Vector/Pathogen/Host Interaction, Transmission

Retention of \textit{Campylobacter} (Campylobacterales: Campylobacteraceae) in the House Fly (Diptera: Muscidae)

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ABSTRACT The house fly (\textit{Musca domestica} L.) may transmit \textit{Campylobacter} to broiler flocks. We assessed the retention time of house flies for \textit{Campylobacter jejuni} at five temperatures and three doses. Flies were inoculated individually at their proboscis with \(1.6 \times 10^7\) CFU (colony forming units) of \textit{C. jejuni} and incubated at 15, 20, 25, 30, and 35°C. Furthermore, a dose experiment was conducted at 25°C where individual flies were inoculated in three series: \(6.5 \times 10^6, 6.0 \times 10^4,\) and \(8.2 \times 10^2\) \textit{C. jejuni} CFU. Whole flies were tested for \textit{C. jejuni} carriage at 0, 6, 12, 18, and 24 h by initial preenrichment in Bolton broth, which afterwards was streaked on modified mCCDA agar plates and incubated under micro-aerobic conditions. The results showed that the time \textit{C. jejuni} remained in flies declined over time with ascending temperatures and when reducing the inoculation dose. All flies stayed \textit{Campylobacter} positive 24 h post inoculation at 15°C whereas only one-third of the flies were positive at 20°C and few to none at 25, 30, and 35°C. When combinations of temperature and retention time were expressed as accumulated day-degrees, data could be adequately fitted using a generalized linear mixed model that included a linear effect of day-degrees and the difference between the lowest and the two highest doses. Based on model predictions of selected combinations of temperature and dose, the time for 50% and 1% of flies containing \textit{Campylobacter} was calculated. It is suggested that house flies are mainly short distance carriers of \textit{C. jejuni}.

KEY WORDS \textit{Campylobacter jejuni, Musca domestica, broilers, retention time, temperature}

According to several risk analyses the main source of human \textit{Campylobacter} infections in the industrialized world come from contaminated food, and most importantly poultry meat (Craven et al. 2000, Katsma et al. 2007, Nauta et al. 2007). The incidence of \textit{Campylobacter} infections of both poultry and humans peak every summer in Northern temperate climates (Nylen et al. 2002, Patrick et al. 2004). Because this coincides with the peak season for flies, it has led to investigations of the importance of this group of insects in connection with contamination of specific parts of the human food chain, in particular broilers (Hald et al. 2004, 2007, 2008). It has been reported as well, that temperature related variables based on fly activity might be valuable to predict the risk of \textit{Campylobacter} colonization of broiler flocks (Guerin et al. 2008).

The synanthropic and omnivorous house fly (\textit{Musca domestica} L.) (Diptera: Muscidae) and other filth living flies forage and develop in animal feces from dairy cattle and pigs (Busvine 1993). During foraging, flies regurgitate to liquefy their food and defecate remains of previous meals. This predisposes the house fly as an important transmitter of \textit{Campylobacter} spp. (Rosen and Kapperud 1983, Berndtson et al. 1996, Hald et al. 2004, Elkdahl et al. 2005, Nichols 2005, Wales et al. 2010).

Earlier studies conducted under laboratory conditions have shown transmission of \textit{C. jejuni} to chickens by flies. Thus, Shane et al. (1985) showed that house flies exposed to \textit{C. jejuni} positive chickens became carriers and subsequently transmitted \textit{Campylobacter} to an uninfected group of chickens. Field transmission of \textit{Campylobacter} by flies has been reported by Hald et al. (2004), who showed that house flies carrying a \textit{C. jejuni} strain likely picked it up from pastured sheep in the outskirts of a broiler farm. The flies were suggested as the most likely transmitters as the broiler flock was found \textit{C. jejuni} positive with an identical strain. That result was followed by an intervention study, where mesh screens were placed on 20 broiler houses to prevent flies from entering during the study. This significantly reduced the prevalence of \textit{Campylobacter} positive flocks over a period of 7 mo from 51.4% in control houses to 15.4% in the intervention houses (Hald et al. 2007).

A few studies exist where flies around livestock farms have been examined for \textit{Campylobacter} (Hald et al. 2004, 2008; Szalanski et al. 2004). \textit{Campylobacter} positive flies identified in those three studies were the
house fly, the stable fly (Stomoxys calcitrans L.), the black dump fly (Hydrotaea aeneascens, Wiedemann), the false stable fly (Muscina stabulans, Fallen), and the noon fly (Mesembrina meridiana, L.). These all belong to the dipteran family Muscidae that contain many species that are associated with feces from confined livestock (Gregor et al. 2002). In the study by Hald et al. (2008) it was found that 90.3% of *Campylobacter* positive flies were from the family Muscidae and 67.7% were house flies. Positive flies were also found among blow flies (Diptera: Calliphoridae), however with a very low prevalence; one green-bottle fly *Lucilia caesar* (L.) out of 488 collected individuals. Likewise and with low prevalence, in the study by Szalanski et al. (2004) root-maggot flies of the family Anthomyiidae and green-bottle flies of the species *L. caesar* were *Campylobacter* positive.

Although there seems little doubt that house flies and other flies can be carriers of *Campylobacter*, only little information is available about their potential to host the bacteria. In particular, the duration that *Campylobacter* is retained on either the flies’ external surfaces or within their alimentary tract after having consumed food contaminated with *Campylobacter* needs to be examined. This information is important for understanding at what distance house flies can be carriers of *Campylobacter* and become a risk for broiler production. The subject was addressed in a study by Berndtson et al. (1991) where single flies and pools of flies were examined for survival of different strains of *C. jejuni* at 20°C and 32°C. Overall, for both temperatures and single flies’ survival of *C. jejuni* was few hours postinoculation and for pools of flies, up to 2 d. However, the interaction between the dose of *Campylobacter* ingested and temperature on retention time was not addressed in the study.

The aim of current study was to determine the duration *C. jejuni* will remain in laboratory reared house flies after they were individually inoculated at their lapping proboscis with three doses of *C. jejuni* and incubated for up to 24 h at five temperatures.

**Materials and Methods**

**Rearing of House Flies.** The house flies used were established from a dairy cattle farm in 1989 and bred for >225 generations at the Department of Agroecology, University of Aarhus. Newly laid eggs were transferred to fresh larval medium consisting of wheat bran (24.6%), alfalfa (12.3%), yeast (0.6%), melted sugar (0.9%), and tap water (61.6%). After pupation, fly puparia were separated from the medium by water flotation and transferred to a plastic container (length 30 cm, width 20 cm, and height 30 cm) with perforated walls for ventilation. Adult flies emerging were supplied ad lib with sucrose and tap water.

**Preparation of *C. jejuni* Inocula.** A stock suspension was prepared in the morning on the experimental day from a *C. jejuni* culture grown on blood-agar plates at 42°C in a micro aerobic atmosphere (6% O₂, 6% CO₂, 4% H₂ in N₂) for 24 h according to recommendations by the Nordic Committee on Food Analyses (NMKL; www.nmkl.org/Engelsk/index.htm). *Campylobacter* culture was harvested from an appropriate number of agar plates, and suspended in 100 ml 0.9% NaCl shaken on ice for ~2 h. The density of the stock suspension was intended to reach 10⁹ CFU/ml, which was checked at an optical density of 0.540 and measured at wavelength 620 nm in a Multiscan EX Primary EIA v.2.1–0 ELISA-reader (Lab systems, Helsinki, Finland). Vials containing 5 ml suspensions were subsequently made for inocula by appropriate dilutions of the stock suspension in sterile 0.9% NaCl, and kept on wet ice at 2–5°C throughout the day of experiment. From the stock suspension and from each vial of inoculum 1 ml was withdrawn in the morning for estimation of CFU/ml and likewise 1 ml from each vial of inoculum suspension after the experiment. CFU/ml was estimated in duplicate from dilution series in saline, plated onto blood agar, incubated in a micro aerobic atmosphere at 42°C, and colony counting at 24 h of incubation. The factual inoculation dose in 1 μl suspension was subsequently calculated as the average of CFU/ml divided by 1.000 before and after the experiment.

**Inoculation of Flies.** Containers with an appropriate number of house flies were flushed with CO₂ to anesthetize the flies. Female flies were then transferred singly to small sterilized cages made of paper cylinders (diameter 10 cm, height 15 cm) that fit exactly into the bottom of a petri dish, where the top of the dish was used as lid. These cages offered the possibility to flush CO₂ into the cage by penetrating the paper wall with a 19G hypodermic needle. Furthermore the clear lid allowed visual inspection of the individual flies during the entire experiment. Flies were starved for 3–4 h in the cages and were then again anesthetized with CO₂ removed from the cage and fixed individually inside a sterile pipette tip that allowed the fly’s head and the lapping proboscis to protrude. By carefully approaching the fly’s labela with the tip of a pipette containing the *C. jejuni* suspension, most flies ingested the 1 μl suspension of *C. jejuni*. Flies that declined or stopped feeding were eliminated from the study. After the end of the inoculation, each fly was released from its pipette tip, and placed in a new sterilized paper cage. During the rest of the study the flies fed ad lib on sterilized nonhomo-mogenized organic milk soaked into cotton wool in a glass tube.

**The Temperature Experiment.** The inoculation was performed at room temperature, 20–22°C and 50–60% RH with a dose of 1.6 × 10⁷ (±0.35 SE) CFU of *C. jejuni*. The flies were placed in incubators at 15, 20, 25, 30, and 35°C (25 individually caged flies per temperature series). 70% RH and constant light from a fluorescent tube (Philips, PL-L 24 W/840/4P). In a pilot study it was found that the flies did not stay alive for the needed experimental period at 40–42°C, which is the growth optimum for *C. jejuni*. Thus, the maximum experimental temperature was set at 35°C.

At 6, 12, 18, and 24 h postinoculation five randomly chosen cages per temperature series, each with a single fly, were removed from the incubators and the
Campylobacter status (positive or negative) of the flies was determined as described below. For negative controls and before the inoculation experiment, five flies were selected randomly and examined for Campylobacter. For positive controls of the C. jejuni inoculum and controls of inoculated flies (time = 0), five times 1 µl of the C. jejuni suspension and five individually inoculated flies per temperature series were incubated in Bolton broth (see below). The temperature experiment was repeated three times, and comprised a total of 300 (five temperature series × 4 time points × 5 flies × 3 replicates) inoculated flies, plus 15 flies for the negative controls and 75 flies for positive controls.

The Dose Experiment. To examine the relationship between the dose of C. jejuni ingested and the corresponding retention in the fly, three suspensions of \(8.2 \times 10^2\) (±0.22 SE), \(6.0 \times 10^4\) (±0.31 SE), and \(6.5 \times 10^6\) (±0.23 SE) CFU of C. jejuni were prepared. For each of the three series, the inoculation procedure described above was applied. The flies were incubated at 25°C and 70% RH. The dose experiment was repeated tree times and comprised a total of 180 inoculated flies (three doses × 4 time points × 5 flies × 3 replicates) plus 15 flies for the negative controls and 45 flies for positive controls of inoculum (see above).

Culturing of C. jejuni. Anesthetized flies were picked in their cages with sterile forceps and dropped into 1.5 ml Bolton broth (CM9083 with SR0183 and SR0048) (Oxoid, Basingstoke, UK). The abdominal part of each fly was crushed in the broth with the forceps, and the tubes with flies were vortexed to ensure quick release of intestinal content before being incubated micro aerobic at 42°C. At 48 h, 10 µl of broth was streaked onto modified CCDA agar (mCCDA) blood-free agar base supplemented with (CM739 and SR155) (Oxoid, Basingstoke, UK), incubated micro aerobic at 42°C for 48 h and read visually as Campylobacter positive or negative.

Statistics. The relative number of flies with Campylobacter is expected to be approximately binomially distributed and therefore generalized linear mixed models were used to analyze the data (see e.g., McCulloch and Searle 2001). However, a parameter describing the factor by which the variance might be larger (or smaller) than expected from a binomial distribution was calculated and taken into account when testing for differences among treatment and exposure time. This factor is usually called an over dispersion (under dispersion) factor. In the analyses the linear model was applied to the logit of the means.

Firstly, each of the two experiments was analyzed separately using a model that included the fixed effects of treatment (temperature and dose), incubation time and the interaction between these. The number of replicates was included as a random factor. Mathematically the model may be written as:

\[
P_{\text{ter}} = \frac{Y_{\text{ter}}}{N_{\text{ter}}} \approx \text{Binomially distributed}, \ Bi(\eta_{\text{ter}}, N_{\text{ter}})
\]

where

\[
\log \left( \frac{\eta_{\text{ter}}}{1 - \eta_{\text{ter}}} \right) = \mu + \delta_{r} + \gamma_{e} + (\delta \gamma)_{e} + D_{r}
\]  \[1\]

\(Y_{\text{ter}}\) is the observed number of flies with Campylobacter for treatment \(t\) at incubation time \(e\) in replicate \(r\)

\(N_{\text{ter}}\) is the total number of flies at treatment \(t\) at incubation time \(e\) in replicate \(r\)

\(P_{\text{ter}}\) is the relative number of flies with Campylobacter for treatment \(t\) at incubation time \(e\) in replicate \(r\)

\(\mu\), \(\delta_{r}\), \(\gamma_{e}\) and \((\delta \gamma)_{e}\) are the fixed effects of general level, treatment, incubation time and interaction between treatment and incubation time

\(D_{r}\) is the random effect of replicate \(r\). \(D_{r}\) is assumed to be normally distributed with a mean of 0 and a variance of \(\sigma_{D}^{2}\)

Convergence problems arose for data from the temperature experiments at 15°C as several combinations of temperature and incubation time approached infinity at the logit-scale. Thus all observations from the treatment at 15°C were omitted from the analyses.

Secondly, the two experiments were analyzed jointly in a mixed model where the effects of dose and day-degrees were included as fixed effects. The number of day-degrees was calculated from the formula:

\[
\text{Day-degrees} = ((\text{Temperature} - \text{basis temperature}) \times \text{Incubation time/24}).
\]

A basis temperature of 4°C below which Campylobacter was expected to stay alive for an extended period of time was found to give the best fit to the data (see also Blankenship and Craven 1982). Here the experiment and replicate number were included as random effects.

Mathematically the model may be written as:

\[
P_{\text{ster}} = \frac{Y_{\text{ster}}}{N_{\text{ster}}} \approx \text{Binomially distributed}, \ Bi(\eta_{\text{ster}}, N_{\text{ster}})
\]

where

\[
\log \left( \frac{\eta_{\text{ster}}}{1 - \eta_{\text{ster}}} \right) = \alpha_{d} + \beta_{d_{ster}} + C_{s} + D_{r_{ster}}
\]  \[2\]

\(\eta_{\text{ster}}\) is the calculated number of day-degrees for treatment \(t\) at incubation time \(e\) in replicate \(r\) of experiment \(x\)

\(\alpha_{d}\) is the effect of dose \(d\), and \(\beta\) is the effect of day degrees

\(C_{s}\) is the random effect of experiment \(x\). \(C_{s}\) is assumed to be normally distributed with a mean of 0 and a variance of \(\sigma_{C}^{2}\)

Other terms have the same definitions as above.

The model fit the data adequately using a linear effect of day-degrees and an effect of the difference between the lowest dose and the higher doses. The model parameter estimates are shown in Table 1.

The model could then be used for predicting the proportion of flies with Campylobacter at a given temperature, dose and incubation time based on the formula:

\[
P = \frac{e^{\eta}}{1 + e^{\eta}}
\]  \[3\]
where $L$ is the linear predictor on the logit scale, $L = \hat{\alpha}_d + \hat{\beta}g$.  
$\hat{\alpha}_d$ and $\hat{\beta}$ are the estimates of $\alpha_d$ and $\beta$ in model 2.  
The estimated relative number of flies with *Campylobacter* in each combination of treatment and incubation time was calculated by back transforming the estimates on the logit-scale. The 95% confidence intervals (CI) of the relative numbers were likewise calculated by back transforming the intervals on the logit-scale.

From this, the number of day-degrees required to reduce the proportion of flies with *Campylobacter* to a desired percentage ($\text{Pct}$), can be calculated as:

$$\hat{d}_d = (\hat{\alpha}_d - \ln(\text{Pct} / (100 - \text{Pct})) / \hat{\beta} \quad [4]$$

Based on this, the number of hours by which the flies with *Campylobacter* is reduced to $\text{Pct}$ can be calculated as:

$$\hat{h}_d = 24\hat{d}_d / (t - 4) \quad [5]$$

Approximate variances on the predicted number of hours were calculated using the delta method (see e.g., Cox, 1998). From these variances 95% CI were estimated for the calculated hours.

All analyses were carried out using the procedure Glimmix of SAS version 9.2 (see the chapter on PROC GLIMMIX in SAS Institute, 2009).

### Results

#### The Temperature Experiment.

Almost all flies of the positive controls (74 out of 75) in the temperature experiment were positive at 0 h at all tested temperatures. Furthermore, all flies used for negative controls were negative for *Campylobacter*.

The analysis showed a significant decline in the number of *C. jejuni* positive flies in relation to ascending temperature ($F = 7.75, df = 3,39, P = 0.014$) and increasing incubation time ($F = 12.77, df = 3,39, P < 0.001$), but no interaction between temperature and incubation time ($F = 1.01, df = 9,30, P = 0.456$). The predicted proportions of positive flies (back transformed *logit* means) for each of the five constant temperatures and incubation time, together with the corresponding 95% CI are shown along with the observed proportions in Fig. 1.

#### The Dose Experiment.  
All positive control flies (45) were *Campylobacter* positive at 0 h at all three doses. The analysis showed a significant decline in the proportion of *Campylobacter* positive flies with increasing incubation time ($F = 20.27, df = 3,30, P < 0.001$) and with decreasing inoculation dose ($F = 14.66, df = 2,30, P < 0.001$), but no significant interaction between dose and incubation time ($F = 0.07, df = 6,24, P = 0.998$). The separate analysis of the doses showed significant differences between the low dose and the two higher doses ($t = 4.68$ and $5.06$, $P$ was in both cases <0.001), but no significant differences between the two higher doses ($t = 0.64, P > 0.527$). The predicted proportions of *Campylobacter* positive flies at any dose and incubation time, calculated by the back transformed means and with the corresponding 95% CI are shown along with the observed proportions in Fig. 2.

#### Both Experiments Combined.

The mixed model analysis using a linear effect of day-degrees on the basis of the experimental temperatures and an effect of the difference between the lowest dose and the higher doses fitted data adequately with a significant effect of day-degrees ($F = 95.99, df = 1,87, P < 0.001$) and for dose ($F = 7.06, df = 3,87, P = 0.003$). The estimated parameters of the model are shown in Table 1. The estimates show that the intercept (on the logit-scale) for the high doses was almost twice that for the low dose and that the number of flies with *Campylobacter* (on the logit scale) increased 0.322 for each day-degree.

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**Table 1.** Estimated mixed model parameters for calculating the proportion of flies with *Campylobacter* for given day-degrees (Eq. 3)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_d$ Constant value for low dose</td>
<td>2.69 (1.70–3.40)</td>
</tr>
<tr>
<td>$\alpha_d$ Constant value for high doses</td>
<td>4.92 (3.97–5.87)</td>
</tr>
<tr>
<td>$\beta$ Common slope</td>
<td>0.322 (0.267–0.378)</td>
</tr>
</tbody>
</table>

Figures in brackets are 95% CI.

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**Fig. 1.** Predicted proportion of *C. jejuni* positive house flies in relation to temperature. Predicted proportion (solid line) of *C. jejuni* positive house flies with 95% CI (dotted lines) at 0, 6, 12, 18, and 24 h of incubation after inoculation with $1.6 \times 10^7$ CFU and incubation at 15, 20, 25, 30, and 35°C. The observed proportions of *Campylobacter* positive flies in the three replicates of five flies each, at the respective temperatures and incubation times are represented by a cross. Data for the positive controls at 0 h are included as well.
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tion decreased with increasing temperature, again with the value for the low dose being considerably lower than the other two doses at 25℃. The lowest predicted proportion <1%, was found at 35℃ after incubation at 24 h.

The day-degrees required to reduce the proportion of flies with Campylobacter to 50% was calculated to be 8.35 for the low dose and 15.28 for the high doses, meaning that the halving time for the high doses was almost twice as high as for the low one.

Fig. 3 shows the reductive time based on model predictions until the proportion of C. jejuni positive flies remained in the flies, the two factors were combined in a statistical model. Based on the model predictions e.g., at 25℃, 50% of a group of flies can be expected to carry viable Campylobacter 20 h after.

Discussion

The results clearly demonstrated that the prevalence of Campylobacter positive flies declined over time with ascending temperatures and when the inoculation dose was reduced. In fact, only a few flies stayed positive 24 h post inoculation at 25, 30, and 35℃ despite a high inoculation dose of Campylobacter. This result, however, was in contrast to those at 15 and 20℃, where a high number of the flies remained Campylobacter positive throughout most of the 24 h incubation period (Fig. 1).

Because the temperature and the inoculation dose both had a significant effect on the time Campylobacter remained in the flies, the two factors were combined in a statistical model. Based on the model predictions e.g., at 25℃, 50% of a group of flies can be expected to carry viable Campylobacter 20 h after.

Using the model, the proportions of flies with C. jejuni for each combination of treatment and incubation time could be calculated (Table 2). The predicted proportions were above 99% at incubation time 0 h (except for the low dose where the proportion was ~94%) and the confidence limits were rather narrow.

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having ingested a high dose and 10 h if the dose is low (Fig. 3).

House flies and other filth living flies harboring *Campylobacter* may be an immediate risk for most broiler farms in Northern Europe when outdoor temperatures reach levels above 20°C and droppings from pastured farm animals are present (Hald et al. 2004, 2008; Szalanski et al. 2004). However, with the relatively short retention time found in the current study, the role of house flies as long distance carriers of *Campylobacter* is probably small. Actually, based on the results it seems the prerequisites for a successful transfer of *Campylobacter* by house flies are (1) short distances from source to the broilers, (2) a constantly high turnover rate of new flies acquiring *Campylobacter*.

Indications for house flies as short distance carriers of *Campylobacter* was found in a study by Hald et al. (2008) where flies collected near broiler farms and examined for *Campylobacter* showed a notably higher prevalence on one of the farms, most likely because this farm had a swine operating unit located in its close vicinity (see also Hald et al. 2004). Furthermore, despite their high activity and mobility, adult house flies generally remain within a perimeter of a few hundred meters from the sites of their development (Schoof 1959, Lysyk and Axtell 1986, Boase 2007, Stafford 2008). Only under unfavorable conditions are they disposed to make migratory flights. Such movements of single or groups of house flies, with the risk of disseminating *Campylobacter* to sites situated further away from the source, are generally triggered by density dependent factors such as competition for food or lack of proper oviposition sites. It is especially pronounced on dairy cattle, swine farms, and poultry facilities when the population density of flies attains high levels in the warm summer period (Stafford and Bay 1994, Skovgård and Nachman 2004).

Based on laboratory findings, the flight velocity of 5 d old house flies is ≈0.6 m per second at 26°C and flight duration is ≈90 min, giving a maximum distance of ≈3 km (Shepard et al. 1972). In a recent paper by Nazni et al. (2005) based on a mark-release recapture study, a single fly managed to travel 7 km in 2 d whereas the overall picture from the few recaptures was flight ranges of 2–5 km with a travel time of 1 to 4 d. Therefore, if a house fly acquires *Campylobacter* from an outdoor or indoor source, and immediately takes flight in a straight line, in most cases it will contribute to a horizontal dissemination of *Campylobacter* over a few kilometers only.

Temperatures below 15°C were not included here as it was assumed a priori that house flies as carriers of *Campylobacter* will be of insignificant importance, primarily because house flies are generally inactive. This phenomenon was observed in the current study and described as well by Keiding (1986). However, despite house flies’ sluggish behavior at low temperatures, survival of *Campylobacter* is expected to increase. Consequently, during periods of cool weather the flies might switch from being active carriers of *Campylobacter* and instead become in-active reservoir organisms for the bacteria. The significance of house flies as reservoir for *Campylobacter* at low temperature is unknown, but may be of some significance because in-
activated house flies can stay alive for at least 2 mo at low temperatures (Casagrande and Hansens 1969).

In line with low temperatures, diurnal temperature oscillations should be considered as well. For example, early mornings and late evenings in spring and autumn in Northern-Europe are generally cool with temperatures below 10–15°C. Midday temperatures normally increase however, allowing the flies to forage and move actively. Therefore, if flies pick up Campylobacter during midday they can become carriers for an extended period when temperatures decrease, and might become a risk for the broiler production in the proximinity.

Most experimental doses of C. jejuni used in the current study exceeded the ones a house fly may take up in a natural environment. A house fly can take up 2–4 μl liquefied food in one meal (Kobayashi et al. 2009). House flies forage preferably on freshly voided livestock manure, which may contain between 10^3 to 10^7 CFU of Campylobacter per gram (Hutchison et al. 2004, Sinton et al. 2007, Krueger et al. 2008, Gilpin et al. 2009). Thus, an estimate of the number of Campylobacter a house fly could take up in one meal would be up to 10^8 CFU. However, given the opportunity, house flies in the environment probably will take up a meal several times during the day, but this may not automatically lead to an increased number of Campylobacter a fly can hold. It is well known that foraging house flies simultaneously and consistently regurgitate and defecate resulting in the excretion of a high number of Campylobacter as well. Furthermore, the destruction of Campylobacter cells within the alimentary tract because of the exposure to digestive enzymes, low pH levels and antagonistic effects from the natural bacterial flora will probably contribute further to decrease the number of Campylobacter cells (Greenberg 1973, Espinoza-Fuentes and Terra 1987, Terra and Regel 1995). This research area, however, should be given attention as the load of Campylobacter in the surface of flies might become a risk for the broiler production in the proximinity.

In conclusion, this study showed that the time C. jejuni was retained after inoculation with a single dose in laboratory reared house flies could be described by the number of day degrees with a base temperature of 4°C. This means that at low temperature (15°C) about half of the flies will still be carriers after 15–35 h, depending on the dose. At high temperature (35°C) and again depending on the dose, about half the flies will be carriers after ≈6–12 h. So overall, the retention time of Campylobacter declined significantly with ascending temperature and reduced doses.

Based on the results, the house fly is suggested to mainly be a short distance carrier of Campylobacter, which further stresses the importance of an efficient on-farm control of house flies and other fliest production facilities.

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