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# Risk factors for *Listeria monocytogenes* contamination in French laying hens and broiler flocks

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# ABSTRACT

The objective of this study was to identify potential risk factors for *Listeria monocytogenes* contamination in French poultry production. Eighty-four flocks of layer hens kept in cages and 142 broiler flocks were included in this study. For each production type, a questionnaire was submitted to farmers and fecal samples were taken to assess the *L. monocytogenes* status of the flocks during a single visit to the farm. Two logistic regression models (specific to each production) were used to assess the association between management practices and the risk of *L. monocytogenes* contamination of the flock.

The prevalence of *L. monocytogenes*-positive flocks was 30.9% (95% CI: 21.0; 40.9) and 31.7% (95% CI: 24.0; 39.4) for cage-layers and broiler flocks, respectively. For layer flocks, the risk of *L. monocytogenes* contamination was increased when pets were present on the production site. When droppings were evacuated by conveyor belt with deep pit storage, the risk of *L. monocytogenes* contamination decreased significantly. Feed meal was found to be associated with a higher risk of *L. monocytogenes* contamination than feed crumb. For broiler flocks, the risk of *L. monocytogenes* contamination was increased when farmers did not respect the principle of two areas (clean and dirty) at the poultry house entrance. A first disinfection by thermal fogging and the absence of pest control of the poultry house before the arrival of the next flock was found to increase the risk of contamination. When litter was not protected during storage and when farm staff also took care of other broiler chicken houses, the risk of *L. monocytogenes* contamination increased significantly. In the case of the watering system, nipples with cups were found to decrease the risk of contamination.

# 1. Introduction

*Listeria monocytogenes* is recognized as an important foodborne pathogen in many industrialized countries. The consumption of food contaminated by *L. monocytogenes* 

has been identified as the main transmission route for this pathogen in both humans and animals. In humans, Listeriosis is a rare but serious illness that may lead to abortion or serious cases of meningitis or encephalitis, and even death. Cases are observed especially in vulnerable and immunocompromised subjects such as newborn infants, pregnant women, cancer or AIDS patients and the elderly. Because of the high fatality rate (20–30%), Listeriosis ranks among the most frequent causes of death due to foodborne illnesses (Vaillant et al., 2005; EFSA,

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2009). Since 2006, an increasing number of Listeriosis cases have been observed in several European Union countries, including France, predominantly in people of more than 60 years of age (Denny and McLauchlin, 2008; Goulet et al., 2008a). In 2007, the incidence of Listeriosis in France reached 5.0 cases per million inhabitants (Goulet et al., 2008b).

L. monocytogenes is widely disseminated in the environment (soil, surface water, plants, and infected animals). The ubiquitous character of the pathogen inevitably results in the contamination of numerous food products (such as milk and dairy products, raw vegetables, meat and meat products and seafood). Poultry, poultry products, eggs and egg products have rarely been reported to be involved in L. monocytogenes outbreaks (Gottlieb et al., 2006). In most studies, the contamination of poultry meat occurs during the slaughtering and processing phases (Lawrence and Gilmour, 1994; Chasseignaux et al., 2002; Rorvik et al., 2003; Barbalho et al., 2005; Loura et al., 2005). Very few studies have reported an incidence of L. monocytogenes at the farm level (Chemaly et al., 2008). Our study, as part of a European project "Poultryflorgut", is a continuation of Chemaly's study, aiming to investigate the potential risk factors for L. monocytogenes contamination in poultry production.

While many published studies have focused on the risk factors associated with Salmonella and Campylobacter contamination in poultry flocks, data concerning risk factors for L. monocytogenes contamination in poultry flocks has not been published to date. The main risk factors for poultry contamination by those pathogens included (a) the type of housing (Methner et al., 2006; Namata et al., 2008; Huneau-Salaün et al., 2009; Van Hoorebeke et al., 2010), (b) large flock sizes (Mollenhorst et al., 2005; Huneau-Salaün et al., 2009), (c) farms with hens of varying ages (Huneau-Salaün et al., 2009), (d) delivery trucks passing near the entrance of the poultry house (Huneau-Salaün et al., 2009), (e) the type of sample (Huneau-Salaün et al., 2009), (f) the season (Kapperud et al., 1993; Angen et al., 1996; Refrégier-Petton et al., 2001; Bouwknegt et al., 2004; Huneau-Salaün et al., 2007; McDowell et al., 2008; Ellis-Iversen et al., 2009), (g) the presence of rodents (Kapperud et al., 1993; Refrégier-Petton et al., 2001; Huneau-Salaün et al., 2007; McDowell et al., 2008), (h) inadequate levels of hygiene in the poultry house (Kapperud et al., 1993; Van de Giessen et al., 1996; Evans and Sayers, 2000; Hald et al., 2000; Gibbens et al., 2001; McDowell et al., 2008; Aury et al., 2010), (i) the number of poultry houses on the farm (Angen et al., 1996; Refrégier-Petton et al., 2001; Bouwknegt et al., 2004; McDowell et al., 2008), (j) the presence of other animals on the farm (Kapperud et al., 1993; Van de Giessen et al., 1996; Van de Giessen et al., 1998; Bouwknegt et al., 2004; Cardinale et al., 2004), and (k) farm staff caring for several houses (Kapperud et al., 1993).

The aim of our study was to assess the relationships between poultry farm characteristics and management practices with the presence or absence of *L. monocytogenes* contamination of laying hen and broiler flocks at the end of the production period.

#### 2. Materials and methods

#### 2.1. Study design

Two studies were carried out in France, the first one between October 2004 and September 2005 for laying hens, the second one between October 2005 and September 2006 for broiler flocks. French commercial holdings listed by the French ministry of Agriculture, with at least 1000 laying hens producing table eggs and 5000 broiler chickens were included in these studies. The flocks were investigated in the second half of production within a maximum of 9 weeks and 3 weeks before depopulation for laying hens and broiler flocks, respectively. The number of flocks to be sampled was based on an expected flock-prevalence of 10% for broilers and 15% for layers with an accuracy of 5% and 95% confidence limits. Holdings to be visited were randomly selected, regardless of the housing system. If more than one flock were concerned per holding, only one was randomly selected. The epidemiological unit was the flock, defined as a group of birds kept in the same poultry house.

#### 2.2. Data collection

Each flock was visited once. For cage-layers, the *L. mono-cytogenes* status of the flock was assessed by taking five samples of faeces (pooled samples inside the cage) and two dust samples (from egg belts and from underneath the cages). While for broiler chickens, sampling consisted of five different pairs of boot swabs per flock inside the poultry house wherein floor area was divided into five equal sectors for sampling.

Exposure to potential risk factors was evaluated with the help of a questionnaire specific to each production type (laying hen or broiler), submitted to the farmer by a technician from the French Veterinary Authorities at the same time samples were collected. To standardize the conditions of collection of answers, a guideline was provided to the technician. Both questionnaires (available upon request) were structured in five parts, and covered the parameters listed in Table 1. The laying hen flock questionnaire also included questions regarding egg production characteristics. Most of the questions were in the form of a checklist or multiple-choice, with the exception of those relative to dates or precise numbers (age at slaughter, etc.) which were open-ended. Technical documents presents on the poultry-house the day of visit (husbandry register, food delivery order, etc.) were checked, and the rearing characteristics and sanitary events occurring during the rearing period were also recorded. The questionnaires for laying hens and broilers consisted of 110 and 147 questions, respectively (34% and 30% open-ended questions, respectively). These questionnaires were pre-tested at the beginning of the study and validated by several French Veterinary Authorities and poultry veterinarians. Samples and questionnaires were sent to the French Agency for food, environmental and occupational health safety laboratory (Anses Ploufragan-Plouzané) on the day of sampling and no later than 7 days after sampling for holdings located far from the laboratory. Informations from these ques-

Table 1

Parameters covered by the questionnaires and the number of questions related for each production type (laying hens and broilers) in each section.

		Number of questions		
		Laying hens	Broilers	
Farm characteristics	Location, poultry production on the farm (size, type, number of poultry houses), other animal productions, domestic animals, farm staff characteristics	24	30	
Poultry house characteristics	Age of the poultry house, ventilation system, size, changing room, flooring material in poultry room and changing room, footbath, poultry house entrance, hand washing, clothes and shoes	18	44	
Management practices	Flock size, type of production, sex, genetic strain, stocking density, litter material and litter condition, heating system, feeder and drinker systems (origin, treatments)	22	34	
Biosecurity	Access to facilities and surroundings, hygiene procedures, cleaning and disinfection procedures, control of rodents and insects	18	20	
Clinical parameters and treatments For laying hens only	Antibiotic treatments, occurrence of clinical disease, peak of mortality, medication	5	19	
Eggs production	Production type, stocking eggs, packaging eggs	23		

tionnaires were recorded by a technician from the French Agency for food safety laboratory in a Microsoft ACCESS 2000 database.

2.3. L. monocytogenes isolation and identification

All the samples were analysed for L. monocytogenes detection following a modified protocol based on the NF EN ISO11290-1 standard. In the case of laying hen samples, the first enrichment consisted of diluting faeces or dust (1/10) in half strength Fraser broth and incubating for  $24\pm2h$  at 30 °C. In the case of broiler samples, the first enrichment consisted of suspending 25 ml taken from the initial broth where the boot swabs were enriched (buffered peptone water) into 225 ml (1/10) of half strength Fraser broth and incubating for  $24\pm2h$  at  $30^{\circ}C$ . A first isolation was carried out on ALOA agar (AES, Bruz, France) and five characteristic colonies were purified on TSAYe for identification and serotyping. For the second enrichment, 0.1 ml of half strength Fraser broth transferred into Fraser broth (AES, Bruz, France) and incubated for  $48\pm2h$  at 37 °C. A second isolation was carried out on ALOA agar and five characteristic colonies were isolated on TSAYe for identification and serotyping. A total of 10 characteristic colonies per positive sample were identified according to the miniaturized method developed by the laboratory of Anses Ploufragan (Toquin and Lahellec, 1987). In order to identify characteristic colonies, we performed sugar fermentation (xylose, rhamnose), hemolysis and the camp test. All the characteristic strains isolated were serotyped with sera purchased from Eurobio, Les Ulis, France.

# 2.4. Definition of the outcome variable

The epidemiological unit was the flock. A flock was considered contaminated by *L. monocytogenes* if at least one sample taken from the poultry house tested positive. The outcome variable "*Listeria monocytogenes status*" was dichotomous (contaminated flock versus non-contaminated flock). Statistical analyses were carried out

using SAS 9.1 software. Prevalence of positive flocks for laying hens and broilers and the associated 95% exact CI were computed with the FREQ procedure of SAS 9.1.

#### 2.5. Statistical procedure

Two logistic regression models were built: one for laying hens reared in cages and one for broiler flocks. All explanatory variables were coded categorically. Whenever possible, the number of categories was limited to ensure a category frequency higher than 10%.

A two-stage procedure was used for both analyses in order to assess the relationship between explanatory variables and the *L. monocytogenes* status of the flock. In a first step, a univariate analysis was performed to relate *L. monocytogenes* contamination of the flock to each explanatory variable, using the Chi-square test or Fisher exact test. Only factors associated with *L. monocytogenes* contamination of the flock ( $\chi^2$  test, p < 0.25) were selected for the multivariate model. All bilateral relationships between the selected variables were evaluated by Chi-square test or Fisher exact test to evaluate a potential colinearity. When relationships between variables showed a strong statistical association (p < 0.05), only one of the two variables of interest (i.e., the one most closely statistically related to the outcome variable) was chosen.

In the second step, all factors retained in the previous step were included in a logistic multiple regression model (proc LOGISTIC; SAS Institute Inc.). Multiple logistic regression models were built using a backward elimination procedure based on the Wald test, using p > 0.05 as a criterion for elimination. The variable with the highest p was removed and the model was rerun until all remaining factors were significant (p < 0.05). Variables were only removed if this did not affect the coefficients of other variables included in the model by more than 30%. Goodness of fit for the final model was assessed using the following tests: Pearson  $\chi^2$ , deviance and Hosmer-Lemeshow (Hosmer and Lemeshow, 1989). Odds ratios were converted into relative risks according to the method proposed by Beaudeau and Fourichon (1998). Interactions between

# Table 2

Distribution of *Listeria monocytogenes* positive samples among the 7 samples collected in 26 cage-reared hens flocks positive for *L. monocytogenes* at the end of the production period, France, 2004–2005.

Number of positive samples	1	2	3	4	5	6	7	Total
Number of positive flocks % <i>L. monocytogenes</i> positive flocks	12	6	5	2	1	0	0	26
	46.2	23.1	19.2	7.7	3.8	0.0	0.0	100.0

the independent variables in the final model were as well tested.

# 3. Results

In the case of cage flocks, the median age of hens at sampling was 62 weeks (mean 64; standard deviation (SD) 7.2; inter-quartile range (IR) 5). The median number of hens per cage was 4 (mean 6; SD 3.8; IR 2), with a median area per bird of 572 cm<sup>2</sup>/bird (mean 595; SD 93.9; IR 78.5).

Overall, 26.8% (38 of 142) of the broiler farms included in the study had a single broiler house, while 38.7% (55) had two houses, 12.7% (18) three houses, and 21.8% (31) four or more houses. The majority of the farms (92; 64.8%) also had other animal species (cattle, pork, sheep) and 26.1% had other avian species (laying hens, turkeys, ducks). The median number of broilers per flock (broiler house) was 13,085 (mean 14,568; SD 11570; IR 19,264), while the median number on each farm was 16,386 (mean 25,917; SD 26668; IR 26,358). The median age of the broilers at sampling was 48 days (mean 56; SD 23.7; IR 44).

In 26 (31%) of the 84 cage-layer flocks studied, *L. mono-cytogenes* was isolated in at least one sample. The number of *L. monocytogenes*-positive samples varied between flocks. Of the 26 positive cage flocks, 46.2% presented only 1 positive sample, and none presented samples that were all positive (Table 2). Three risk factors were significantly associated with *L. monocytogenes* contamination at the end of the laying period (Table 3). Presence of pets on the production site was found to increase the risk of contamination. Concerning dropping evacuation, the risk of *L. monocytogenes* contamination decreased when droppings were evacuated by conveyor belt with deep pit storage or deep pit only. Lastly, feed meal was found to be associated with

a higher risk of *L. monocytogenes* contamination than feed crumb.

Among the 142 broiler flocks studied, 45 tested positive for *L. monocytogenes*. The prevalence of *L. monocytogenes* contamination in broiler flocks was 31.7% (95% Cl: 24.0; 39.4). The number of *L. monocytogenes*-positive samples also varied between flocks. Among the 45 positive broiler flocks, 35.6% had only 1 positive sample whereas all 5 samples were positive in 8.9% of the flocks (Table 4).

Six variables were significantly associated (p < 0.05)with the status of *L. monocytogenes* in the broiler flock at the end of the rearing period (Table 5). The risk of L. monocytogenes contamination increased (i) when farmers did not respect the principle of two areas (clean and dirty) at the poultry house entrance, (ii) when first disinfection was not carried out by spraying, (iii) when there was an absence of pest control of the poultry house before the arrival of the next flock, (iv) when litter was not protected during storage, and (v) when farm staff cared for other broiler chicken houses. The risk of L. monocytogenes contamination decreased when the watering system consisted of nipples with cups. Thus, the variables retained for the final model were mostly related to the hygienic status of the poultry house and the sanitary measures applied to the flocks.

# 4. Discussion

This is the first time that an epidemiological study of risk factors for *L. monocytogenes* contamination in laying hen and broiler flocks was carried out in France at the national level.

#### Table 3

Variables and variable categories in the final logistic-regression model of risk factors for *L. monocytogenes* contamination of 84 French cage flocks at the end of the production period.

Variables	% L. monocytogenes	Logistic regression model <sup>a</sup>					
	positive flocks	OR <sup>b</sup>	95% CI <sup>d</sup> (OR)	RR <sup>c</sup>	95% CI (RR)	р	
Dropping storage system							
Deep pit	16.7	0.24	0.06-1.01	0.36	0.10-1.01	0.05	
Evacuated by conveyor belt with deep pit storage	11.8	0.13	0.02-0.69	0.21	0.04-0.78	0.02	
Evacuated by conveyor belt with dunghill storage	42.9	1		1			
Feed							
Feed meal	36.9	6.11	1.19-31.31	4.20	1.13-19.37	0.03	
Feed crumb	10.5	1		1			
Pets							
Yes	44.4	3.20	1.05-9.71	2.13	1.04-4.15	0.04	
No	24.6	1		1			

<sup>a</sup> Intercept = -2.1304 (p = 0.0096), model d.f. = 4, Hosmer-Lemeshow goodness-of-fit test  $\chi^2 = 1.33$  (p = 0.97).

<sup>b</sup> Odds ratio.

<sup>c</sup> Relative risks.

<sup>d</sup> Confidence interval.

#### Table 4

Distribution of *L. monocytogenes* positive samples among the 5 samples collected in 45 broiler flocks positive for *L. monocytogenes* at the end of the rearing period, France, 2005–2006.

Number of positive samples	1	2	3	4	5	Total
Number of positive flocks	16	12	8	5	4	45
% L. monocytogenes positive flocks	35.6	26.7	17.8	11.0	8.9	100.0

#### Table 5

Variables and variable categories in the final logistic-regression model of risk factors for *L. monocytogenes* contamination of 142 French broiler flocks at the end of the rearing period.

Variables	% L. monocytogenes	Logistic regression model <sup>a</sup>					
	positive flocks	OR <sup>b</sup>	95% CI <sup>d</sup> (OR)	RR <sup>c</sup>	95% CI (RR)	р	
Watering system							
Nipples with cups	22.9	0.17	0.05-0.65	0.38	0.28-0.79	0.009	
Nipples without cups	58.8	1		1			
Drinkers	34.6	0.34	0.09-1.23	0.53	0.35-1.12	0.100	
Respect of the principle of th	e two areas (clean and dirty) a	it the poultry ho	use entrance				
Yes	6.9	1		1			
No	38.1	10.54	1.97-56.40	6.85	1.66-34.50	0.006	
First disinfection							
By spraying	27.8	1		1			
By thermal fogging	48.2	3.76	1.33-10.66	2.21	1.19-3.46	0.013	
Pest control of the poultry-h	ouse						
Yes	26.7	1		1			
No	53.9	3.69	1.32-10.32	2.17	1.19-3.37	0.013	
Litter storage protected							
No	43.2	3.99	1.56-10.25	2.41	1.37-4.12	0.004	
Yes	26.5	1		1			
Farm staff takes care of othe	r broiler chicken houses in the	holding					
Yes	37.8	2.55	1.01-6.48	1.98	1.01-4.09	0.048	
No	21.2	1					

<sup>a</sup> Intercept = -3.2425 (p = 0.0032), model d.f. = 7, Hosmer-Lemeshow goodness-of-fit test  $\chi^2 = 8.34$  (p = 0.50).

<sup>b</sup> Odds ratio.

<sup>c</sup> Relative risks.

<sup>d</sup> Confidence interval.

## 4.1. Caged hens

The presence of pets on the production site was found to increase the risk of *L. monocytogenes* contamination in laying hen flocks. *L. monocytogenes* is commonly found in the excrement of a large number of animal species (Larpent, 2000), but few studies report the incidence of *L. monocytogenes* in pets (Weber et al., 1995). Pets can then be vectors and play a role in the multiplication of *Listeria* by excreting a great number into the environment. A study carried out in broiler flocks showed that pets around the poultry house (within 5–10 m) associated with the absence of sanitary barriers at the poultry house entrance, was significantly associated with an increased risk of *Campylobacter* contamination (Hald et al., 2000).

Feed meal was also found to increase the risk of *L. monocytogenes* contamination in laying hen flocks. Various sources of contamination (during food manufacturing, storage, or distribution) can have an impact on the birds' health. According to Skovgaard (1990), feed can play a role in the transmission of *Listeria*, which has been found in poultry in pellet form (Blank et al., 1996; Whyte et al., 2003).

Some studies carried out in pigs have shown that wet feed was significantly related to an increased risk of *L. monocytogenes* contamination (Skovgaard and Norrung, 1989; Beloeil et al., 2003).

The evacuation of droppings by conveyor belt with deep pit storage was found to decrease the risk of *L. monocytogenes* in laying hen flocks. Droppings or manure can play a role in the transmission of *L. monocytogenes* when development conditions are favorable. *L. monocytogenes* belongs to the terrestrial ecosystem and has an important incidence on the environment (Skovgaard, 1990).

#### 4.2. Broiler flocks

Nipples with cups appear to have a protective effect against L. monocytogenes contamination of broiler flocks. Nipples with cups avoid wasting water and also help maintain the litter in good condition, i.e., dry, healthy, and crumbly with minimal ammonia release. The drinking water is thus protected against microbial contamination caused by bird beaks soaking in the traditional drinking troughs. Conversely, the absence of cups or badly regulated troughs facilitates moisture in the litter (i.e., fermentation and ammonia release) as well as the formation of a crust that constitutes an ideal outbreak site for various infectious agents, thus increasing health risks. Moisture in the litter is also influenced by the nature of the litter itself (e.g., straw, wood shavings), since each material has a different absorption rate. In our study, the type of litter was not significantly related to the watering system used.

*L. monocytogenes* is commonly found in soil and on plant material, particularly that undergoing decay, support of development of high numbers of *L. monocytogenes* (Rocourt and Seeliger, 1985; Fenlon, 1999). Survival of *L. monocytogenes* in soil depends on the soil type and its moisture content (Welshimer, 1960). Thus, humid organic material favors *L. monocytogenes* growth.

In terms of biosecurity measures, we found that the risk of *L. monocytogenes* contamination in broiler flocks increased when the farmer did not respect the principle of having two areas at the poultry house entrance (dirty area where farm staff and visitors must change clothes and shoes/clean area where specific clothes and shoes must be used). The presence of a changing room at the poultry house entrance limits the risk of contamination. The changing room has to be clean, tidy, and disinfected between each flock.

Some studies carried out on broiler chickens reported a reduced risk of *Salmonella* (Henken et al., 1992; Davies et al., 1997; Tablante et al., 2002) and *Campylobacter* flock contamination (Van de Giessen et al., 1996; Evans and Sayers, 2000; Hald et al., 2000; Gibbens et al., 2001; McDowell et al., 2008) if specific hygienic measures (use of disinfectant foot dips, washing of hands, changing clothes and shoes, respect of the two areas) were taken by people entering the broiler house. Farm workers were thus considered as mechanical vectors of contamination from the environment. A study carried out on pigs showed that the absence of a changing room at the entrance of pig rearing facilities was significantly associated with an increased risk of *L. monocytogenes* contamination (Beloeil et al., 2003).

Spraying at the first disinfection appears to have a protective effect against *L. monocytogenes* contamination in broiler chickens. Disinfection is one of the essential hygiene practices classically stipulated in farm guidelines. This biosecurity measure aims to protect animals from infection by pathogens and to prevent their spread beyond a contaminated flock. In France, it is generally carried out in two times: the first by spraying and the second by thermal fogging. In this survey, the number of disinfections was significantly related to the disinfection procedure used (p = 0.0002). Davies and Wray (1996) have shown that successive treatments by spraying and thermal fogging eliminate the maximum of germs.

*Listeria* is not particularly resistant to disinfectants (Larpent, 2000). However, Best et al. (1990) shows that disinfection is inefficient in presence of organic matters. In order to obtain optimal disinfection, it is thus necessary to respect cleaning and disinfection procedures. Moreover, the ability of *L. monocytogenes* to develop biofilms on a variety of surfaces makes a disinfection treatment difficult. Many studies have shown *L. monocytogenes* resistance on the action of disinfection of biofilms (Mah and O'Toole, 2001; Moretro and Langsrud, 2004; Robbins et al., 2005).

Absence of pest control at the poultry house before the arrival of the next flock was found to increase the risk of *L. monocytogenes* contamination in broiler flocks. Poultry houses associated with animal diversity, and favorable temperature and humidity conditions with organic matter facilitate insect development (litter beetles, flies), which can serve as vectors for various pathogens. Poultry fae-

ces are a privileged substrate for the development of many species. Thus, good sanitary practices at the poultry house within the flock and between flocks limit insect development. Several studies have shown that an inadequate pest control system favors the persistence of *Campylobacter* (Shane et al., 1984; Refrégier-Petton et al., 2001; Hald et al., 2004) and its transmission between two consecutive flocks in the poultry house.

Failure to properly protect litter during storage was found to increase the risk of *L. monocytogenes* contamination in broiler flocks. Litter stored in poor conditions (without protection, open house, not covered, on the floor) will more easily come in contact with wild or domestic animals. Wild birds can also be vectors of *L. monocytogenes* transmission and play a role in the multiplication and dissemination of *Listeria* (Weis and Seeliger, 1975; Fenlon, 1985; Bouttefroy et al., 1997; Yoshida et al., 2000; Hellstrom et al., 2008). Several studies on silage (Fenlon, 1985; Quessy and Messier, 1992) have also shown the role of wild animals in contamination by *L. monocytogenes*.

Moreover, if litter is not protected from moisture, *L. monocytogenes* can survive and multiply before even entering the poultry house. The bacterium could then be introduced via the litter into the poultry house. Thus, litter should always be stored in an enclosed location, protected from wild birds and with an effective pest control in order to avoid contamination by wild life. It is important to avoid bringing in wet organic material with the new litter.

The risk of *L. monocytogenes* contamination in broiler flocks was found to increase when the farm staff cared for other broiler houses within the holding. The presence of other poultry flocks raised at the same production site probably facilitates the transmission of pathogens between flocks via clothes and shoes of the personnel. Several studies carried out on broilers have also identified an increase in *Campylobacter* contamination when farm personnel cared for other poultry houses on the farm (Kapperud et al., 1993) or when there are many poultry houses on the site (Refrégier-Petton et al., 2001; Bouwknegt et al., 2004; McDowell et al., 2008).

# 5. Conclusion

Our investigation showed high prevalence (around 30%) of *L. monocytogenes* in both laying hens and broiler flocks. Differences observed in risk factors could be related to flock management practices within each poultry production. These factors were mostly related to the hygienic status of the house and sanitary practices observed at the farm. Most of these factors have already been reported in studies related to *Salmonella* and *Campylobacter* in poultry flocks, but this is the first time such results are presented for *L. monocytogenes*.

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