

## ***Campylobacter* infections in children exposed to infected backyard poultry in Egypt**

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### **SUMMARY**

*Campylobacteriosis* is a zoonotic disease which has a worldwide public health impact. The disease is endemic in Egypt; however, the epidemiology in animals and humans has not been fully characterized. The objective of this study was to compare the risk of *Campylobacter* faecal carriage in children exposed to *Campylobacter*-infected vs. non-infected backyard poultry and to identify risk factors for a backyard being classified as infected. A total of 103 households which owned backyard poultry were sampled from a rural community in Egypt. Within these households 379 poultry and 106 children were tested for *C. jejuni* and *C. coli*; 23·5% and 5·5% of poultry were positive for *C. jejuni* and *C. coli*, respectively. In the studied households; 12·3% of children were positive for *C. jejuni*, and 2·8% were positive for *C. coli*. Using logistic regression, households with poultry positive for *C. jejuni* had 3·86 (95% confidence interval 1·0–15·0) times the odds of having children positive for *C. jejuni* compared to those housed with poultry which all tested negative. Backyard poultry may present a transmission route of *C. jejuni* to children. Backyards with poor cleaning and disinfection, wet litter and manure disposed of within the backyard had increased odds of being positive for *C. jejuni*. Enhancing biosecurity and management in poultry backyards may reduce the risk of the disease.

**Key words:** Backyard poultry, *Campylobacter*, logistic regression, MAT, seroepidemiology.

### **INTRODUCTION**

*Campylobacter* species are the most frequent bacterial aetiology of gastroenteritis associated with foodborne diseases worldwide [1–3]. Symptoms of campylobacteriosis include diarrhoea, stomach pain and fever [4]. The illness is usually self-limiting and symptoms should subside within 1 week; however, infection

may lead to complications such as dehydration, bacteraemia, hepatitis and neurological damage [5]. Death is rare but occasionally occurs in young children, the elderly and immunocompromised individuals [6]. Incubation periods range from 2 to 7 days [7, 8] and average shedding is about 1–3 weeks following the incubation period depending on the setting [9–12]. In humans, *C. jejuni* and *C. coli* are the most commonly isolated species of *Campylobacter* in cases of gastrointestinal diseases [13–15]. Humans acquire *Campylobacter* infections chiefly through handling and/or consumption of contaminated food or drinks;

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mainly poultry, untreated water and unpasteurized milk [14, 16, 17]. Direct and indirect contact with infected animals (including poultry) and/or their faeces is also a risk factor for acquisition of infection by *Campylobacter* species [18]. In developing countries, *Campylobacter* infections occur most frequently in children as older children and adults acquire a level of protective immunity following exposure [9, 19, 20].

Few data exist regarding the epidemiology of *Campylobacter* species in poultry and children in Egypt. In a survey conducted in Alexandria, Egypt, *Campylobacter* species were isolated from 16.8% of 880 children suffering from diarrhoea compared to 6.4% of 1079 healthy children [21]. In a hospital-based study in Cairo, *Campylobacter* were isolated from 37/143 (25.9%) of diarrhoeic children compared to 20/132 (15.2%) of non-diarrhoeic children ( $P < 0.05$ ) [22].

The aim of this study was to investigate whether children exposed to backyard poultry infected with *Campylobacter* were at higher risk of being infected by *Campylobacter* compared to those that were exposed to backyard poultry which were *Campylobacter* negative. Risk factors for backyards being classified as positive were then investigated. To the best of our knowledge this is the first study assessing the risk to children posed by *Campylobacter* species colonization of backyard poultry.

## METHODS

### Study design

This study was conducted during April 2011 to May 2012. The study area consisted of four villages located in Gharbia governorate in the Nile Delta region of Egypt, ~100 km north of Cairo (Fig. 1).

The sample size was 100 households; which would be sufficient to detect a difference in proportions with a confidence of 95% and power of 80%, assuming the proportion of households positive for *Campylobacter* infection was 30% in the exposed group (*Campylobacter* +ve backyard poultry) compared to 5% of households positive for *Campylobacter* in the unexposed group. It was decided that four villages would be sampled in order to ensure that any effects observed were not specific to one village. After consultation with the General Authority for Veterinary Services (Gharbia Branch), a suitable list of villages was obtained in rural communities of the

governorate where most households raised chickens, ducks and/or turkeys on a small scale to supplement their income. Estimates of the number of households were obtained and sampling proportional to size was used to select four villages; with larger villages having a higher probability of being selected. Households were then selected using systematic sampling.

The target sample size for each village was 25 households; the sampling interval for each village was obtained by dividing the number of households by the target sample size. A road leading from the village centre was randomly sampled and systematic sampling began from this road, the first house sampled was dependent on the sampling interval. Sampling continued either side of the road until the border of the village was reached; and the first road clockwise was taken back towards the village centre. Next, another road opposite to the first road was selected and sampling continued in this manner. Households were sampled if they met the following two criteria: (1) owned between 10 and 100 head of backyard poultry; (2) presence of at least one child aged between 7 and 15 years in frequent contact with live poultry (i.e. involved in feeding or cleaning the backyard). If a household did not meet these criteria they were excluded and a neighbouring household was visited, until a suitable household was found.

Within poultry backyards those with  $\leq 50$  birds had three birds sampled, those with  $> 50$  birds had four birds sampled. Birds were selected for sampling using simple random sampling; cloacal swabs were collected from each selected poultry. The date of sampling, location, household ID and poultry species were recorded. All available children who were in contact with live backyard poultry aged between 7 and 15 years in each household were sampled. Children's stool samples were collected in individual sterile containers and the date of sampling, location, household ID and the age and sex of each child were recorded.

Samples from children and backyard poultry were collected on the same day. All samples were stored on ice and transported to the laboratory within 2 h of collection. Bacterial isolation and identification of *C. jejuni* and *C. coli* was performed. The laboratory work was performed at El-Slam Laboratory, Tanta, Egypt and the Faculty of Veterinary Medicine–Kafrelsheikh University (FVM-KU) Laboratory. During the sample collection, data on biosecurity and management measures was recorded by observation of the examined backyards and discussions

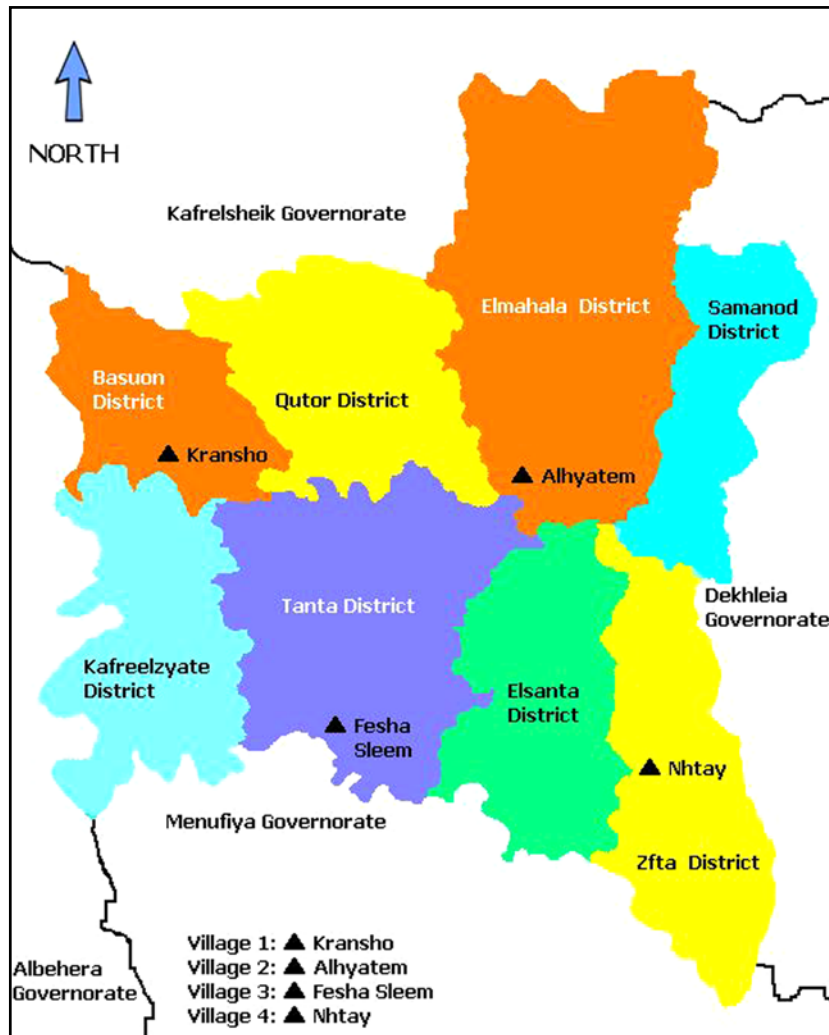


Fig. 1 [colour online]. The study area of the Gharbia governorate in the Nile Delta region of Egypt.

with livestock owners. Data collected included: (1) whether the poultry were housed in similar or different age groups; (2) whether poultry were housed with different poultry species; (3) presence or absence of other livestock in the same poultry backyard; (4) regularity of drinking water sanitation; (5) frequency of feeding sanitation; (6) observations on the cleaning and disinfection of poultry house, surrounding areas and utensils; (7) the litter quality, i.e. was there presence of wet litter; (8) whether the manure disposal was inside or outside the backyard; (9) whether ill birds were isolated from the flock.

Ethical approval was obtained by a committee from the Ministry of Health and Population – Gharbia Directorate, for institutional care and concern, for children, while sampling of poultry was approved by a committee from FVM-KU for institutional bird care and use.

### Isolation of *Campylobacter* species

*Campylobacter* selective agar (Preston) was used for selective isolation of *C. jejuni* and *C. coli* from children and poultry samples. The selective media was prepared from *Campylobacter* agar base (CM0689, Oxoid Ltd, UK), Preston *Campylobacter* Selective Supplement (SR0117, Oxoid Ltd, UK) and lysed horse blood (SR0048, Oxoid Ltd, UK). Each cloacal swab or 0.5 g children's stool was emulsified in 2 ml of 0.1% peptone water and then inoculated onto the selective medium by cotton-tipped swab. The inoculated plates were loaded in anaerobic jars (HP0011, Oxoid Ltd, UK) and incubated at 42 °C for 24–48 h under microaerophilic conditions. The Oxoid Gas Generating kit for *Campylobacter* species (BR0056, Oxoid Ltd, UK) and an active catalyst were used to obtain a microaerophilic atmosphere of about

Table 1. Percentage of backyard poultry positive for *C. jejuni* and *C. coli*

Village no.	No. of backyards sampled	No. of poultry sampled	No. of positive poultry		
			<i>C. jejuni</i>	<i>C. coli</i>	Mixed <i>C. jejuni</i> and <i>C. coli</i>
Village 1	26	96	25 (26%)	5 (5.2%)	4 (4.2%)
Village 2	25	92	23 (25%)	6 (6.5%)	3 (3.3%)
Village 3	25	92	24 (26.1%)	3 (3.3%)	5 (5.4%)
Village 4	27	99	17 (17.2%)	7 (7.1%)	6 (6.1%)
Total	103	379	89 (23.5%)	21 (5.5%)	18 (4.7%)

5–6% oxygen, 10% carbon dioxide and 84–85% nitrogen.

### Identification of *Campylobacter* species

The positive control was *C. jejuni* ATCC® 29428 and the main characteristic features were good growth with grey-brown coloured colonies. The negative control was *Escherichia coli* ATCC® 25922 with inhibited growth. All colonies exhibiting morphology and motility typical of *Campylobacter* species, Gram staining and the oxidase test were used for the identification of *C. jejuni* and *C. coli*. Biochemical tests, i.e. hippurate hydrolysis and indoxyl acetate hydrolysis, were applied for phenotypic characterization of selected thermophilic *Campylobacter* species [23, 24]. *C. jejuni* was positive for hydrolysis of hippurate and indoxyl acetate, whereas *C. coli* was negative for hydrolysis of hippurate and positive for hydrolysis of indoxyl acetate.

### Statistical analysis

All analyses were performed at the household level and *C. jejuni* and *C. coli* were analysed separately; presence of at least one positive poultry sample resulted in the backyard being classified as positive for *Campylobacter* and the contact children considered exposed. The percentage of poultry and children positive for each *Campylobacter* species was calculated. *C. coli* infections in children were rare, therefore further analysis focused on *C. jejuni*.

Initially, analysis was performed using  $\chi^2$  tests; any variables associated with outcome with a *P* value  $\leq 0.2$  were then retained for further analysis. Logistic regression models were used to calculate odds ratios and confidence intervals for the association between households with poultry positive to *C. jejuni*

(exposure) and the presence of children positive to *C. jejuni* within these households (outcome). Risk factors for the presence of *C. jejuni* in backyard poultry were then investigated; all variables retained from the first analysis step were included in the logistic regression model in order to control for potential confounding; variables which were associated with the outcome with a *P* value  $>0.05$  were then removed from the final model. In both models village was included as a fixed effect to control for the correlation of households within villages; and to assess whether there were significant differences in *C. jejuni* prevalence between villages. Due to collinearity between management practices and limited sample size; only univariate analysis was performed, therefore the results of the current study have not been controlled for confounding. All analyses were performed using Stata v. 11 (StataCorp., USA).

### RESULTS

A total of 1383, 1276, 1297 and 1387 poultry were housed in 26, 25, 25 and 27 backyards in the studied villages, respectively. The numbers of poultry (mean  $\pm$  s.d.) from the examined backyards in villages 1, 2, 3 and 4 were  $53.19 \pm 22.40$ ,  $51.04 \pm 22.73$ ,  $51.88 \pm 22.09$  and  $51.37 \pm 21.00$ , respectively. Of the 379 birds sampled, *C. jejuni*, *C. coli* and mixed infections were present in 89 (23.5%), 21 (5.5%) and 18 (4.7%) birds, respectively (Table 1). This equated to 57 (55.3%) and 25 (24.3%) backyards being classified as positive for *C. jejuni* and *C. coli*, respectively. Thirty-five backyards were negative for both species.

A total of 27, 26, 25 and 28 children were sampled from 26, 25, 25 and 27 households, respectively. *C. jejuni*, *C. coli* and *C. jejuni*+*C. coli* combined were isolated from 13 (12.3%), three (2.8%) and three (2.8%) children, respectively (Table 2). *Campylobacter*

Table 2. Percentage of stool samples positive for *C. jejuni* and *C. coli* in children

Village no.	No. of households sampled	No. of children sampled	No. of positive children		
			<i>C. jejuni</i>	<i>C. coli</i>	Mixed <i>C. jejuni</i> and <i>C. coli</i>
Village 1	26	27	6 (22.2%)	0 (0%)	0 (0%)
Village 2	25	26	4 (15.4%)	1 (3.8%)	0 (0%)
Village 3	25	25	2 (8%)	0 (0%)	1 (4%)
Village 4	27	28	1 (3.6%)	2 (7.1%)	2 (7.1%)
Total	103	106	13 (12.3%)	3 (2.8%)	3 (2.8%)

Table 3. *Campylobacter* infection and exposure status of children

Backyard status	<i>Campylobacter</i>			
	<i>C. jejuni</i> +ve	<i>C. coli</i> +ve	Mixed +ve	<i>Campylobacter</i> –ve
<i>C. jejuni</i> +ve	9	0	0	35
<i>C. coli</i> +ve	0	1	0	11
Mixed +ve	1	0	3	10
<i>Campylobacter</i> –ve	3	2	0	31
Totals	13	3	3	87

Table 4. Poultry biosecurity and management factors, according to household *C. coli* and *C. jejuni* status

Variable	Households observed, n (%)		Households observed, n (%)	
	<i>C. coli</i> –ve	<i>C. coli</i> +ve	<i>C. jejuni</i> –ve	<i>C. jejuni</i> +ve
Different age groups	64 (82.0)	24 (96.0)	38 (82.6)	50 (87.7)
Mixed species poultry	50 (64.1)	24 (96.0)	20 (43.5)	54 (94.7)
Mixed species livestock	40 (51.3)	11 (44.8)	23 (50)	28 (49.1)
Infrequent drinking water sanitation	55 (70.5)	21 (84.0)	29 (63.0)	47 (82.5)
Infrequent feeding sanitation	56 (71.8)	23 (92.0)	26 (56.5)	53 (92.9)
Poor cleaning and disinfection	42 (53.6)	20 (80.0)	17 (37.0)	45 (79.0)
Wet litter	50 (64.1)	25 (100)	20 (43.5)	55 (96.5)
Manure inside the backyard	42 (53.9)	20 (80.0)	11 (23.9)	51 (89.5)
Ill birds inside the backyard	32 (41.0)	15 (60.0)	15 (32.6)	32 (56.1)

infection in children and the status of backyards is given in Table 3.

Using logistic regression children from households with at least one bird positive for *C. jejuni* had 3.86 (95% confidence interval 1.0–15.0) times the odds of being positive for *C. jejuni* compared to children from households no birds positive. There was no statistically significant association between gender of the child and *Campylobacter* status ( $P=0.42$ ).

Biosecurity and management factors which may be associated with *Campylobacter* species are presented in Table 4. Mixing species of poultry groups (71.8%)

was common as was infrequent feeding sanitation (76.7%), poor cleaning and disinfection (60.2%), presence of wet litter (72.8%), manure inside the backyard (60.2%) and the presence of ill birds inside the backyard (45.6%). Univariate analysis suggested all the above-mentioned management factors were associated with the likelihood of a household being classified as positive for *C. jejuni* ( $P<0.05$  for all factors). However, the final multivariable logistic regression model only identified poor cleaning and disinfection, presence of wet litter, and manure inside the backyard as significant risk factors ( $P<0.05$ ) (Table 5).

Table 5. Risk factors associated with poultry testing positive for *C. jejuni*

Variable	OR (95% CI)
Village 3 (baseline)	1
Village 1	2.90 (0.50–16.9)
Village 2	2.09 (0.35–12.4)
Village 4	2.70 (0.40–18.0)
Poor cleaning and disinfection	8.35 (2.07–33.7)
Wet litter	13.5 (1.00–95.3)
Manure inside the backyard	24.5 (5.85–102.8)

OR, Odds ratio; CI, confidence interval.

## DISCUSSION

This is the first study to assess the risk of *Campylobacter* infection in children exposed to infected backyard poultry in Egypt. Our results indicate that *C. jejuni* infection is more common than *C. coli* in both poultry and children in the area. Very limited data are available regarding the prevalence of *Campylobacter* species in Egypt. A previous study of *C. jejuni* infection in poultry found isolation rates varied with the organ sampled, with 29% of chicken livers yielding *C. jejuni* compared to 18.5% from the gizzard, 8.5% from the spleen and 5% from the heart. Higher rates were recorded from chicken giblets (23.5%) compared to duck (19%), turkey (14.5%) and squab (4%) [25].

Previous studies in other countries have also identified *C. jejuni* as the most common isolate in poultry with *C. coli* being more frequently isolated from pigs and sheep [14, 26–29]. Generally, the population of pigs in Egypt is much lower than the poultry populations due to the popular consumption of poultry and the large-scale government culling of around 300 000 pigs during the 2009 H1N1 ‘swine flu’ pandemic [30].

The current study demonstrates a positive association between the presences of *Campylobacter*-infected backyard poultry and *Campylobacter* infection in children. This may represent a potential route of infection. A previous study in Peru using molecular typing (PFGE) suggested that children and poultry from the same household shared similar if not identical strains of *C. jejuni* suggesting the existence of an infection route from poultry to child [31]. Larger studies including molecular typing and collection of potential confounders, e.g. other livestock, eating habits, other dietary sources, etc. would need to be

performed to investigate this potential infection route further in Egypt.

Three children in the current study were infected with both species, as were their backyards. Moreover, *C. jejuni* was found more frequently than *C. coli* in backyard poultry and children. Finally, the proportion of obtained isolates was higher in poultry compared to children. Three children were infected with *C. jejuni* and two with *C. coli* but these were not isolated from their respective poultry flocks. However, since only four birds were sampled per flock it is likely that there was misclassification with some infected flocks being defined as ‘negative’ on sampling results. Other exposure routes may also account for infection of these children namely ingestion of contaminated water, milk, food and exposure via other livestock or other households’ backyard poultry [2, 18, 32, 33].

Our results indicated low biosecurity and management measures in the examined backyards which may enhance the spread of *Campylobacter* species and increase the risk of exposure to children. Putative risk factors identified included mixing species groups of poultry, poor cleaning and disinfection, high presence of manure and wet litter, presence of ill birds and poor feeding biosafety. However multivariable regression only identified poor cleaning and disinfection, presence of wet litter and manure as risk factors in the present study. Further larger studies in this area are required to identify risk factors and thereby allow development of potential interventions for control.

A previous study found raising of poultry with other species, e.g. cattle and sheep, poor cleaning and disinfection of surrounding area of poultry houses, and presence of manure inside the farm were risk factors for *Campylobacter* infection in Senegalese broiler flocks [34]. The risk of *Campylobacter* infection may be increase by up to twofold in the presence of wet litter [35]. Furthermore; *Campylobacter* may be found in the water, feed, litter and air of poultry houses [36].

In developing countries many guidelines for good biosecurity in backyard poultry are impractical and have low adoption rates [37]. Information needs to be disseminated to households regarding the risk of *Campylobacter* to children and methods of reducing exposure, these methods need to be specific, easy to implement and cost-effective, e.g. hand washing, separation of different animal species and ages, isolation of ill birds and improved disinfection practices and the success monitored.

## CONCLUSIONS

The findings from this preliminary study indicate that exposure of children to infected backyard poultry may present a route of transmission for *Campylobacter* infection. Good biosecurity and management must be applied to prevent the transmission of *Campylobacter* from poultry to children. Increasing public health awareness of households may help to control this zoonotic problem.

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## DECLARATION OF INTEREST

None.

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