text)	No/yes, heavy rain/yes, other	14/11/19

n.a., Not available.

Direct Examination. This measured the prevalence of *H. meleagridis* detected through microscopic examination in aliquots of caecal content from the necropsied 1–3 individuals (number of animals that carried *H. meleagridis*/number examined).

Lesion Index. This was an average indicator of the intensity of gross lesions in necropsied turkeys for one farm. The lesions consistent with histomoniasis recorded on the same necropsied individuals were scored macroscopically [from 0 (no lesion) to 4 (severe lesions)] separately for the two caeca and for liver [11, 12]. LI was calculated as the mean score for the three organs for each turkey:

$$\mathrm{LI} = \frac{\sum_{k=1}^{n} (C1_k + C2_k + L_k)}{n},$$

where $C1$ is the first caecum lesion score (0–4), $C2$ is the second caecum lesion score (0–4), L is the liver lesion score (0–4) and n is the number of turkeys autopsied from the farm. LI indicated the *Histomonas* dissemination and pathogenicity within birds. We confirmed the diagnosis of blackhead disease only in flocks where the presence of *H. meleagridis* was detected by DE and typical gross lesions were observed on caeca or/and liver with $\mathrm{LI} > 1$.

Morbidity. This is the frequency of sick birds that the farmer suspects of being related to histomoniasis during the entire rearing period (number of sick birds/total flock size at the beginning).

Mortality. This measures the frequency of dead birds in similar conditions (number of dead birds/total flock size). Mortality and Morbidity were thus considered as a sign of *Histomonas* pathogenicity at flock level. By comparison, the death losses during the rearing period without any specific diseases are estimated on average at 10% in standard turkey production (between 3·5% and 12%) [13]. Therefore, we regarded as small an outbreak with Mortality $< 10\%$, moderate for between 10% and 20% and severe at $> 20\%$.

In the questionnaire, farmers were asked to give their own estimate of mortality related to histomoniasis, considered as the leading factor of morbidity and mortality. We acknowledged that the observed values may include other factors of mortality; however, it is a good representation of the mortality that farmers believed was due to histomoniasis, which is an important criterion for their farm management. We hypothesized that the declared losses were generally related to histomoniasis but we tested their relationship with the true presence of *H. meleagridis* by our analyses.

Statistical analysis

Flock management associations

We first described flock management in order to (i) explore the relationship between variables describing flock management and (ii) reduce the number of independent variables to be tested for association with histomoniasis.

We used multiple correspondence analysis (MCA), which is a form of multivariate descriptive analysis that has been developed for qualitative variables [14]. Here, the analysis was used to reveal several possible types in farms based on common traits. MCA also keeps track of the associations between factorial axes and original variables in the form of correlation ratios that can be used to graphically represent these associations [15]. The first factorial axes may be considered as the most important independent variables that can be extracted from the dataset.

We treated missing observations in the following way: when a variable was missing for only one farm (surface, topography), we replaced the variable by the modality observed most frequently. When a variable was often missing (hygiene, pH, *Eimeria*, *Capillaria*, *Heterakis*) we created a modality 'not available'.

We performed respectively three MCAs for Farming, Preventive measures and Sanitary situation datasets. We hypothesized that farming, preventive measures and sanitary situation, although potentially correlated, had separate effects on the risk of histomoniasis. Finally, separating the three sets of variables allowed us to explore only the relationships of interest; it avoided highlighting meaningless correlations that would necessarily emerge from the multivariate dataset. For each MCA, we arbitrarily considered as many factorial axes as necessary to explain at least two thirds of the original variability. To interpret factorial axes, we considered variables whose correlation ratios with factorial axes were the highest, i.e. we retained variables whose correlation ratio was at least twice the mean correlation ratio with this axis, or at least the two most important variables when less than two variables respected this criterion.

Relationship between the detection of H. meleagridis *and histomoniasis*

We tested for the correlations between the four variables describing *H. meleagridis* and histomoniasis using Pearson correlation coefficients.

Relationship between flock management and histomoniasis

We first selected factorial axes of the MCAs most correlated to each variable describing histomoniasis, using Pearson correlation coefficients. We considered all axes with significant correlation at the $P=0{\cdot}2$ level. Then, in order to assess the direct relationship between the original variables and disease, we tested for the relationship between the variables defining selected factorial axes and variables describing histomoniasis using analyses of variance (ANOVAs). With this procedure, only variables that explained important factorial axes were tested, which avoided performing large numbers of tests.

RESULTS

The survey was performed on 14 farms in 2002 and on 30 farms in 2003. Ten farms were sampled twice, which we considered as two distinct farms because many factors varied between the two years, including flock management practices. All 44 farms were used to establish the typology, to maximize the representativeness of the sample (the sample included 32% in 2002 and 83% in 2003). Only the 38 farms that provided birds for post-mortem examinations were used to study histomoniasis and the relationship with flock management.

Flock typology

For each of the three MCAs, Table 2 shows the retained factorial axes with their proportion of explained variance and gives the variables most correlated with each factorial axis. Table 2 also gives the modalities which were typically associated.

Regarding Farming, 10 factorial axes were necessary to describe more than two thirds of the original variability, whereas six axes described Preventive measures and six axes described Sanitary situation. These large numbers meant that there were no homogenous groups in the studied farms.

Detection of *H. meleagridis* and histomoniasis

Of the 44 farms, 15 provided three individuals, 11 gave two, 12 gave one and six gave none. We considered whether this variability influenced the estimation of disease status in the farms: farmers that were more concerned by histomoniasis may have provided more information. However, when two or three individuals from the same farm were examined, their DE results were more similar than expected by chance. Moreover, the mean LI and the mean DE scores were similar whatever the number of turkeys provided, showing that no bias existed towards more infected farms providing more information. Of the 38 farms that provided animals for post-mortem examination, DE revealed the presence of *H. meleagridis* from 26 flocks (68·4%). Gross lesions were observed in animals from 20 farms. The mean LI of the 20 non-negative farms was $4{\cdot}90 \pm 2{\cdot}5$.

Farmers declared a non-null Morbidity in 38/44 cases (86·4%). In these flocks, mean Morbidity was 11·17% (from 0·4% to 47·1%) and mean Mortality was 8·74% (from 0·12% to 47·1%).

In the 38 farms that provided complete information, two correlations in the four variables were significant between Morbidity and Mortality and between DE and LI (Fig. 1). There was no significant relationship between Morbidity or Mortality and LI or DE (all $r<0{\cdot}366$); for example high LI were sometimes observed in farms with low Mortality. Therefore, four groups of flocks can be separated on the basis of their mortality, and LI values, illustrating the different possible combinations between these variables (Fig. 2):

- group A: 16 flocks with low mortality (mean 1·70%) and low LI (equals 0),
- group B: 13 flocks with low mortality (mean 2·37%) and high LI (mean 4·94),
- group C: three flocks with high mortality (mean 20·67%) and low LI (mean 0·11),
- group D: six flocks with high mortality (mean 22·41%) and high LI (mean 5·44).

Relationship between flock management and histomoniasis

Presence of H. meleagridis *at DE*

The presence of parasites at DE was related to both Farming and Preventive measures (Table 3): DE was correlated at the $P=0{\cdot}20$ level (Pearson's $r>0{\cdot}213$)

Table 2. *Results from the multiple correspondence analyses performed on the three sets: (a) Farming, (b) Preventive measures and (c) Sanitary situation. The table gives the proportion and cumulative proportion of variance of the original dataset explained by each factorial axis, the variables most correlated to the axis (see text for definition) and the modalities associated on the axis*

Axis	Proportion	Cumulative proportion	Variables most correlated (modalities that are associated)
(*a*) Farming			
F1	0·102	0·102	Freq-litter, Nb-build, Field surface, Sharing, Soil-field (>once per week/$\geqslant$3 buildings/ up to 40 m^2/turkey/successively/clay field)
F2	0·094	0·196	Cover, Topography, Chickens, Year, Field surface (no tree cover/slope field/absence of chickens/2003/20–40 m^2 per turkey)
F3	0·085	0·281	Time-farm, Water-field, Puddles, Nb-turkey (farming for >5 years/dry field/no puddle/>1000 turkeys)
F4	0·077	0·358	Sharing, Origin-build, Field surface (sharing with other species successively/ origin: poultry/>40 m^2 per turkey)
F5	0·071	0·429	Origin-build, Time-build, Freq-litter (origin: turkey/building <5 years old/ changing litter <once per month)
F6	0·067	0·496	Origin-build, Perches, Cover (origin: other species/presence of perches/tree cover)
F7	0·061	0·557	Year, Origin-water, Field surface (2003/municipal water/<20 m^2 per turkey)
F8	0·057	0·614	Litter, Freq-litter, Nb-turkey, Period (litter: other/changing litter less than once per month/<500 turkeys/May)
F9	0·050	0·664	Nb-build, Nb-turkey, Time-farm (1 or 2 buildings/up to 1000 turkeys/farming for >5 years)
F10	0·045	0·709	Ext-water, Chickens, Origin-water, Puddle, Freq-litter (drinking troughs in field/presence of chickens/municipal water/presence of puddles/once per week to once per month)
(*b*) Preventive measures			
P1	0·166	0·166	Year, pH (2002/acid pH)
P2	0·133	0·300	Treatment, investigations (treatment applied/veterinary investigations)
P3	0·116	0·415	Hygiene, prophylaxis (hygiene poor or intermediate/no prophylaxis)
P4	0·105	0·520	Hygiene, investigations (hygiene: bad/farmer investigations)
P5	0·086	0·606	Hygiene, acidification (hygiene: poor/no acidification)
P6	0·073	0·679	Hygiene, investigations (hygiene: intermediate/no investigations)
(*c*) Sanitary situation			
S1	0·205	0·205	*Capillaria, Eimeria, Heterakis* (*Capillaria* n.a., *Eimeria* n.a., *Heterakis* n.a.)
S2	0·168	0·373	Diarrhoea, Date-diarrhoea (no diarrhoea, date n.a.)
S3	0·112	0·485	Stress, Mycotoxin (stress by rain, presence of mycotoxins)
S4	0·097	0·582	Before, *Heterakis* (no histomoniasis before, presence of *Heterakis*)
S5	0·076	0·658	*Capillaria, Eimeria* (presence of *Capillaria*/absence or numerous *Eimeria*)
S6	0·071	0·729	*Eimeria*, Date-diarrhoea (presence of *Eimeria*/diarrhoea in Sept./Oct.)

n.a., Not available.

to factorial axes involved in Farming (axes F2, F6, F9), and Preventive measures (axes P1, P4). When tested separately, DE was highest in 2002 ($P=0·016$), when field surface was $<20\ m^2$ per individual ($P=0·012$) and when the field was covered by trees ($P=0·015$). DE frequency also tended to be high when turkeys had perches ($P=0·123$). Regarding Preventive measures, the relationship with pH was significant ($P=0·006$), but the presence of parasites was lower with neutral than with acidic pH. Finally, DE frequency was high when general hygiene was poor ($P=0·043$). Because of the effect of the year on DE, there might be a confounding between the effect of year and the effects of other variables. We thus performed a number of two-way ANOVAs to test for the effects of field surface, tree cover, pH and hygiene after taking into account the year effect, and to test for interactions between year and other variables (Table 4). After taking into account the effect of year, only surface remained a significant effect ($P=0·018$). Other variables and interactions were no longer significant (all $P>0·05$).

Table 3. *Modalities of the three sets of variables* (*a*) *Farming* (*b*) *Preventive measures and* (*c*) *Sanitary situation positively associated to the four variables used to quantify the presence of* H. meleagridis *recorded at the laboratory* (*Direct Examination and Lesion Index*) *and the severity of histomoniasis provided by the farmers* (*Morbidity and Mortality*) *and P value of the corresponding ANOVA tests. We reported significant relationships when P value* $<0{\cdot}05$ (*in bold type*) *and relevant trends when P value between 0·05 and 0·2* (*in normal type*)

	(*a*) Farming	(*b*) Preventive measures	(*c*) Sanitary situation
Direct examination (DE)	• Year 2002 (**$P=0.016$**) • Field surface <20 m² (**$P=0.004$**) • Tree cover in field (**$P=0.015$**) • Presence of perches ($P=0.123$)	• Year 2002 (**$P=0{\cdot}016$**) • Acid pH (**$P=0{\cdot}006$**) • Poor hygiene in building (**$P=0{\cdot}043$**)	• Year 2002 (**$P=0{\cdot}016$**)
Lesion index (LI)	• Field surface 20–40 m² (**$P=0{\cdot}046$**) • Field shared simultaneously with other poultry ($P=0{\cdot}076$)	—	• Presence of diarrhoea (**$P=0{\cdot}031$**) • Diarrhoea in Aug.–Oct. ($P=0{\cdot}125$)
Morbidity	• Water from municipal network ($P=0{\cdot}106$) • Litter changed less than once per week ($P=0{\cdot}152$)	—	• Presence of diarrhoea (**$P=0{\cdot}040$**) • Diarrhoea in June or July (**$P=0{\cdot}046$**)
Mortality	• Buildings always used for turkeys ($P=0{\cdot}128$)	—	• Presence of diarrhoea ($P=0{\cdot}054$) • Diarrhoea in June or July (**$P=0{\cdot}041$**)

Lesion Index

Although DE frequency and LI were correlated, LI was related to other factors than DE frequency: it was correlated with factorial axes F1, F9, P3, S2 and S5. LI was highest on farms where the field surface was intermediate ($P=0{\cdot}046$) and tended to be high when simultaneous sharing occurred ($P=0{\cdot}076$) (Table 3). Regarding Sanitary situation, LI was related to the presence and date of diarrhoea and was high when diarrhoea was reported ($P=0{\cdot}031$). LI also tended to be high when diarrhoea occurred from August to October ($P=0{\cdot}125$).

Morbidity

Morbidity was related to factorial axes: F7, F8, S2 and S3. Only origin of water had a relationship close to significance with Morbidity, with high morbidity when water only originated from the municipal network ($P=0{\cdot}106$) (Table 3). Morbidity tended to be low when the litter was changed at least once per week ($P=0{\cdot}152$). Finally, Morbidity was related to diarrhoea ($P=0{\cdot}040$) and date of diarrhoea ($P=0{\cdot}046$): the highest morbidity occurred when diarrhoea was observed in June or July.

Mortality

Mortality was related to similar factorial axes as Morbidity (F7, F8, S2, S3) but was also correlated to axes F6 and F9. Origin of the building was the variable closest to significance ($P=0{\cdot}128$), with lowest mortality when buildings had previously been used for species other than poultry, and highest mortality when buildings had always been used for turkeys (Table 3). Like Morbidity, Mortality was related to axis S2, i.e. diarrhoea ($P=0{\cdot}054$) and date of diarrhoea ($P=0{\cdot}041$), high mortality being observed when diarrhoea was observed at the beginning of the season.

DISCUSSION

This work represents a first step for identification of risk factors of histomoniasis in free-range turkey production.

Flock management

We detected a high diversity of management practices and no particular typology with homogenous groups of farms according to their practices in PDO Bresse

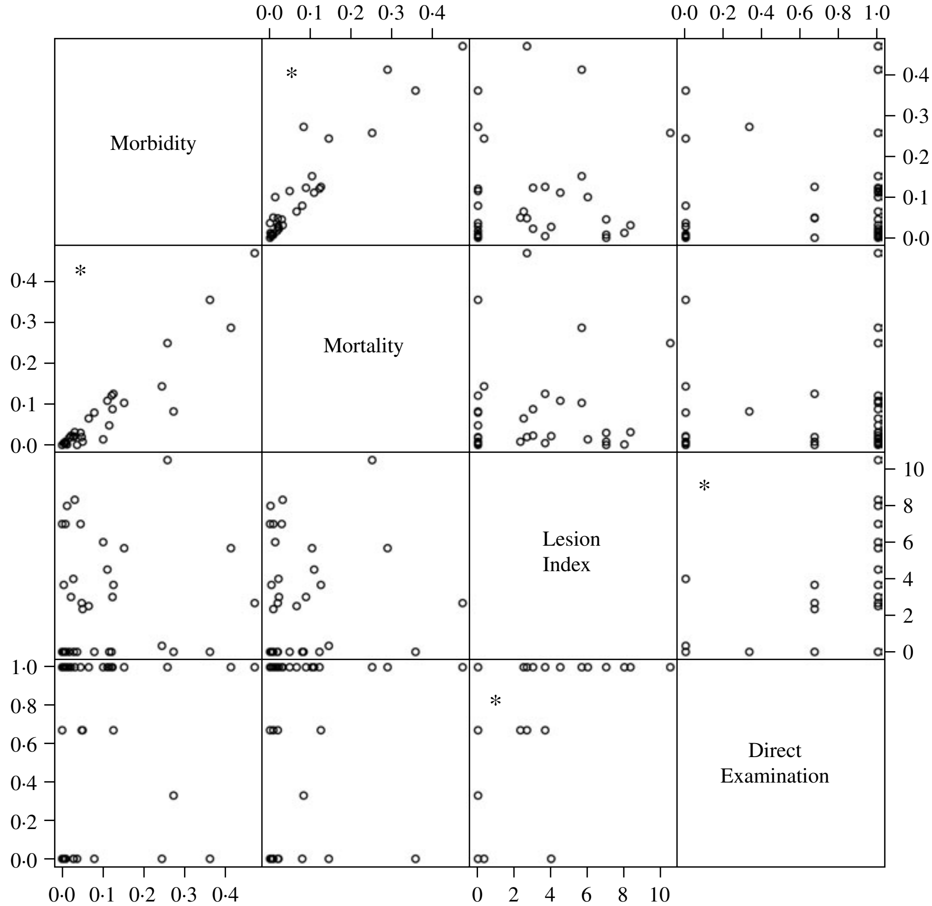

Fig. 1. Relations between the four variables describing the presence of *H. meleagridis* and severity of histomoniasis visualized in a scatterplot matrix. Two significant correlations are revealed [highlighted by an asterisk (*)]: between Morbidity and Mortality ($r=0{\cdot}942$, $P<0{\cdot}001$), and between Lesion Index and Direct Examination ($r=0{\cdot}554$, $P<0{\cdot}001$).

turkey production. In particular, no common traits were shared by large flocks (>1000 birds) compared to small flocks (<500 birds). This may be due to the fact that, within the constraints imposed by the PDO guarantee, farmers are free to follow their own usual practices.

Presence of *H. meleagridis* and severity of histomoniasis

The relationship between Morbidity and Mortality was strong because, in several farms, only mortality was detected by farmers. This relationship also means that morbidity was rarely observed alone. The specific indicators of blackhead disease, LI and DE, were not correlated to the levels of morbidity and mortality reported by farmers during rearing. In three flocks, high death losses were reported without confirmed histomoniasis diagnosis but with high coproscopic excretion of *Eimeria* oocysts. These cases may be due to misdiagnosis by farmers, based mainly on presence of diarrhoea. Typical sulphur-coloured droppings are early clinical signs of histomoniasis [9], although watery or dark-coloured diarrhoea is also observed in coccidiosis and in necrotic enteritis [16, 17]. Moreover, caecal and liver lesions can be confused with other diseases [1]. The presence of *H. meleagridis* at DE in 7/16 flocks without clinical signs and death losses supports the hypothesis of frequent asymptomatic circulation of the parasite, previously proposed by Zenner *et al.* [10]. Finally, in the 19 flocks with clinically confirmed histomoniasis, only six exhibited the moderate or severe outbreaks commonly described in the literature; the others suggested that *H. meleagridis* may be present and may cause important lesions with death losses <10%. A similar variability of mortality was reported in 113 standard turkey flocks with histomoniasis in France [2]. Our

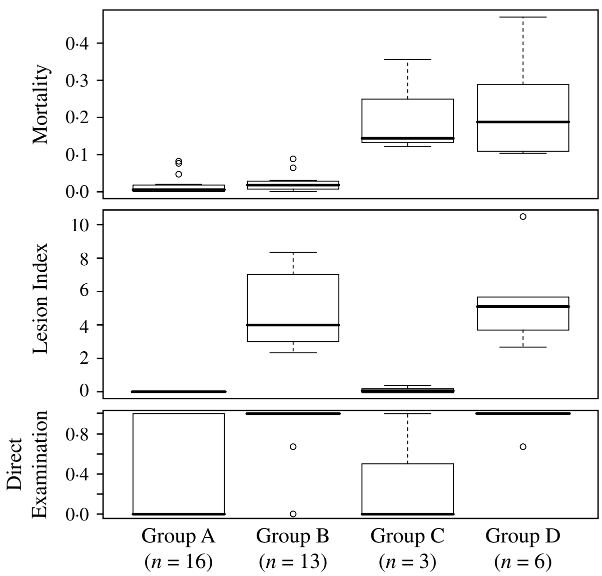

Fig. 2. Boxplot representations of Mortality, Lesion Index and Direct Examination for the 38 flocks rearranged in four groups according to the diagnosis of histomoniasis and the severity of the disease: group A without histomoniasis and rare presence of *H. meleagridis*; group B with small histomoniasis outbreaks; group C without confirmed histomoniasis but with other causes of death; group D with moderate and severe histomoniasis outbreaks. The 25th, 50th and 75th percentiles and extreme values are shown.

Table 4. *Two-way ANOVAs testing the effects of surface, cover, pH and hygiene on the presence of* H. meleagridis *by Direct Examination, after taking into account the effect of year*

Factor	D.F.	SS	*P*
Year	1	1·1629	0·010
Surface	2	1·4103	0·018
Year × surface	2	0·2109	0·513
Residual	32	4·9494	
Year	1	1·1629	0·017
Cover	1	0·1349	0·400
Year × cover	1	0·1189	0·429
Residual	34	6·3167	
Year	1	1·1629	0·013
pH	2	0·4469	0·086
Year × pH	1	0·0908	0·469
Residual	33	5·5860	
Year	1	1·1629	0·011
Hygiene	3	1·1124	0·093
Year × hygiene	2	0·5408	0·198
Residual	31	4·9174	

d.f., Degrees of freedom; SS, sum of squares.

observations cannot be compared with those reported previously [1, 9, 18, 19], because these authors had only reported the maximal mortality observed in severe outbreaks, or the total mortality attributed to blackhead in a country. When an infectious disease is present, the observed mortality rate in a population is determined by the equilibrium between host resistance, parasite virulence and/or environmental factors. Host resistance and parasite virulence were not investigated during this survey, but they obviously play an important part in differences of pathogenicity of isolates. The study of genotype variability of *H. meleagridis* has only recently started [20, 21].

Relationship between flock management and histomoniasis

We found statistically significant relationships, but also several non-significant trends that could be biologically relevant. Because of the small size of our dataset, we advocate that both results deserve consideration. DE frequency was significantly highest during 2002 and when small field surface per turkey was used, and tended to be high when low hygiene and acidic pH were observed; LI was high when field surface was intermediate and tended to be high when the field was shared simultaneously with other poultry species and when diarrhoea was reported at the end of the growth period. Morbidity was mostly related to the presence of diarrhoea at the beginning of the growth period, tended to decrease when litter was changed frequently and varied with origin of drinking water. Finally, Mortality was related to diarrhoea at the beginning of the season and tended to be high when buildings were always used for turkeys.

Between years variability

This was observed through high presence of *H. meleagridis* during 2002, without relation to losses at flock level. Many aspects of flock management differed between the two years of study (e.g. flock size, field surface or diagnosis of other disease). The use of nifursol in 2002, before its ban in April 2003, should have contributed to decreased parasite prevalence but it probably did not affect sufficient flocks (5/14 in 2002) to have any detectable effect. Alternatively, a true year-to-year variability effect may exist, for example due to meteorological conditions. This is plausible since the summer of 2003 was particularly hot and dry throughout the whole Europe.

Hygiene in buildings

As previously described, hygiene in buildings estimated by general cleanliness and litter quality is related to the presence of *H. meleagridis* and in the severity of histomoniasis. Poor hygiene in the building, wet litter and infrequent litter change probably increased the contact between birds and their droppings. Moreover, direct transmission from turkey-to-turkey was experimentally demonstrated [22] by cloacal drinking phenomenon [23]. Several factors have been identified in poultry for the occurrence of wet litter, including feed components, drinker design, depth of litter, temperature and relative humidity of the building, and clinical diseases in which diarrhoea is an important sign, in particular coccidiosis [24]. Presence of diarrhoea, often highlighted in our study, was associated on the one hand with blackhead disease diagnosis and lesions while on the other, diarrhoea observed at the beginning of the growth period (June or July), may cause rapid deterioration of litter quality. Therefore, all parameters that produce moisture in buildings, particularly general hygiene, wet litter and diarrhoea, contribute to increase the direct transmission to *H. meleagridis* and severity of histomoniasis.

Sharing a field simultaneously with other poultry species, especially chickens

Sharing a field is related to more serious caecal and liver lesions. This may be clinical reflection of cross-transmission of *H. meleagridis* between chickens and turkeys. Chickens are the major host for *Heterakis gallinarum*, the caecal worm used by *Histomonas* to survive from one flock to the next [1] and the best reservoir of infection [25, 26]. They probably contaminate fields with many worm eggs carrying histomonads. Therefore, severity of turkey lesions may be due to heaviest contamination and/or high pathogenicity of *Heterakis*-transmitted *Histomonas*. Our finding confirms previous results that sharing a field area is a risk factor for turkeys [1].

Acidic drinking water

Contrary to previous thinking, acidic drinking water has consistent association with the presence of *H. meleagridis* in turkey caeca. Drinking water is a prominent vehicle for pathogens in poultry flocks [27–29]. Physical and chemical water characteristics affect its bacteriological quality. Therefore, administration of organic acids via the drinking water to adjust the pH between 4 and 6 is considered an easy decontamination method, without creating residue problem [29]. Moreover, this practice is widespread in the poultry industry as a tool for improving bird performance [30], although the optimal pH range recommended for poultry is 6·8–7·5 [31]. In free-range turkey production, water acidification with acetic acid or vinegar is widely used to prevent histomoniasis, without scientific assessment. However, if the level of hygiene is low, drinking water may be intensively contaminated with dirty organic matter such as feed, faeces, soil or unchanged litter. For a long time, *H. meleagridis* was considered to have low environmental resistance, to be unable to form cysts or resistant forms [1], but cyst-like stages have recently been described from cultures [32]. Therefore, they should be involved in further studies on histomonad transmission, in particular by water-distribution systems.

This investigation of potential risk factors related to histomoniasis on free-range turkey farms is a first step to a good understanding of the epidemiology of this disease. The lack of treatment legally available in Europe for this type of study could lead to new ways and practices to improve the environment and management to decrease the occurrence of the disease. A point to be specifically investigated is the possible adverse effect of the acidification of drinking water in poultry productions.

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DECLARATION OF INTEREST

None.

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