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Campylobacter infections in four poultry species in respect of frequency, onset of infection and seasonality

Campylobacter-Infektionen bei vier verschiedenen Geflügelarten unter Berücksichtigung von Häufigkeit, Infektionsbeginn und Jahreszeit

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Summary

Over a seven-year period (2001–2007) flocks of four poultry species, 52 broiler flocks, 46 Pekin duck flocks, 22 Muscovy duck flocks, 20 turkey flocks, which were kept on the same farm, were continuously investigated for *Campylobacter* (*C.*). Altogether 76.1% of the broiler flocks, 59.6% of the Pekin duck flocks, 68.2% of the Muscovy duck flocks and 90.0% turkey flocks were *Campylobacter* positive. The prevalence during the course of the fattening period increased steadily. There was no specific point of time for the onset of infection. More detailed examination over a one-year period showed the highest isolation rates of *C. coli* from July to September and a higher isolation rate of the same agent with increasing age, in all species except Muscovy ducks. Moreover, *C. coli* was isolated more often from the lungs of broilers and Muscovy ducks than from the other two bird species. Flocks of all species housed during the summer months featured a higher prevalence of *Campylobacter* colonisation than those housed in winter. This was statistically significant for broilers. Another approach for evaluating the seasonality of *Campylobacter* colonisation was to compare the age of the respective poultry species when the onset occurred in summer and in winter. All poultry species were younger when infection was introduced into a flock in summer. This was statistically significant for broilers and for Pekin ducks.

Keywords: *Campylobacter*, broiler, turkey, Pekin duck, Muskovy duck, season

Zusammenfassung

Während eines ununterbrochenen Zeitraumes von sieben Jahren (2001–2007) wurden 52 Broilerherden, 46 Pekingentenherden, 22 Moschusentenherden und 20 Putenherden an einem Standort auf das Auftreten von *Campylobacter* (*C.*)-Infektionen untersucht. Der Anteil *Campylobacter*-positiver Herden betrug bei Broilern 76,1 %, bei Pekingenten 59,6 %, bei Moschusenten 68,2 % und bei Puten 90 %. Stellt man die ersten *Campylobacter*-Nachweise dem Alter der jeweiligen Tiergruppe gegenüber, so ergibt sich eine stetig steigende Kurve. Der Verlauf dieser Kurven weist für jede Geflügelart eine andere Steigung auf, die durch die Haltungsbedingungen beeinflusst sein könnte. Ein bestimmter Zeitpunkt, an dem sich die Herden infizieren ergibt sich nicht. Eine Differenzierung der *Campylobacter*-Spezies während eines Jahres ergab eine häufigere Isolierung von *C. coli* in der Zeit von Juli bis September als in anderen Monaten. Parallel dazu konnte *C. coli* häufiger bei längerer Lebensdauer der Geflügelart nachgewiesen werden mit Ausnahme bei Moschusenten. Außerdem ließ sich *C. coli* häufiger bei Broilern und Moschusenten aus der Lunge isolieren als bei Pekingenten und Puten. Herden aller Geflügelarten, die im Sommer eingestallt wurden, wiesen durchgehend eine höhere *Campylobacter*-Prävalenz auf als solche mit Einstallung im Winter. Für Broiler war dies statistisch signifikant. Eine andere Herangehensweise zur Bewertung saisonaler Zusammenhänge ist, das Alter der Herde beim erstmaligen Nachweis der *Campylobacter*-Infektion heranzuziehen. Grundsätzlich waren die Herden aller Geflügelarten zum Zeitpunkt des Infektionsbeginns im Sommer jünger als bei Infektion im Winter. Dies ließ sich für Broiler und für Pekingenten statistisch absichern.

Schlüsselwörter: *Campylobacter*, Broiler, Pute, Pekingente, Moschusente, Jahreszeit

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Introduction

Campylobacter (*C.*) continues to be the most commonly reported zoonotic bacterial pathogen in humans in the EU. The trend in confirmed cases of human campylobacteriosis shows an increase in the last years with a constant marked seasonality, in which most cases are reported during the summer months from June to August and a gradually decrease from September to December. Handling, preparation and consumption of poultry meat and especially broiler meat is considered to be the main food-borne source of infection and may account for 20% to 40% of human campylobacteriosis cases. An additional 50% to 80% of human cases may be assigned to direct or environmental transmission of *Campylobacter* originated from chicken as the main *Campylobacter* reservoir (EFSA, 2013).

A vertical transmission of *Campylobacter* spp. from breeder flocks via the ovary and fertile egg to the progeny is unlikely to occur (Shane et al., 1986; Shanker et al., 1986; Evans, 1992; Jacobs-Reitsma et al., 1995; Gregory et al., 1997; Newell and Wagenaar, 2000; Callicott et al., 2006). *Campylobacter* positive laying hens which were faecal shedders did not produce infected eggs (Shane et al., 1986; Baker et al., 1987) although *Campylobacter* can be detected in the lower and upper reproductive tract (Cox et al., 2009). Due to findings of the same type of *Camplyobacter* in breeders and the hatchery (Byrd et al., 2007) or their progeny (Pearson et al., 1996; Cox et al., 2002) the possibility of vertical transmission has been suggested.

The horizontal transmission from the environment is most relevant for the infection of poultry (Shanker et al., 1986; van de Giessen et al., 1996; Jacobs-Reitsma, 1997). Potential vectors are beetles (Jacobs-Reitsma et al., 1995; Hazeleger et al., 2008), although they do not seem to play a significant role as a reservoir of *Campylobacter* from one fattening cycle to the next (Skov et al., 2004). Also fleas and mites (Lindblom et al., 1986), flies (Rosef and Kapperud, 1983; Shane et al., 1985; Hald et al., 2008) and rodents (Kapperud et al., 1993; Berndtson et al., 1994; Meerburg et al., 2006) have been identified as vectors. Infected livestock and free-living animals including wild birds can be considered as infective sources especially in free-range poultry (Glünder et al., 1988; van de Giessen et al., 1996; Gregory et al., 1997; Stern et al., 1999). Both, *Campylobacter* contaminated equipment being used by staff and thinning of broiler flocks have also been identified as risks for *Campylobacter* transmission (Hald et al., 2000; Allen et al., 2008). Water has been described by several authors as an important source of *Camplyobacter* infection (Engvall et al., 1986; Kapperud et al., 1993; Ogden et al., 2007; Messens et al., 2009). A horizontal transmission also seems to be possible by aerosols originating from the cleaning of neighbouring houses (Berndtson et al., 1996; Posch et al., 2006).

The influence of the production system can be assumed from the finding that organic and free-range chickens are more often *Campylobacter* positive than intensively reared birds, and that layers kept in floor pen systems are more frequently colonised compared to layers kept in diverse cage systems, possibly due to increased environmental exposure (Kazwala et al., 1993; Rivoal et al., 1999; Heuer et al., 2001; Weber et al., 2003; El-Shibiny et al., 2005; Luangtongkum et al., 2006; van Overbeke et al., 2006; Huneau-Salaün et al., 2007; Näther et al., 2009; Allen et al., 2011; Wassenaar, 2011).

The timepoint of infection can be delayed under excellent hygienic conditions and it even appeared to be feasible to grow broilers free of *Campylobacter* (Munroe et al., 1983; Neill et al., 1984; Smitherman et al., 1984; Altmeyer et al., 1985). However, a single individual bird infected with low numbers of *Campylobacter* can be sufficient for the initial infection of a flock (Stern et al., 2001). *Campylobacter* counts in the intestine are very high (Altmeyer et al., 1985; Weber, 2000) and the majority of birds rapidly become *Campylobacter* positive by faecally contaminated litter, feed and water within a few weeks or even days (Smitherman et al., 1984; Shanker et al., 1990; Gregory et al., 1997; Evans and Sayers, 2000; Shreeve et al., 2000; Newell and Fearnley, 2003; Nauta et al., 2009; van Gerwe et al., 2009; Wassenaar, 2011). Thus, broilers as well as layers become infected very early in life and are generally *Campylobacter* positive at an age of two to four weeks (Kazwala et al., 1990; Jacobs-Reitsma et al., 1995; Berndtson et al., 1996). The probability of *Campylobacter* colonisation increases with age and duration of the keeping period (Rosef et al., 1984; Altmeyer et al., 1985; Lindblom et al., 1986; Jacobs-Reitsma, 1997).

Since newly hatched chicks are free of *Camplyobacter* and in most field investigations chickens become *Campylobacter* positive only at an age of two to three weeks Newell and Fearnly (2003) considered flock colonisation to be age-dependent. They regarded the period up until the first evidence of *Campylobacter* infection as the so-called "lag phase", this being an inherent property of the chick. Various reasons are mentioned for this delay of infection: Maternal antibodies, antibiotic feed additives, altered feed compositions, shifts in the intestinal microflora and the maturation of mucosal immunity (Stern et al., 1988; Newell and Wagenaar, 2000; Sahin et al., 2002; Newell and Fearnly, 2003). *Campylobacter* infection induces specific serum antibodies within three weeks after experimental inoculation (Myszewski and Stern, 1990; Cawthraw et al., 1994; Widders et al., 1996) that can be transferred as maternal antibodies to the progeny and may be conducive to the lag phase (Sahin et al., 2001, 2003; Cawthraw and Newell, 2010). In contrast, Ringoir et al. (2007) showed that two-day-old chicks are more susceptible than two-week-old birds and there are also inconsistent reports concerning an age-dependent susceptibility for *Campylobacter* infection described by Wassenaar (2011).

A seasonal influence on the *Campylobacter* prevalence in broilers has been described in several studies. *Campylobacter* isolations from broilers were higher during the summer and autumn (Kapperud et al., 1993; Jacobs-Reitsma et al., 1994; Wedderkopp et al., 2001; Reich, 2007). In a study by Jore et al. (2010) a concordant seasonality in the incidence of *Campylobacter* colonisation in broiler flocks and the incidence of campylobacteriosis in humans was demonstrated, whereas this seasonal variation in humans in tropical climates is not present (Allos, 2001). Any season-dependent patterns of *Campylobacter* prevalence in turkeys as well as Pekin and Muscovy ducks have not been described.

There is a linear relationship between the *Campylobacter* prevalence in broiler flocks and the public health risk (EFSA, 2011), and consequently research has been focused on elucidating the epidemiology of broiler colonisation. There are notably fewer data available

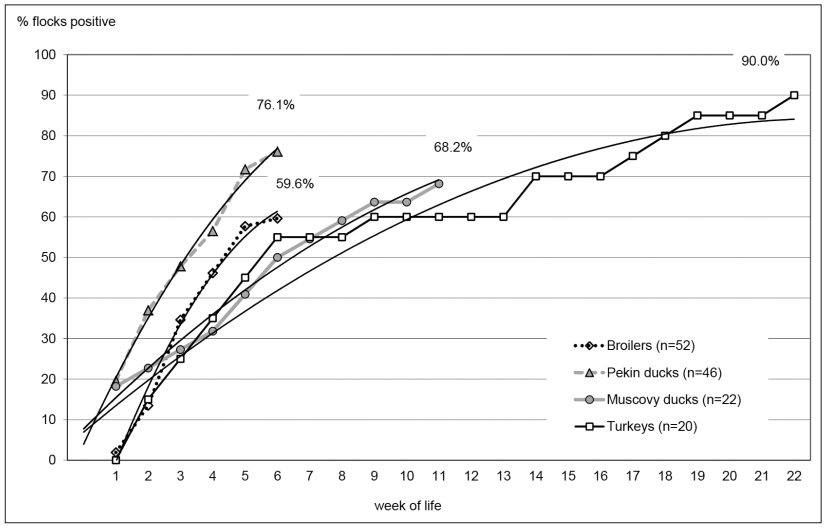

FIGURE 1: *Cumulative diagram of Campylobacter positive flocks per week in the course of the fattening period. The regression coefficient (R^2) of the respective regression line for broilers = 0.987, for Pekin ducks = 0.991, for Muscovy ducks 0.984 and for turkeys 0.962.*

about turkeys and ducks kept in houses without access to free range. The objective of the present study was to determine *Campylobacter* prevalence in four different poultry species in the course of seven years in respect of onset of infection and seasonality.

Material and Methods

Farm management and poultry flocks

This study considers poultry flocks of four avian species which were reared on the farm for Education and Research of the University of Veterinary Medicine Hannover. Over a period of seven years, beginning from January 2001 until September 2007, altogether 46 Pekin duck flocks, 22 Muscovy duck flocks, 20 turkey flocks and 52 broiler flocks were investigated. All birds were bred in commercial hatcheries and placed into four separate poultry houses on day of hatch. Broilers, turkeys and Pekin ducks were kept on litter, whereas Muscovy ducks were raised on plastic grits. All flocks were subsequently reared under commercial conditions, including feed from the same feed mills, which also supply other flocks of the respective commercial poultry integrations. The keeping density, the feeding programme, temperature and lighting conditions were in accordance with the general regulations for all other farmers of the same integrations fattening poultry. Information on flock size, room size and average slaughter age of the individual flocks is given in Table 1.

TABLE 1: *Details of sampled poultry flocks*

Poultry species	No. of flocks	No. of animals per flock	Room size	Litter	Slaughter age (d)
Pekin ducks	46	3,400	425 m^2*	straw	39–50
Muscovy ducks	22	1,840–3,550	425 m^2	plastic grits with manure pit below	60–82
Turkeys	20	3,100–3,300	945 m^2	wood shavings and straw	112–147
Broilers	52	18,500–18,800	945 m^2	wood shavings	30–41

* Additional winter garden (173 m^2) from the 3rd week of life onwards until slaughter

A distance of 26 m between the houses, separate clothing for animal keepers and a physical barrier between the inside and outside area of the entrance room including disinfection of hands and boots prevented direct transmission of infectious agents by the personnel. During the service period which varied from seven to 36 days the cleaning and disinfection measures were carried out by an external commercial company. Rodents were controlled along the outside of the houses with commercially available cumarin preparations.

Sampling methods

Each poultry house was checked at least twice a day. All dead ducks and turkeys were collected and stored at 4°C to 7°C until necropsy was performed. Twice a week five to ten broilers, which had died the previous night were chosen for necropsy. Moribund individuals, killed for reasons of animal welfare were also included in the examinations. The abdominal cavity was aseptically opened for bacteriological sampling. Freshly sterilised instruments were used for removal of the liver, lungs and digestive tract and were also exchanged between the necropsy of each bird. Individual organ and caecal samples were placed into separate sterile plastic bags and transported directly to the laboratory.

Laboratory testing

Caeca were opened and swabs with caecal material were streaked directly onto modified *Campylobacter* charcoal differential agar (mCCDA, Oxoid, Wesel, Germany). Media were incubated for 48 h at 37°C microaerobically in anaerobic jars (Oxoid), using the CampyGen Kit (Oxoid). Up to three *Campylobacter*-presumptive colonies from each sample were subcultured on mCCDA and subsequently on Columbia agar supplemented with 7% sheep blood (Oxoid) and then being incubated for 48 h at 37°C under microaerobic conditions. Colonies were identified as *Campylobacter* spp. by typical morphology and motility, Gram-stain, catalase and oxidase reaction and no growth under aerobic and anaerobic conditions. *Campylobacter* isolates from the caeca, liver and lungs, which had been collected during a one-year period from November 2004 to November 2005 were additionally identified by PCR as already described (Alter et al., 2011).

Evaluation and statistics

For analysis of seasonal influences the months from October to March were classified as winter and the period from April to September as summer. Data were represented as the number of positive samples over the total number of samples taken. The Chi-Square test (SigmaStat 3.1, Systat Software, San Jose, California, USA) was used to evaluate differences in *Campylobacter* spp. prevalence by flock, sample site, *Campylobacter* species and season. For comparing the age at the time point of onset of infection the t-test was applied. Significance of data was set at $p \leq 0.05$. Different letters in the figures and tables indicate statistically significant differences.

Results

Frequency and onset of *Campylobacter* colonisation in poultry flocks

Altogether 2444 Pekin ducks, 1847 Muscovy ducks, 3034 turkeys and 5524 broilers were tested for the presence of *Campylobacter*. Throughout the investigation some poultry flocks of any species remained *Campylobacter* negative (Tab. 2). The colonisation occurred the earliest in Pekin and Muskovy ducks from first day of life. In contrast one turkey flock was colonized as late as one day prior to slaughter (day 150). Positive and negative flocks appeared with no identifiable system, indicating that there is no specific factor leading to *Campylobacter* positive or negative flocks. During fattening more and more flocks have been colonised by *Campylobacter* in which differences could be seen between species. Figure 1 shows the percentage of *Camplyobacter* positive flocks of the investigated poultry species. Nearly 20% of the flocks of both duck species were already infected within the first week of life, while turkey flocks and broiler flocks became positive at a comparable level about one week later. In general, the curves of the four poultry species showed a similar increase during their fattening periods. The maximum infection rates stopped at 59.6%, 68.2% and 76.1% for broilers, Muscovy and Pekin ducks, respectively due to slaughter at the end of the fattening period. The overall *Campylobacter* detection rate based on combined results from male and female turkey flocks did not exceed 90% since two flocks of female turkeys had already been slaughtered with a negative *Campylobacter* status in the 16th week of life (Fig. 1).

Interrelation between season and *Campylobacter* colonisation

Figure 2 describes the *Campylobacter* status of flocks kept either in the summer or winter season. A flock initially housed in one of the two seasons was therefore assigned to the respective season regardless of life span. It became obvious that statistically significantly more broiler flocks were *Campylobacter* carriers in the summer than in the winter period. The majority of two duck species and turkey flocks were also positive in summer but the difference was not statistically significant. Coincidentally, it appeared that the two negative female turkey flocks were housed and reared in the winter season.

Another approach was to compare the age of the birds when the first individual became positive within a flock during the summer or the winter season (Fig. 3). Infection of broilers and Pekin ducks with *Campylobacter* occurred significantly earlier in life in flocks reared in summer than in flocks reared in winter. The numerically greatest difference with 38 days in summer versus 51 days in

TABLE 2: *Overview of Campylobacter isolations from poultry flocks indicating the duration of the fattening period and the day of the first proof of Campylobacter in the respective flock*

Flock serial-No.	Broilers		Pekin ducks		Muskovy ducks		Turkeys	
	First proof (day)*	Period (days) **	First proof (day)	Period (days)	First proof (day)	Period (days)	First proof (day)	Period (days)
1	–	36	20	50	39	79	–	113
2	29	36	42	50	39	81	36	117
3	27	36	–	47	30	74	21	143
4	20	36	–	48	32	67	15	137
5	–	36	33	48	59	79	35	143
6	–	36	19	49	14	69	115	146
7	–	36	13	48	–	66	123	147
8	39	40	7	45	–	67	26	146
9	11	41	6	47	–	76	129	147
10	24	37	24	47	23	73	40	146
11	16	35	24	47	4	60	30	147
12	20	40	28	47	–	68	–	112
13	–	36	10	45	54	74	96	114
14	–	36	3	44	3	67	94	149
15	11	35	34	45	74	76	23	146
16	–	36	29	42	–	74	150	151
17	–	36	9	41	7	77	10	112
18	16	37	–	41	1	80	11	113
19	26	37	30	42	–	81	61	146
20	26	36	14	44	–	82	13	128
21	–	37	16	43	15	77		
22	–	37	34	43	44	76		
23	–	37	–	39				
24	30	37	31	45				
25	20	36	33	46				
26	20	36	2	49				
27	2	30	6	49				
28	–	37	9	49				
29	23	37	3	45				
30	–	36	1	44				
31	–	36	–	44				
32	–	36	–	44				
33	29	35	–	39				
34	21	35	–	39				
35	27	34	–	39				
36	17	34	–	39				
37	10	34	20	45				
38	11	35	26	45				
39	–	34	1	42				
40	31	32	8	42				
41	29	34	–	45				
42	18	34	36	41				
43	14	34	2	42				
44	–	33	19	47				
45	–	34	9	46				
46	–	33	13	46				
47	19	35						
48	35	36						
49	19	34						
50	10	34						
51	–	36						
52	–	39						

* Age in days of the particular poultry species, when *Campylobacter* was isolated the first time in respective flock;
** days of keeping period (until slaughter); – no *Campylobacter* isolation during the entire fattening period

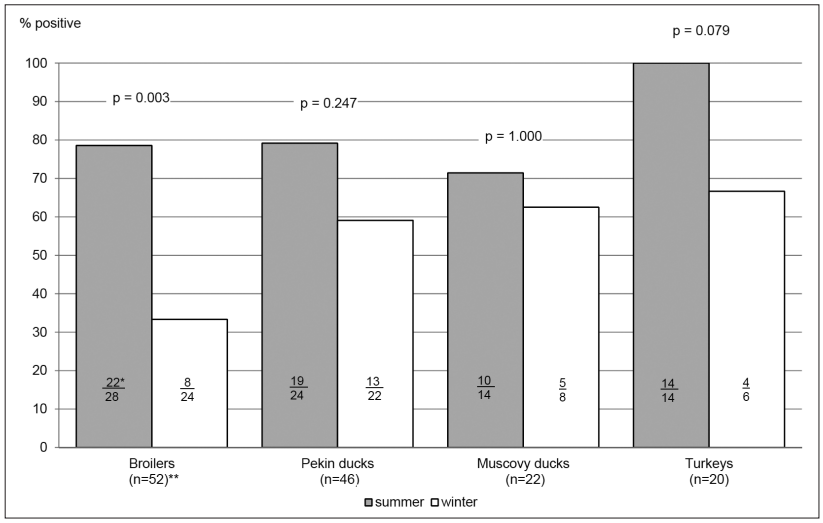

FIGURE 2: *Seasonal occurrence of Campylobacter in poultry flocks (% positive), * number of flocks positive/number of flocks tested during the respective season, ** total number of flocks examined.*

winter for the onset of *Campylobacter* infection was found in Muscovies, although this variation was not statistically significant.

Distribution pattern of *Campylobacter* species

During a one-year period three *Campylobacter* isolates per caecal sample were differentiated to the species level. All isolates belonged either to the species *C. jejuni* or *C. coli*. The proportion of *C. coli* isolates (Tab. 3) was generally higher in poultry species with a longer fattening period except for Muscovy ducks. In relation to the season, *C. coli* (Fig. 4) was only isolated from July to December with a peak in August and September. All birds were additionally examined for the presence of *Campylobacter* species in the liver and lung during this period (Tab. 4). Both, *C. jejuni* and *C. coli* could be isolated from liver and lung 35 times in total out of 410 *Campylobacter* positive birds (8.5%). The proof of 25 isolates from the lung differed significantly (p = 0.016) from the overall ten isolates from the liver. Furthermore, broilers and Muscovies showed the highest occurrence of the agent in extra-intestinal sites (12.6% and 16.4%) when compared with the other species (Pekin ducks 2.1%, turkeys 1.7%). Both, liver and lung were infected simultaneously in only two individuals of both, broilers and Muscovy ducks. In all other cases the agent could be found in either the lung or the liver. There was no evidence that these organs become infected at a certain time point after the first proof of *Campylobacter* in the flock.

TABLE 3: *Correlation of C. coli isolations in percent of all Campylobacter isolations with mean age of the respective poultry species; only flocks were included with isolation of both Campylobacter spp. Comparison of the ratio of C. coli: Br vs. Pe p = 0.015, Br vs. Mu p = 0.138, Br vs. Tu p = 0.001, Pe vs. Mu p = 0.001, Pe vs. Tu p = 0.387*

	Age (mean at slaughter)	*C. jejuni* (n)	*C. coli* (n)	% *C. coli* of all *Campylobacter* isolates
Broilers (Br)	36	121	40	24.8[a]
Pekin ducks (Pe)	46	51	35	40.7[b]
Muscovy ducks (Mu)	66	91	18	16.5[a]
Turkeys (Tu)	145	108	96	47.1[b]

[a, b] Indicate significant differences

Discussion

Frequency and onset of *Campylobacter* infections in poultry flocks

The percentage of *Campylobacter* positive broiler flocks varies from 15% in Iceland (Barrios et al., 2006) to around 40% in Ireland and Germany (McDowell et al., 2008; Näther et al., 2009) up to nearly 100% in Great Britain (Evans and Sayers, 2000). Thus, 60% *Campylobacter* positive broiler flocks found in this study is in accordance with findings of others.

Only very little information exists about the percentage of *Campylobacter* positive turkey flocks. Wallace et al. (1998) reported a colonisation rate of 100% in five investigated broods of poults by day 21 of life, 66% of the flocks in Germany (EFSA, 2012) and 41.8% of the slaughter batches in Slowenia were tested positive (EFSA, 2012) as well as 50% of 6 commercial flocks in Finland (Perko-Mäkelä et al., 2009). The own findings of 90% *Campylobacter* positive flocks of a total of 20 flocks is in accordance with the reported range in the literature and confirms nearly exactly an earlier study on the *Campylobacter* prevalence in turkeys carried out on several different farms in Northern Germany where 89% of 19 flocks were found to be *Campylobacter* positive (Glünder and Windhaus, 1998).

Currently only few data are available about the *Campylobacter* prevalence in domestic ducks kept in commercial larger-scale production and there is virtually no further information about the duck species or the housing system. Nonga and Muhairwa (2010) reported from Tanzania a *Campylobacter* prevalence of 80% in commercial free-range Muscovy duck flocks. Tsai and Hsiang (2005) found a flock prevalence of 92% out of 100 duck farms including 44 cross-breed mule duck farms, nine native Tsaiya duck farms, 36 Muskovy duck farms, two Cherry Valley duck and one mallard duck farm. Prevalences for the single flocks were not reported.

The onset of *Campylobacter* colonisation was found in two flocks of Pekin ducks and one flock of Muscovy ducks already on the first day of life and on the second day in one broiler flock (Tab. 1). As described above vertical transmission is unlikely to occur but it cannot be ruled out that *Campylobacter* contaminated crates and vehicles used for transportation are a possible source of colonisation (Wassenaar, 2011). In the course of the fattening period more and more flocks of each poultry species became *Campylobacter* positive. There is, however, no evidence of a special point of time when flocks are preferably infected. The curves which describe the percentage of *Campylobacter* positive flocks of the respective poultry species show a rising curve with increasing age. The curves' steepest increase was related to Pekin ducks, followed by broilers. The increase was less pronounced for Muscovy ducks and turkeys. The rapid colonisation of Pekin ducks could be due to the

fact that they are kept on straw containing enormous amounts of wasted drinking water which can support the survival of *Campylobacter* introduced into the house and the free access to the winter garden from the third week of life onwards during the entire year. Broilers were kept in a closed house on litter which was not renewed during the entire fattening period. This may enhance the humidity inside, thereby supporting the survival of *Campylobacter* as it additionally facilitates the birds` direct contact to faeces. In contrast to Pekin ducks, Muscovy ducks were kept on grids and in this way the individuals are less exposed to their faeces in comparison to broilers. The slower *Campylobacter* colonisation in Muskovies could be due to the fact that they were kept under blue light and comparably dark to prevent cannibalism. This did in fact attract fewer amounts of flies from outside. Turkeys are kept in a dry surrounding on wood shavings, which are less favorable for the survival of *Campylobacter* (Egen and Glünder, 2001). Furthermore, the weekly adding of fresh litter material also reduces the contact to faeces and by this the uptake of *Campylobacter* contaminated material. From other reports it is well known that the housing system can influence the prevalence of *Campylobacter* (Näther et al. 2009; Allen et al., 2011; Wassenaar, 2011). Partial depopulation of a flock is a known risk of introduction of *Campylobacter* into a flock (Allen et al., 2008) but could not contribute to results of the present study because thinning was generally not carried out.

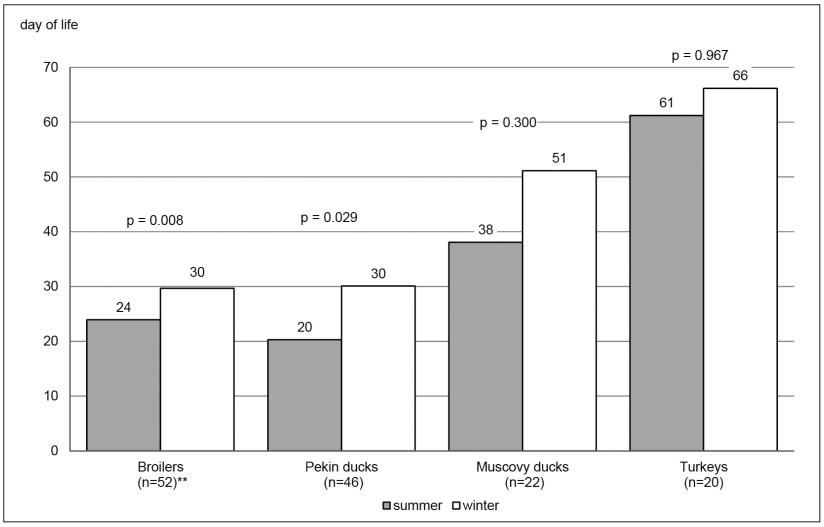

FIGURE 3: *Seasonal influence on first incidence of Campylobacter in a flock (day of life of the individual from which Campylobacter could be isolated the first time).*

In this study no evidence is given for a lag phase which is regarded to be caused among others by maternal antibodies (Newell and Wagenaar, 2000; Sahin et al., 2001, 2002, 2003). It can be assumed that all parent breeder flocks were or had been infected during their life and transferred humoral antibodies to their progeny. In this case colonisation of chicks and poults during their first three weeks of life should have been prevented which was not the case. Furthermore, it could be shown that high titers of humoral antibodies induced after immunisation with an inactivated *Campylobacter* vaccine were no able to protect against colonisation after a challenge infection with either the homologous or a heterologous *Campylobacter* strain (Glünder et al., 1994). Also, a previous colonisation with one strain does not necessarily protect against a secondary infection with another strain; at least it could be shown under laboratory conditions that *Campylobacter* strain-dependent mutual colonisation inhibiting effects occur. These findings were confirmed for *Campylobacter* isolates obtained under field conditions for different poultry species (Glünder et al., 1994; Weber, 2000; Alter et al., 2011). Wallace et al. (1998) reported the beginning of *Campylobacter* colonisation of poults within the first seven days. They also considered the isolation procedure important for actual *Campylobacter* detection.

C. jejuni/*C. coli* distribution pattern

C. jejuni is the dominant species in most studies (Vandeplas et al., 2010; EFSA, 2010, 2012, 2013; McDowell et al., 2008). In a study by Näther (2006) 77% of the *Campylobacter* isolates from broiler flocks at slaughter were *C. jejuni* and 23% *C. coli*. It was observed that 14% of the isolates from conventionally reared flocks, 36% from flocks kept in Lousiana sheds and 65% and 78% were *C. coli* in free-range and organic flocks. Findings of *C. coli* could be related to the higher age of broilers in free-range and organic flocks (Näther, 2006). Also, other authors reported that poultry was first colonised by *C. jejuni*, followed by *C. coli* in older birds (El-Shibiny et al., 2005; Humphrey et al., 2005). This is also in accordance with findings in young gulls which predominantly carried *C. jejuni* while older gulls tended to be *C. coli* positive (Glünder et al., 1991). The correlation between age of the bird and the higher probability of isolation of *C. coli* can also be underlined by our

TABLE 4: *Isolation of Campylobacter from liver and lung of poultry species tested Campylobacter positive in the caeca during a one-year period (Nov. 2004–Nov. 2005); * a, b: Statistics for interspecies isolation from the lung: Br vs. Pe = 0.007, Br vs. Mu p = 0.844, Br vs. Tu p = 0.006, Pe vs. Mu p = 0.019, Pe vs. Tu p = 0.842, Mu vs. Tu p = 0.022, c, d liver vs. lung p = 0.016; ** two individuals had a combined infection of both organs*

Poultry species	*Campylobacter* positive (n)	Liver					Lung				
		C. jejuni	*C. coli*	*C. jejuni* + *C. coli*	Σ		*C. jejuni*	*C. coli*	*C. jejuni* + *C. coli*	Σ	
Broilers (Br)	143	2	1	1	4	**	13	3	0	16	a*
Pekin ducks (Pe)	94	0	1	0	1		0	1	0	1	b
Muscovy ducks (Mu)	55	3	2	0	5	**	4	0	2	6	a
Turkeys (Tu)	118	0	0	0	0		2	0	0	2	b
Σ	410				10[c]					25[d]	

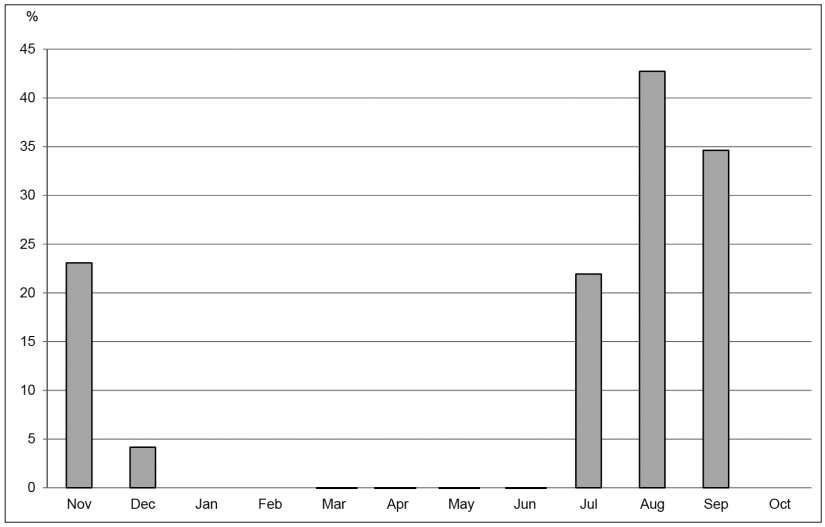

FIGURE 4: *C. coli isolations in percent of Campylobacter isolations in the course of one year (Nov. 2004–Nov. 2005)*

own findings (Tab. 3). With the exception of Muscovy ducks, the percentage of *C. coli* isolates increased with the duration of the fattening period. Recently, even an increased risk of *C. coli* infection in older people was reported (Roux et al., 2013).

The highest isolation rate of *C. coli* during July to September (Fig. 4) in our study is in accordance with results of Williams and Oyarzabal (2012) and Lawes et al. (2012) who also found *C. coli* most often in the same months. Not astonishingly Roux et al. (2013) also proved an increased risk for *C. coli* infection of people during the summer months.

Extra-intestinal *Campylobacter*

Furthermore, broilers and Muscovy ducks showed the highest occurrence of the agent in extra-intestinal sites (12.6% and 16.4%) when compared with the other species (Pekin ducks 2.1%, turkeys 1.7%). An explanation for the relatively low isolation rate from the lung of Pekin ducks and turkeys could be that Pekin ducks had an environment with extremely low concentrations of ammonia or dust because they had free access to the winter garden from the third week onwards. Concerning turkeys they were often given fresh litter and probably had better ventilation because of a row of windows being opened according to the outside weather conditions. In contrast to that, broilers were kept without fresh litter material in a warm humid house which might have led to increased ammonia and dust concentrations. Muscovy ducks were kept on grids over a manure pit and they were thus possibly in intensive contact with the noxious gaseous evaporations from the manure pit. From investigations of laying hens it is known that stress factors such as a coincident Salmonella infection can support the presence of *Campylobacter* in the liver (Glünder et al., 1998). A higher isolation rate of *Campylobacter* from extraintestinal organs of broilers was reported by Cox et al. (2007) after transportation of the broilers and after going through the slaughter and the defeathering and cooling process.

Seasonality of *Campylobacter* prevalence

In contrast to the few authors who found no correlation between *Campylobacter* prevalence and season in broilers (Humphrey et al., 1993; Evans and Sayers, 2000) a higher prevalence of *Campylobacter* during summer is reported by many others reviewed by Newell and Fearnly (2003) and Näther et al. (2009). Own investigations on the seasonal occurrence of *Campylobacter* demonstrate that not only more flocks were found to be positive for the bacterium in summer (Fig. 2) but also the onset of infection was earlier in the warm season (Fig. 3).

While the seasonal influence on *Campylobacter* infection in broiler flocks is well examined and statistically confirmed (Bouwknegt et al., 2004; McDowell et al., 2008; Lawes et al., 2012) only few studies on the prevalence in turkey flocks exist and no studies at all on the prevalence in duck flocks kept commerically under modern conditions.

The short fattening period of broilers enables a clear assignment of a flock to a season. The fattening period (Tab. 2) for Pekin ducks was up to 50 days, for Muscovy ducks up to 82 days and for turkeys up to 151 days, respectively. Thus, it could for example be possible that a turkey flock which was housed during the winter months became positive in summer. The evaluation using this classification (Fig. 2) clearly showed a statistically significant higher prevalence of *Campylobacter* in summer for broilers and even a higher but not significant prevalence also for the other three poultry species. Due to these uncertainties another approach was tried and the onset of infection chosen for grouping in the season (Fig. 3). The age of the respective flocks at the time point of the first isolation in summer was compared to those obtained during the winter season. The obtained results demonstrate that the onset of infection is earlier in summer than in winter. This difference proved to be statistically significant not only for broilers but also for Pekin ducks.

The earlier incidence of *Campylobacter* infection during the warmer period of the year can be supported by higher temperatures coincident with higher ventilation rates (Newell and Fearnley, 2003). Insects, rodents and wild birds which represent important vectors (Shane et al., 1985; Kapperud et al., 1993; Vandeplas et al., 2010; Hald et al., 2008; Hazeleger et al., 2008; McDowell et al., 2008) are more active and have a higher reproduction rate during summer and autumn and can therefore increase the chances of introducing *Campylobacter* into the poultry houses. Control measures such as fly screens caused a sustained suppressed prevalence of *Campylobacter* spp. among poultry (Bahrndorff et al., 2013). Recently, the seasonal variation in the feed-producing process has been discussed in relation to a likely contribution to the seasonality of *Campylobacter* infections (Üffing, 2012).

In conclusion, the present study indicates that there is not only a seasonal influence on a flock's *Campylobacter* prevalence but also on the timepoint of onset of infection. Onset of colonisation does not appear to correlate with a specific age, but rather every day provides an opportunity to introduce *Campylobacter* into a poultry flock, only influenced by the presence and activity of vectors, environmental and seasonal factors.

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