Associations between animal welfare indicators and *Campylobacter* spp. in broiler chickens under commercial settings: A case study

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Highlights

• Welfare and health indicators may be used to identify *Campylobacter*-positive flocks.

• Predictors are a high prevalence of severe lesions on the footpad and arthritis.

• Future studies could assess welfare indicators for predicting *Campylobacter* status in a broiler flock.

Abstract

Few studies have previously investigated how poor animal welfare might be associated with infection of zoonotic pathogens in humans. This paper assesses the predictive value of the presence of *Campylobacter* spp. in broiler chicken flocks when animal-based measures related to footpad dermatitis, hock burns, body lesions and arthritis are identified under commercial conditions (high density). The study population included 32 flocks analysed on farm and at slaughter, slaughtered between April and August 2008 in six different slaughter plants in Brittany, France. Welfare and health indicators are those indicated by the European legislation and sampling was carried out in the framework of the European baseline survey on the prevalence of *Campylobacter* in broiler chicken. Caecal contents, sampled both on farm and at slaughter, and carcass skin samples from the neck and breast at slaughter, were investigated for the presence of *Campylobacter* spp. Logistic models/classification trees were used to estimate the probability of the presence (or absence) of a specific foodborne pathogen in a flock based on specific animal-based measures (or combinations of measures) in order to study the potential relationship between welfare indicators and foodborne pathogen prevalence/incidence levels. On farm, flocks with more than 25% animals with severe lesions on between 25 and 50% of the footpad are predicted to be *Campylobacter*-positive whereas flocks where less than 13 individuals have arthritis are predicted to be *Campylobacter*-negative. The error rate on farm and at slaughter was 10 and 4% respectively indicating good predicting abilities. A poor welfare environment may result in stress, which reduces chicken immunocompetence making them more susceptible to *Campylobacter* spp. An infection with *Campylobacter* spp may lead to impaired defence and susceptibility to other pathogens which may result in greater intestinal excretion. Poor welfare and high growing rate lead to digestive troubles that lead to litter humidity. Litter humidity that, among other things, causes footpad dermatitis may also influence the horizontal transmission of the *Campylobacter* spp. infection due to the normal coprophagic behaviour of poultry. Reducing welfare problems by a better management of rearing conditions would not only improve broiler welfare, but it would also decrease the risks of *Campylobacter* contamination, of carcass condemnations and of economic loss for the poultry industry.

1. Introduction

It has been suggested that animal-based welfare measures should be used to assess the most relevant welfare concerns in broiler farming (EFSA, 2012). They indicate the health status and wellbeing of the animals, but their predictive value for food safety remains largely uninvestigated. The Welfare Quality® project focused on animal-based measures to be monitored at slaughter to assess levels of welfare in a standardised way (Keeling, 2009). Some animal-based measures such as footpad dermatitis, hock burns and breast burns, are currently collected in fulfilment of European legislation (Council Directive 2007/43/EC). These animal-based measures are visually detected and recorded at slaughter (EFSA, 2012); however, different scoring categories and definitions are used e.g. for contact dermatitis (Algers and Berg, 2001; Allain et al., 2009; Michel et al., 2012) and significant variability exists in the measures and related scoring system used (Butterworth et al., 2015). According to the Directive 2007/43/EC, Annex III states that information regarding mortality rate on farm should be recorded at the slaughter plant. Moreover, in the post-mortem inspection, the official veterinarian has to identify any possible indications of poor welfare, such as abnormal levels of contact dermatitis, parasitism and systemic illness. In the case of findings consistent with poor welfare, the official veterinarian has to communicate these to the owner and to the local competent authorities. Although resources and management inputs may be correctly applied according to legislations, this does not guarantee high standards of animal welfare.

Competent authorities are also responsible for monitoring relevant food safety parameters on carcasses according to Regulation EC 2073/2005 and may have national voluntary monitoring programmes in place. Therefore, databases at slaughter plants can be exploited for data containing both chicken welfare and food safety information.

In Europe, thermotolerant *Campylobacter* continues to be the most reported gastrointestinal bacterial pathogen in humans (71 per 100,000 habitants in 2014). More than 80% of the human *Campylobacter*iosis in Europe is caused by C. jejuni while C. coli is responsible for 7% of the human cases (EFSA and ECDC, 2015). Infection by thermotolerant *Campylobacter* is not considered a health problem in poultry (Wagenaar et al., 2013). No clinical signs or mortality are noticeable in *Campylobacter* infected poultry. Therefore, the current meat inspection system, relying on Food Chain Information analysis and on visual inspection of birds and carcasses, is of no use for detecting poultry contaminated with *Campylobacter*. It is also known that intensive farming favours shedding and horizontal transmission of *Campylobacter* spp. in poultry (Cogan et al., 2007; Bull et al., 2008; Verbrugghe et al., 2012). Bull et al. (2008) found that *Campylobacter*-positive flocks were associated both with a higher level of rejections at slaughter, because of gross pathology lesions due to general infections, and because of digital dermatitis. The increased incidence of hock marks and footpad dermatitis in fast-growing breeds infected with *Campylobacter* (Williams et al., 2013) affects welfare. Hock marks and footpad dermatitis are painful conditions, which reduce welfare in broiler chickens (EFSA, 2012). Infectious and non-infectious arthritis are also painful conditions in broilers and can cause lameness (EFSA, 2012). Infectious arthritis may be caused by a number of different pathogens (virus, bacterial, mycoplasma). It can also be the consequence of systemic infection or cellulites of the hock caused by contact with faecal material (i.e. litter related) (Xavier et al., 2010). Non-infectious arthritis is related to genetic selection in broiler chicken (Bradshaw et al., 2002).

The objective of this paper is to assess the predictive value for the presence of *Campylobacter* spp. in flocks when animal-based measures related to footpad dermatitis, hock burns and body lesions are identified within flocks from commercial settings. The article is intended as a case study to generate hypotheses. Based on our findings we provide recommendations on the best animal-based measures to estimate the probability of flocks being *Campylobacter*-positive.

2. Material and methods

2.1. Study population and study design

Sampling was carried out in January-December 2008, within the framework of the EU baseline survey on the prevalence of *Campylobacter* in broiler chicken. The epidemiologic unit was the slaughtered flock, previously placed in the house at the same time. The study population included 32 flocks of broiler chicken analysed both on farm and at slaughter; the flocks were slaughtered between April and August 2008 in six different slaughter plants in Brittany, France. Each flock belonged to a conventional farm (i.e. no free-range or organic farms) and included on average 27523 ± 9239 (SD) animals (range: 12046–54523). The two main genotypes represented were Hubbard (44%) and Ross (37%). Broiler age at slaughter was on average 38 ± 5 days (range: 28 – 47 days) and the average slaughter weight was 1.77 ± 0.53 kg (range: 1.25–3.19 kg). The average mortality at day 10 of the rearing period was 1.37% ± 0.31 (1.06–1.69 95% CI) and mortality at the end of the rearing period was 2.13% ± 0.39 (range: 1.74% – 2.52%). Flocks were not thinned.

2.2. Sample collection on farm and at slaughter

The method for collection of samples was the same as that described by Allain et al. (2014). On farm, five broilers per flock were euthanized with pentobarbital; their caecal contents were collected and pooled in a sterile bag one week before slaughter.

Sampling collection at the slaughter plant was carried out according to Hue et al. (2011). Caecal content from 10 randomly selected broilers per flock were collected at evisceration and pooled in a sterile bag. From the same flock one randomly-chosen carcass was collected after chilling. From this carcass, neck and breast skin samples were collected. All the samples were investigated for the presence of *Campylobacter* spp.

Bacteriological analyses were carried out within 24 h from sample collection at the French National Reference Laboratory for *Campylobacter* (French Agency for Food, Environmental and Occupational Health- Anses).

2.3. Welfare assessment at slaughter

At the slaughter plant, animal-based welfare measures were scored on a random sample of almost 400 carcasses per flock, inspected on the slaughter line after scalding. Any sign of contact dermatitis was recorded (Allain et al., 2009). Five body areas were defined for welfare scoring: i) footpad, ii) digits, iii) tarsus, iv) ventral side of body and v) dorsal side of body.

Footpad lesions were recorded according to a 10-point scale (Table 1).

Table 1. Scoring system for footpad lesions.

	Extent (%) of the foot pad affected		
Foot lesion	<25	25–50	>50
No lesions	P0		
Keratosis/papilloma	P1	P2	P3
Superficial lesion (crust)	P4	P5	P6
Severe lesion (ulcer)	P7	P8	P9
Healing	P10		
Overall score	OP		

Digit lesions were scored on a 5-point scale where score 0 indicated no dermatitis, score 1–4 depending on the presence of dermatitis and the number of toes affected. Tarsus lesions were recorded on a 7-point scale and arthritis was also recorded (Table 2).

Table 2. Score ranking and description of the lesions of the tarsus.

Tarsus lesion	**Score description**
T0	No tarsus lesion
T4	Presence of a brown coloured lesion <0.25 cm2
T5	Presence of a brown coloured lesion between 0.25–0.5 cm2
T6	Presence of a brown coloured lesion >0.5 cm2
T7	Presence of a black coloured lesion <0.25-cm2
T8	Presence of a black coloured lesion 0.25–0.5 cm2
T9	Presence of a black coloured lesion >0.5 cm2
A	Presence of arthritis
OT	Overall score

The dorsal area of the carcass was inspected to record the presence of scratches and haematoma (Table 3). The ventral area of the carcass was inspected to record signs of swelling, crusts, pustules, haematoma and scratches (Table 4).

Table 3. Scoring and description of the dorsal lesion of the carcass.

Carcass dorsal lesion	Score description
DH	Presence of a haematoma other that red (purple to blue-black)
DHV	Presence of haematoma yellow-green
DG1	Presence of rough scratch(es) or scratch marks
DG2	Presence brown scratch ≤1 cm2
DG3	Presence brown scratch >1 cm2 or multiple
OD	Overall score

Table 4. Scoring and description of the ventral lesion of the carcass.

Carcass ventral lesion	Score description
VA	Presence of swelling >0.5 cm2
VC	Presence of crust(s)
VP1	Presence of a single pustule size ≤0.5 cm2
VP2	Presence of a single pustule size >0.5 cm2
VH	Presence of a haematoma >1 cm2 other than red (violet to blue-black)
VHV	Presence of a haematoma >1 cm2 yellow-green (old)
VG1	Presence of red scratch(es) or trace of scratch
VG2	Presence of brown scratch ≤1 cm2
VG3	Presence of brown scratches >cm2 or multiple
OVG	Overall score for the lesions of the ventral part of the carcass

For footpad, digits, tarsus and carcass body scoring areas, a composite score was also determined, which is described below.

2.4. Overall score of animal-based measures

An Overall Score Measure (OSM) for each of the four types of measures (Tables 1–4) was calculated using the following equation which considers the number of animals in each of the categories, weighted according to the severity and standardized by the number of categories recorded.

$$OSM = \frac{\sum_{t=0}^{J} t \cdot \frac{n_t}{N}}{J}$$

Where nt is the number of animals in category t, N is the total number of animals assessed, t is representing the different categories (scoring for each measure) and J is the total number of categories of the measure to be standardized. The OSM would be 0 in the case that all animals assessed were classified in the category 0 and 1 in the case that all were classified in the highest category.

2.5. Bacteriological analysis

Pooled caecal contents collected on farm and pooled caecal contents collected at slaughter were investigated separately to detect and enumerate thermotolerant *Campylobacter*. Ten microliters of pooled caecal content were plated directly onto modified charcoal-cefoperazone-deoxychocolate agar (mCCDA) (Oxoid, Dardilly, France) and incubated for 44 ± 4 h at 41.5 ± 1 °C under microaerobic conditions (5% O2, 10% CO2 and 85% N2). Up to five typical *Campylobacter* spp. colonies were sub-cultured onto plates of Columbia Agar (AES, Bruz, France) for further characterisation according to the NF EN ISO method 10272-1 (2006). On the carcass, 27 g of skin from the breast and neck were collected and analysed to detect and enumerate *Campylobacter* spp. Quantitative analyses were carried out according to the NF EN ISO method 10272-2 (2006). The limit of enumeration for the pooled caeca was 2.30 log CFU/g and for carcasses it was 1.00 log CFU/g.

In the classification analysis, the outcome used was dichotomous. That is to say, flocks having a positive pooled sample to *Campylobacter* were classified as either positive or negative on the farm and positive or negative at slaughter. For the purpose of the study, the risk between C.jejuni and C.coli was considered equivalent.

2.6. Statistical analysis

Logistic models/classification trees were used to estimate the probability of the presence of a specific foodborne pathogen in a flock and individual animal-based welfare status (the specific welfare indicator selected) in order to study the potential relationship between welfare indicators and foodborne pathogen prevalence/incidence levels. For the problem of classification at hand several classification techniques could be applied (e.g. machine learning techniques such as: random forest, boosting, bagging, support vector machine, classification trees, etc.). A classification trees methodology was chosen because of the ease of interpretation and also considering that the aim was to generate hypotheses in the context of welfare linked to food safety. We focused on binary classification trees which results in a saturated tree. The saturated binary tree was then pruned to an optimal size tree. A brief overview of the different processes is given below.

2.7. Variable importance measure

The variable importance measure used was based on the proposal by Breiman et al. (1984) using the prune tree. The measure was computed as follows:

$$\Im_l^2 = \sum_{t=1}^{J-1} \hat{I}_t^2 \cdot I\left(v\left(t\right) = l\right)$$

measuring the relevance for each predictor variable Xl. The sum is over the J−1 internal nodes of the prune tree. At each such node t, five of the best-input variables Xv(t) that could be used for partitioning the region associated with that node into two sub-regions were chosen; within each, a separate constant was fitted to the response values. The particular variables chosen were the ones that gave maximal estimated improvement Iˆt2 in squared error risk over that for a constant fit over the entire region. The squared relative importance of variable Xl is the sum of such squared improvements over all internal nodes for which it was chosen as the splitting variable.

The model was applied to the flocks where caeca samples were collected on the farm and to the flocks sampled in the caeca at slaughter.

For all the welfare indicators (footpad dermatitis, hock lesions, etc.) each of the classes (e.g. P0, P1, P2…, P7, P8) were transformed to standardised scale and each of them were included in the model in a similar way as when they are included in parametric models considering dummy variables. This implies that each class (e.g. P0, P1, P2…, P7, P8) was having its own effect without assuming proportionality or ordinality.

3. Results

3.1. Welfare indicators

In Table 5 the average prevalence from the scoring of the five body areas are reported for the 32 flocks. The average prevalence of each specific animal-based measure is presented for the individuals observed for the 32 flocks.

Table 5. Average prevalence of individual welfare indicators on the total individuals observed (N = 32 Broiler flocks, France, 2008). For a more detailed explanation of the animal-based measures, see Tables 1–4.

Body Area	**Animal-based Measure**	**General Description**	**Average Prevalence (%)**	**95%CI**
	P0	No lesion	3.6	2.5–4.8
	P1		0.9	0.8–1.2
	P2	Keratosis/papilloma	1.0	0.7–1.2
	P3		1.4	1.15–1.6
	P4		16.9	15.5–18.3
FOOTPAD	P5	Superficial lesion	18.1	16.9–19.3
	P6		9.9	8.9–11.01
	P7		15.9	14.6–17.2
	P8	Severe lesion	21.0	19.5–22.6
	P9		10.3	8.3–12.2
	P10	Healing	0. 9	0.5–1.3
DIGIT	D0	No lesion	52.0	48. 6–55.4
	D1	Presence of dermatitis on 1 digit	16.8	15. 8–17.8

Body Area	Animal-based Measure	General Description	Average Prevalence (%)	95%CI
TARSUS	D2	Presence of dermatitis on 2 digits	14.7	13.5–15.9
	D3	Presence of dermatitis on 3 digits	9.4	8.4–10.5
	D4	Presence of dermatitis on 4 digits	7.2	5.9–8. 5
	T0	No lesion	54.3	52.0–56.59
	T4	Presence of brown lesion	26.8	25.7–27.9
	T5		8.3	7.8–8.8
	T6		3.6	3.2–3.9
	T7	Presence of black lesion	3.1	2.7–3.5
	T8		2.3	2.0–2.6
	T9		1. 7	1.3–2.0
	A	Presence of arthritis	11.8	10.9–12.7
DORSAL SIDE	DG1	Presence of scratches	20.2	19.4.20.9
	DG2		16.2	15.5–16.8
	DG3		18.6	17.5–19.8
	DH	Presence of haematoma	4.6	4.0–5.1
	DHV		0.8	0.7–1.0
VENTRAL SIDE	VA	Presence of swelling	25.0	23.7–26.4
	VC	Presence of crust	3.0	2.4–3.6
	VG1	Presence of scratches	0.3	0.3–0.4
	VG2		0.0	0.1–0.1
	VG3		0.0	0–0.0
	VH	Presence of haematoma	3.2	2.9–3.4
	VHV		0.7	0.6–0.9
	VP1	Presence of pustule	13.9	13.3–14.5
	VP2		17.0	15.8–18.1

Almost half of the broilers had severe lesions on the footpad; only 3.6% of the broilers had no footpad dermatitis. More than half of the broilers had no digit lesions. More than half of the broilers had no tarsus lesions. Almost 39% of the broilers had brown lesions on the tarsus. More than half of the broilers had scratches on the dorsal side of the body area. Almost 30% of the broilers had pustules on the ventral side of the carcass.

3.2. Food safety indicators

Positive flocks, their prevalence and enumeration (SD and 95% CI included) for *Campylobacter* spp., C. jejuni, C. coli and both species are shown in Table 6 for pooled caeca collected on farm. The presence of *Campylobacter* and its species collected from pooled caeca or from skin samples taken from the carcasses at slaughter is presented in Table 6.

Table 6. Prevalence and quantification of the species of Campylobacter in caeca on farm and at slaughter and on carcass at slaughter (N = 32 Broiler flocks, France, 2008).

	Species of *Campylobacter*	**Positive flocks/sampled flocks**	**Prevalence (%)**	**95% CI**	**Quantification (Log10 CFU/g)**
Pooled caeca sampled on farm[a]	*Campylobacter* spp.	25/32	78.1	63.6–2.7	7. 9 ± 1.3
	C. jejuni	16/32	50.0	32.4–67.6	7.7 ± 1.3
	C. coli	6/32	18.8	4.9–32.6	8.5 ± 0.7
	C. jejuni and C. coli	1/32	3.1	0.0–9.2	9.1
Carcass sampled at slaughter	*Campylobacter* spp.	31/32	96.9	90.8–100.0	2.1 ± 1.0
	C. jejuni	28/32	87.5	75.9–99.1	2.0 ± 0.9
	C. coli	3/32	9.4	0–19. 8	2.7 ± 1.4
	C. jejuni and C. coli	12/32	37.5	20.5–54.5	1.9 ± 0.7
Pooled caeca sampled at slaughter	*Campylobacter* spp.	23/32	71.9	57.3–86.4	8.1 ± 0.9
	C. jejuni	16/32	50.0	32.4–67.6	7.7 ± 0.8
	C. coli	7/32	21.9	5. 9–37.9	8.6 ± 0.9
	C. jejuni and C. coli	1/32	3.1	3.0–9.2	8.0

[a]Of the 25 *Campylobacter* species, three strains were not identified.

At slaughter almost all the flocks (96.9%) sampled on the carcass were *Campylobacter*-positive. Over two thirds of all the caeca samples (78.1% on farm and 71.9% at slaughter) were *Campylobacter*-positive. *Campylobacter* jejuni was the most prevalent species both in pooled caeca on farm and at slaughter, and on broiler carcasses.

3.3. Statistical model

Preliminary analysis on the association between the different categories of welfare indicators, including mortality and positivity to *Campylobacter*, showed no clear pattern that distinguished the positive from the negative *Campylobacter* flocks in term of the animal-based measures recorded. Neither was there any clear relationship between mortality at 10 days (as expected, since colonization of *Campylobacter* occurs typically at 2–3 weeks of age) or mortality at the end of the production cycle and positivity to *Campylobacter* on farm or at slaughter. Hence the binary classification tree approach.

The results of the statistical model are presented in Figs. 1–4. Figs. 1 and 3 considered original scores as described in the Material and Methods section, while Figs. 2 and 4 describe the overall score for the proposed welfare indicators. In the figures, scores are sometimes presented as a percentage, depending if the total number of animal observed were provided, sometimes as the number of individuals. The numbers indicated at the bottom of the figures are related to the number of flocks being positive (right side) and negative (left side) to *Campylobacter* spp. The first tree model shown in Fig. 1 indicates that 12 flocks (37.5%) are *Campylobacter*-positive, if the percentage of animals on farm with score P8 (severe lesion on footpad) is greater than 25%. Six flocks (18.7%) are *Campylobacter*-positive, if the percentage of score P8 is below 25%, but the score P1 percentage is above 1.4%. Finally, four flocks (12.5%) are *Campylobacter*-positive when the score P1 is below 1.4% and the percentage of animals with brown scratches on the dorsal carcass below or equal to 1 cm2 (DG2) is below 10%. The most important variables in this analysis were P6 followed by P8.

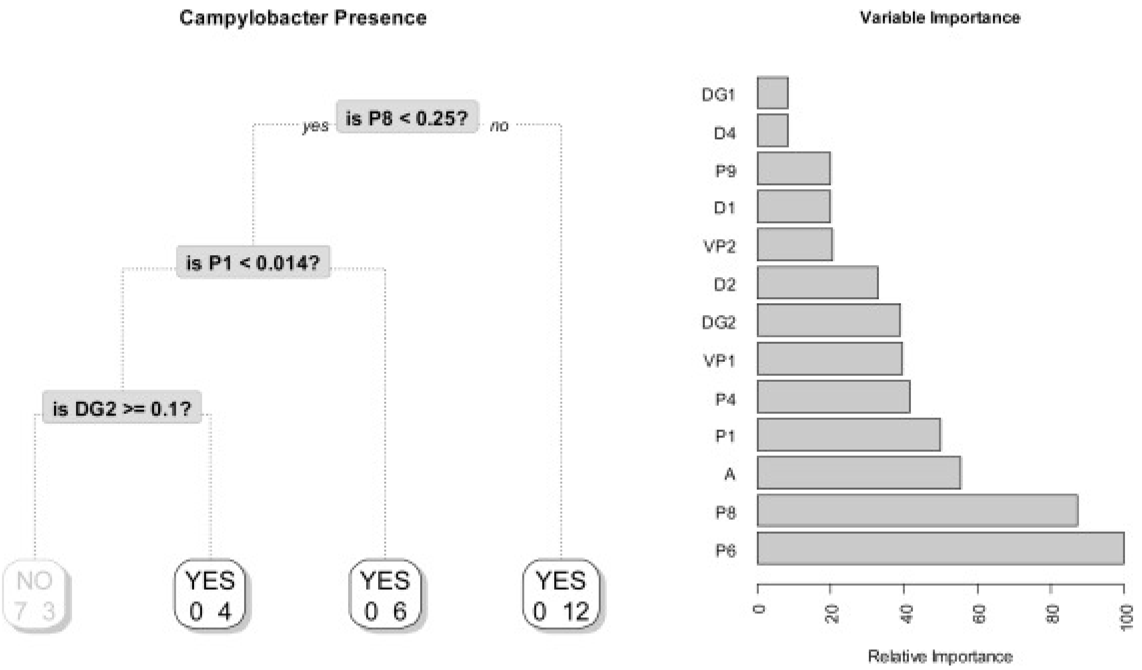

Fig. 1. Potential association of presence of Campylobacter spp. in caeca samples collected on farm and animal-based measures, as well as their importance to classify Campylobacter-positive flocks.

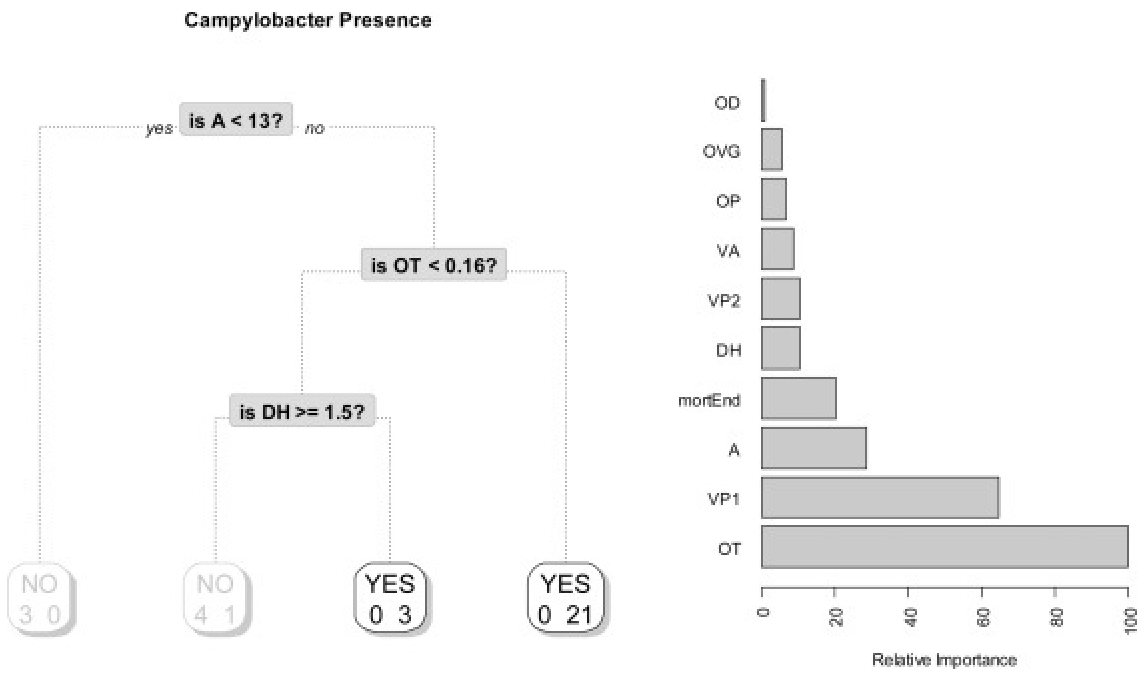

Fig. 2. Potential association of presence of Campylobacter spp. in caeca samples collected on farm and the overall score measure (OSM), based on the number of animals reported in the different degrees of severities, as well as the importance of the animal-based measures to classify Campylobacter-positive flocks.

When the model using the proposed overall score measure is fitted (Fig. 2), three flocks (9.4%) are *Campylobacter*-negative, if the number of animals on farm with arthritis, is below 13. Four flocks (12.9%) are *Campylobacter*-negative, when the number of animals with arthritis is above 13, but the overall tarsus lesion (OT) score is below 16%, and the number of animals with presence of a recent haematoma (purple to blue-black) is above 1. When the number of animals with arthritis is above 13 and the overall score for the tarsus lesions is above 16% then 21 flocks (65.6%) are *Campylobacter*-positive. The most important variable in this analysis was OT.

In Fig. 3, 19 flocks (59.4%) are *Campylobacter*-positive, if the percentage of animals at slaughter with lesion P5 (Superficial lesion: crust, extended between 25 and 50% of the foot) is below 20%. Four flocks (12.5%) are *Campylobacter*-positive, if the percentage of animals with lesion P5 is above 20% and P4 (Superficial lesion: crust, extended below 25%) is below 18%. Finally, six flocks (20%) are *Campylobacter*-negative and two positive, if the percentage of animals with lesion P4, is above 18%, with P5 above or equal to 20%.

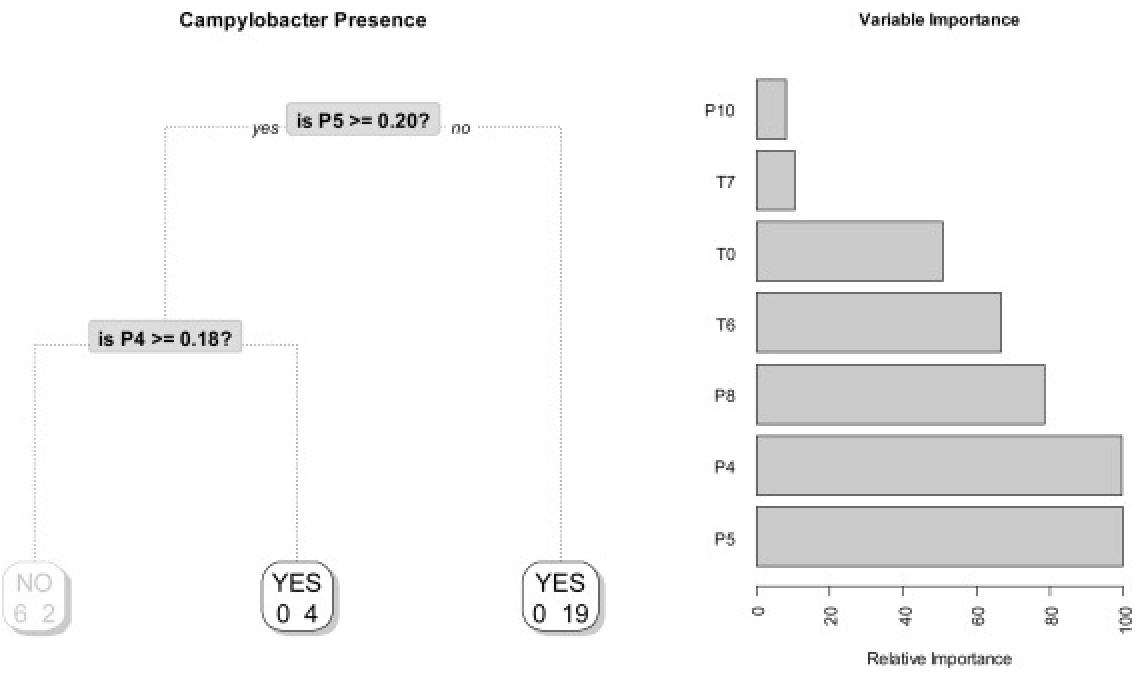

Fig. 3. Potential association of presence of Campylobacter spp. in caeca samples collected at slaughter and animal-based measures, as well as their importance to classify Campylobacter-positive flocks. The number of flocks sampled with the available results for Campylobacter spp. in caeca at slaughter was 31.

When the model using the overall score measure is fitted (Fig. 4), 17 flocks (53.1%) are *Campylobacter*-positive, if the overall score of the foot lesions (OP) is more than 59%. Four flocks (12.5%) are *Campylobacter*-positive, if the overall score of the foot lesions (OP) is below or equal to 59% and more than three animals have dorsal yellow-green haematoma (DHV). Three flocks (3.4%) are *Campylobacter*-positive, if less than three animals have dorsal yellow-green haematoma (DHV) and the overall dorsal lesions score (OD) is less than 1%. On the other hand, six flocks (19.35%) are *Campylobacter*-negative, if foot lesions (OP) is below or equal to 59%, and less than three animals have dorsal yellow-green haematoma (DHV) and the overall dorsal lesion score (OD) is more or equal than 1%.

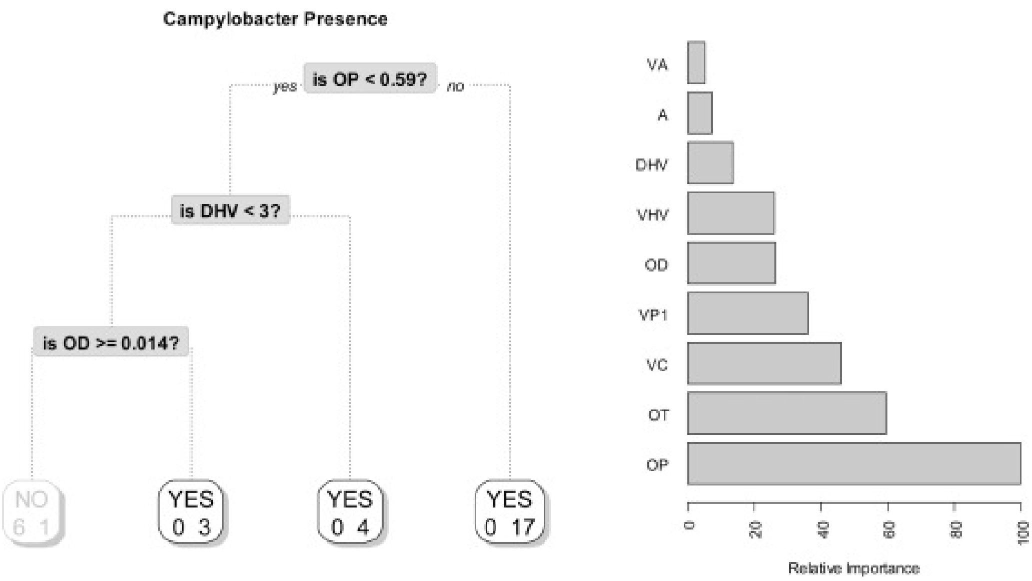

Fig. 4. Potential association of presence of Campylobacter spp. in caeca samples collected at slaughter and the overall score measure (OSM), based on the number of animals reported in the different degrees of severities, as well as the importance of the animal-based measures to classify Campylobacter-positive flocks. The number of flocks sampled with the available results for Campylobacter spp. in caeca at slaughter was 31.

For both (on farm and at slaughter) models when a cross validation method is applied to assess their predicting properties, the error rates obtained are 10 and 4% respectively, indicating good predicting abilities. It was not possible to investigate the potential relationship between quantitative determination for *Campylobacter* spp. and welfare indicators, due to the limited number of farms available in the study (results not shown).

4. Discussion

The present study suggests how welfare and health indicators could be used to identify flocks that are very likely to be positive (or negative) for *Campylobacter* on a routine basis. According to our statistical model, the most useful predictors that a flock is positive are a high prevalence of severe lesions on the footpad and arthritis, a high overall score for foot lesions and a high overall score for tarsus lesions. Flocks with more than 25% animals with severe lesions on between 25 and 50% of the footpad (P8) are predicted to be *Campylobacter*-positive at the end of the rearing period whereas flocks where less than 13 individuals have arthritis (A) are predicted to be *Campylobacter*-negative. Lesions greater than P5 could be identified as predictive welfare measures for being a *Campylobacter*-positive flock at slaughter. The flock could be *Campylobacter*-positive if an overall score for foot lesions at slaughter is more than 59%, that is, animals had a higher score for foot lesion.

Allain et al. (2009) used the same scoring system as in the present study and found slightly lower prevalences for the specific classes of severity. However other scoring systems have also been used. Haslam et al. (2007) used a photographic five-point scale scoring system detecting a moderate to severe average of footpad dermatitis of 11.02% in broiler chickens in the UK. Similarly, Kittelsen et al. (2015) detected an average prevalence of footpad dermatitis of 11.19% and arthritis of 3.3% in 32 flocks of commercial broilers in Norway. De Jong et al. (2012) detected 38.4% of footpad dermatitis using a three-point scale in commercial Dutch fast-growing breeds of boiler chickens. Although automatic video images recording for welfare animal-based measures are a possibility, the scoring system at meat inspection in Europe is not standardised. It is mainly based on photo charts, and at a lesser extent on descriptive charts and combined methods (Butterworth et al., 2015). These authors also indicated that there is some variability in the guidance material between Member states in Europe.

The results of the present study identify the importance of the scoring system when animal-based measures are investigated separately, not only to assess footpad dermatitis and arthritis per se, but also to assess the predictive value for the presence of *Campylobacter* spp. in flocks when animal-based measures related to footpad dermatitis, hock burns and body lesions are identified within flocks from commercial settings. The design of the scoring system permitted us to identify specific scores and the extent of their association with *Campylobacter* spp. We found differences if the aim was to predict *Campylobacter* presence on farm or at slaughter and if we investigated the animal-based measures separately or used an overall score measure based on the number of animals reported in the different degrees of severities. The models fitted in the present study had good predicting abilities, but they should be interpreted with caution since a limited number of flocks were available for analysis and a generalisation to the population might not be appropriate. Moreover, data refers only to poultry flocks in France and as such cannot be generalised to the population of other countries. Larger studies in broilers involving several European Member States with different *Campylobacter* epidemiology are needed to further characterise the association between the prevalence and severity of animal-based measures (e.g. the presence of contact dermatitis, arthritis) and *Campylobacter* infection. Future research studies should include ruling out concomitant infections in order to assess the relationship between welfare lesions and *Campylobacter* only and so to investigate further the mechanism of association. Thus, these results are hypotheses to be tested further. Nevertheless, despite the small sample size of our study, the results of the present statistical model are in line with those of Rushton et al. (2009) and Bull et al. (2008), who carried out their studies in Great Britain. Rushton et al. (2009) in a smaller set of 30 farms observed that footpad dermatitis was a significant predictor of *Campylobacter* infection. Bull et al. (2008) collected health indicators and *Campylobacter* spp. prevalence from 789 flocks and found that if footpad dermatitis affected more than 2% of the flocks, these were more likely to be *Campylobacter* spp. positive and to have a high proportion of the infection. The relationships found in our study between severe lesions of footpad dermatitis and *Campylobacter* infection support this, although the scoring system used by Bull et al. (2008) could not be compared with our scoring system.

Both the on farm analysis and the slaughter plant analysis of the present study confirm the relationship between footpad dermatitis and being a *Campylobacter*-positive flock. To our knowledge, a potential relationship between arthritis and being a *Campylobacter*-positive flock has not been reported before. However, chicken carcass condemnation rate at slaughter was associated with contamination by *Campylobacter* of the broiler skin (Malher et al., 2011) and arthritis is one of the leading causes of chicken carcass condemnation (Lupo et al., 2008), These authors found a lower prevalence of arthritis-polyarthritis (5.5%) compared to the present study (average prevalence 11.8%). Since arthritis is associated with ascites and congestion in the literature, we can assume an infectious origin in these carcasses too (Lupo et al., 2008). Arthritis is a painful condition in broilers (EFSA, 2012). The level of stress that these animals experience may be the cause of immunosuppression with consequent susceptibility to infection (Bull et al., 2008; Cogan et al., 2006). Unfortunately, we could not analyse data for infectious causes of condemnation and eventual co- infections for these flocks.

Footpad dermatitis and arthritis are associated to prolonged contact with wet litter and poor litter quality (Allain et al., 2009; Xavier et al., 2010). Contact dermatitis could be the entrance for the development of arthritis. Wet litter is frequently caused by diarrhoea and intestinal disorders in poultry. The alteration in the intestinal tract in diseased and stressed animals may favour the *Campylobacter* colonization in broilers reared under commercial conditions (high stocking density) exposed to the pathogen. Environmental conditions, such as humidity and temperature, air and litter quality, besides stocking density, are pivotal for the welfare of intensive farming poultry (Jones et al., 2005) and affect the litter microbiological population, including *Campylobacter*. Litter humidity and coprophagic behaviour of poultry both influence the horizontal transmission of the *Campylobacter* spp. infection (Line, 2006). Once infected, chickens eliminate log10 8 cfu/gr of *Campylobacter* with defecation, resulting in a high dose if the faeces are ingested by other chickens (Line, 2006), increasing intra- flock transmission.

Footpad dermatitis and arthritis are concerns for the modern poultry industry for issues of animal welfare, food safety, product downgrade and carcass condemnation (Bradshaw et al., 2002; Knowles et al., 2008; Shepherd and Fairchild, 2010). Chicken pad price has increased due to the high demand for high quality pads (Shepherd and Fairchild, 2010) and footpad dermatitis results in economic losses for the poultry industry. Therefore, prevention of welfare issues like footpad dermatitis would not only increase welfare levels in poultry, but would also decrease condemnations and economic loss for the poultry industry. It should be recommended to monitor especially the proportion of animals in a flock having a high score for footpad dermatitis by recording superficial lesions, such as crusts of the pad, and severe lesions, such as ulcer of the pad as well as the extent of the lesion. Moreover, it is recommended to explore the overall tarsus and pad lesions score of the flock to obtain a prediction of *Campylobacter* infection in a flock on farm and at slaughter.

By controlling welfare and health indicators in broilers, the prevalence of human *Campylobacter*iosis may be potentially reduced. Few authors have investigated how the level of welfare in one species could be associated with the infection of zoonotic pathogens in other species. Alpigiani et al. (2016) conducted a study on 30 batches of finishing pigs, which indicated how animal-based measures, such as mortality and the stress experienced by animals in intensive farming, were associated with Yersinia enterocolitica.

According to Berghaus et al. (2013), the higher prevalence of *Campylobacter* spp. in neck and breast skin at slaughter compared to in the caeca on farm could be the result of the cross contamination during transport and slaughter. In accordance with Hue et al. (2011), we observed a higher prevalence of *Campylobacter* spp. on carcasses than in caeca, although lower quantification of *Campylobacter* was detected on carcasses than in caeca. This might also be explained by cross contamination at slaughter. Moreover, stress, caused by handling of animals and farm management resulting in increased shedding, could be responsible of the increase of *Campylobacter* spp. prevalence in faecal samples (Whyte et al., 2001; Slader et al., 2002).

Predicting *Campylobacter*-positive flocks is of benefit during all the steps of the broiler food chain. The recognised standardised method used to detect *Campylobacter* takes a week to have results, thus the association between welfare indicators and *Campylobacter* in the absence of reliable rapid methods, could help to save time. Monitoring health and welfare indicators could support farmers rearing broilers limit the spread of the infection on farm. It could also support abattoir operators in carrying out preventive measures to limit the load of the pathogen. For example, in countries where the prevalence of *Campylobacter* spp. is low, they could slaughter those flocks likely to be colonised at the end of the slaughter day and eventually carry out any physical treatment of the carcasses. Burfoot et al. (2016) reported a reduction of 1 log10 in *Campylobacter* spp. numbers, on poultry carcasses with rapid surface cooling. Moreover, the application of organic acids (e.g. peracetic acid, lactic acid etc.) resulted in a reduction of *Campylobacter* spp. (Bauermeister et al., 2008).

Quantitative information of the transmission of *Campylobacter* is lacking (Line, 2006). In our study quantification of *Campylobacter* spp. did not turn out to be useful for the statistical modelling. However, quantification of *Campylobacter* spp. in relation to welfare indicators could be useful for *Campylobacter* spp. risk assessment to reduce the risk of transmission of *Campylobacter*iosis from broiler chicken reducing thermotolerant *Campylobacter* load on commercial broiler farms. Using a mathematical model, Rosenquist et al. (2003) indicated that *Campylobacter*iosis could be reduced 30-fold by a decrease of 2-log in the number of *Campylobacter* spp. in broiler carcasses.

Reducing *Campylobacter*iosis involves decreasing the carrier status of poultry flocks by improving on farm biosecurity (Humphrey, 2006). Stress influences the outcome to infection (Alpigiani et al., 2016) through the release of catecholamines, which have an effect on bacterial growth and on bacterial virulence (Cogan et al., 2007). The presence of norepinephrine has been shown to increase the virulence of C. jejuni and the consequently increased shedding and horizontal transmission of *Campylobacter* spp. in poultry (Cogan et al., 2007; Verbrugghe et al., 2012). Farm management (catching, thinning, transport and feed/water deprivation) may also lead to acute and chronic stress (Whyte et al., 2001; Bull et al., 2008). At slaughter, subclinically infected broilers pass undetected through the ante and post-mortem inspection visit. Cross contamination of carcasses at slaughter does seem to occur and, since thermotolerant *Campylobacter* are zoonotic agents, there is an impact on public health (EFSA, 2011).

5. Conclusions and future directions

Severe lesions on footpad and arthritis are identified by our pilot study as predicting *Campylobacter* infected flock. In order to validate the results reported here, a large-scale study involving different Member States should be conducted to assess the value of the identified welfare indicators and their cut-off for predicting *Campylobacter* status in a broiler. Data regarding welfare in poultry are already mandatorily recorded at slaughter and many countries have their own official surveillance on zoonotic agents in place. Therefore, databases at slaughter plants could be better exploited for data containing both chicken welfare and food safety information. To this end, a European platform could be created with the purpose of collating existing data on welfare indicators and zoonotic surveillance monitoring data recorded by competent authorities from different countries and of analysing them to link animal welfare and health indicators to public health.

Specific animal-based measures (e.g. specific severity levels of footpad lesions, arthritis) could be selected and recorded either by farmers or by official veterinarian at slaughter for identifying *Campylobacter*-positive flocks. The scoring system applied for the detection at meat inspection of the gross pathology lesions in broilers (Directive 2007/43/EC), was very detailed and useful for the analysis. However, it would be beneficial for slaughter plant operators and official veterinarians to apply a standardised global scoring system for welfare indicators in order to decrease variations between the different slaughter plants.

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