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Prevalence and antibiotic susceptibility of thermophilic *Campylobacter* isolates from free range domestic duck (*Cairina moschata*) in Morogoro municipality, Tanzania

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Abstract Prevalence and antibiotic susceptibility of thermophilic *Campylobacter* isolated from free-ranging ducks was determined in Morogoro Municipality, Tanzania. Ninety intestinal contents from ducks were screened for thermophilic *Campylobacter* using Skirrow's protocol. Of the *Campylobacter jejuni* isolates, 50 were tested for sensitivity to 12 antibiotics. Overall prevalence of thermophilic *Campylobacter* was 80%. The prevalence of *Campylobacter* in adult ducks (91.3%) was significantly ($p<0.05$) higher than ducklings (68.2%). The isolation rate of *C. jejuni* (81.9%) was significantly ($P<0.001$) higher than *C. coli* (18.1%). All *C. jejuni* isolates were susceptible to streptomycin, nitrofurantoin and amikacin. Forty eight percent, 74% and 82% of isolates were resistant to cefuroxime sodium, tetracycline and ampicillin respectively. Between 20–50% of isolates were resistant to erythromycin, gentamicin, cloxacillin and amoxicillin. Norfloxacin and ciprofloxacin had lower *C. jejuni* resistance of 10% and 16% respectively. *C. jejuni* isolates from adult ducks showed significantly higher rates of resistance ($p< 0.05$) to most antibiotics than did duckling isolates. High prevalence of thermophilic *Campylobacter* in ducks could be of public health significance in Morogoro municipality. The observed multidrug resistance in this study poses a threat of transfer of antibiotic resistance to human pathogens because of the close contact between ducks and human.

Keywords Campylobacter · Prevalence · Ducks · Antibiotics · Resistance · Susceptible

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Introduction

Muscovy ducks (*Cairina moschata*) represent about 5% of the estimated 30 million poultry in the country (MOA 1994), and they are potential source of meat and income to people with the minimum income in Tanzania. Ducks are farmed on small scale backyard flocks; however, the industry is economically important to families with limited resources. The small population of ducks, their acceptability across different cultures and religions and less susceptibility to diseases promises for expansion of duck industry in Tanzania. Generally ducks are managed under free range in most rural and urban areas with no or minimal care, and rarely get veterinary attention. Because of less care, ducks may serve as a potential reservoir of pathogenic infectious organisms like *Campylobacter* to humans and other animals (Jordan and Pattison 1996; Sonaiya and Swan 2004).

Thermophilic *Campylobacter* in particular *Campylobacter jejuni* is recognized as among leading causes of bacterial gastroenteritis in human worldwide (Logan et al. 1999; Aarestrup and Engberg 2001). According to WHO (2001) the incidence of *Campylobacter* infections worldwide has recently surpassed those of salmonellosis and shigellosis. Domestic birds such as ducks are carriers of *Campylobacter* spp. (Yogasundram et al. 1989) and serve as major sources of infection to human (Waldenström et al. 2002). *Campylobacter*-associated gastroenteritis is normally self-limiting, and antimicrobial treatment is reserved for patients with severe and advanced infections (Aarestrup and Engberg 2001; Kurincic et al. 2005). Infections respond to macrolide antibiotics such as erythromycin, fluoroquinolone antibiotics like ciprofloxacin, and also to tetracyclines, cephalosporins, penicillins and sulphonamides (Alfredson et al. 2003, Lin et al. 2007).

There is generally little published information on *Campylobacter* infections ducks in Tanzania, however, studies elsewhere show high prevalence in ducks managed in different management systems (Prescott and Bruin-Mosch 1981; Kasrazadeh and Genigeorgis 1987; Ridsdale et al. 1998). Investigation on antibiotic resistance of *Campylobacter* isolates shows an increased resistance of animal isolates to different antibiotics. Studies by Endtz et al. (1991) and Engberg et al. (2001) showed increased proportion of *Campylobacter* isolates resistant to antibiotics especially fluoroquinolones. It was postulated that introduction of fluoroquinolones in veterinary practice in the 1990s has led into increase in *Campylobacter* isolates that are resistant to fluoroquinolones. Several countries have since then reported increased resistance of *Campylobacter* isolates from humans and animals (Steinbrückner et al. 2001, Feierl et al. 2001). These findings indicate the potential danger of antibiotic resistant *Campylobacter* spp. which could subsequently be transferred to humans through food chain.

Despite the increased interest in *Campylobacter* studies in the world, there is a dearth of information on prevalence and antibiotic susceptibility of isolates from animals and humans in Tanzania. Therefore the purpose of this study was to determine the prevalence of thermophilic *Campylobacter* in clinically normal ducks and to establish antibiotic resistance of *C. jejuni* isolates from backyard duck flocks in Morogoro municipality, Tanzania.

Materials and methods

Study areas

The study was conducted in Morogoro municipality. The municipality is situated at the latitude 5.7 to 10°S and longitude 35.6 to 39.5°E, with an elevation of 500 to 600 m above sea level and is about 200 km west of Dar es Salaam. Although selection of Morogoro municipality, as study area, was based on convenience of accessibility from the laboratory (due to limited funding of the project), it generally represents backyard smallholder duck farming in urban areas of Tanzania. The study duck flocks were from different suburbs of Morogoro municipality namely Chamwino, Kihonda, Kilakala, K'ndege, Konga, Mafiga, Mtoni, Mwanza, Nanenane and Vibandani.

Study population and management of ducks

The population under study comprised of backyard duck (*Cairina moschata*) flocks in Morogoro Municipality. Ducks (*Cairina moschata*) in study areas were housed in backyard and allowed to scavenge freely during day around homestead. Some households had local chickens which scavenged for food together with the ducks. The ducks flock size ranged from 15 to 100 birds. All farmers provided water, table and kitchen remains to their ducks. Occasionally, the ducks were provided with cereals as supplementary feed. No disease preventive measure was recorded to be done in the investigated flocks. Ducks were used in this study because they rank the second in poultry population in Tanzania and the industry constitutes up to 1.5 million birds (MOA 1994).

Sampling and sample size

This was a cross sectional study which was conducted between January and July 2005. The samples were collected from the purposively selected households with backyard duck flocks and willingness of farmers to participate in the study. Different duck flocks had different age groups of ducks and sex category. A total of 15 backyard duck flocks were involved in the study. For sampling purposes the ducks were categorized into ducklings; between two and five months and adult ducks (six months and above). The sample size of 90 ducks was selected to allow a detection

level of 5% with 99% certainty (Canon and Roe 1986). These included 49 females and 41 male ducks of which 46 were adults and 44 ducklings.

Sample collection, isolation and identification of thermophilic *Campylobacter*

The selected ducks were purchased from the owners and transported in cages to Faculty of Veterinary Medicine Public Health laboratory. Each flock was visited once and all the six selected ducks were assessed at the same time. Upon arrival the ducks were examined clinically before were killed by cervical dislocation and subjected to post-mortem examination. The abdominal cavity was opened and the whole gastrointestinal tract (GIT) was removed and straightened to expose the caecum. Using a sterile pair of scissors, a small cut was made through the caecal wall to expose caecal contents. Caecal contents of each duck were separately swabbed by sterile cotton swabs and subsequently placed in sterile universal bottles containing 10 ml of Preston broth (Oxoid Ltd, Basingstoke, UK) with Preston supplements (Oxoid Ltd, Basingstoke, UK) for enrichment. Inoculated universal bottles were subsequently loaded in microaerobic candle jars (Coldstream Engineering Ltd, Arista, Sweden) with a lighting candle and incubated at 37°C for 24 hours as described by Skirrow and Benjamin (1980). After 24 hours of incubation, the universal bottles with enriched samples were shaken and a loopful broth culture was subcultured onto modified charcoal cefoperazone deoxycholate agar (mCCDA) (Oxoid Ltd, Basingstoke, UK) for primary isolation of thermophilic *Campylobacter*. The inoculated petri dishes were incubated at 43°C for 48 hours under microaerobic condition and *Campylobacter* suspect colonies were subcultured on blood agar (Oxoid Ltd, Basingstoke, UK) at 37°C under microaerobic environment for 24 hours. Suspected thermophilic *Campylobacter* colonies on blood agar that were Gram-negative, curved or spiral rods and showed corkscrew like motion, positive to catalase, oxidase and nitrate reduction tests were further tested for hippurate hydrolysis, H_2S production and susceptibility to nalidixic acid and cephalothin. These parameters formed the basis for the identification of *C. jejuni, C. coli* or *C. lari,* as proposed by On (1996).

For the hippurate hydrolysis test, those organisms yielding a positive test were considered *C. jejuni*, while those organisms that showed a negative reaction were considered *C. coli* and *C. lari*. For hydrogen sulfide (H_2S) production test, all the tested *Campylobacter* strains were found to be negative for the test. Susceptibility tests to nalidixic acid (30 μg) (Oxoid Ltd, Basingstoke, UK) and cephalothin (30 μg) (Oxoid Ltd, Basingstoke, UK) were performed for all isolates of thermophilic *Campylobacter* spp. in accordance with the criteria set by the National Committee for Clinical Laboratory Standards (NCCLs) using the disc diffusion method (NCCLs 2002). The isolates were classified as sensitive and/or resistant according to the standardized tables supplied by the NCCLs (2002). *Campylobacter* strains that were sensitive to nalidixic acid but resistant to cephalothin were considered *C. jejuni* and *C. coli*, while strains that were resistant to both drugs were considered *C. lari.*

Antimicrobial agents and susceptibility testing

Antibiotic susceptibility test was performed on Muller Hinton (MH) Agar (Oxoid Ltd, Basingstoke, UK) by disc diffusion method as described by Luangtongkum et al. (2007). The antibiotic susceptibility test was performed only to *C. jejuni* since it had highest prevalence. A total of 50 *C. jejuni* isolates were tested of which 20 were from ducklings and 30 from adult ducks. Briefly, *Campylobacter* suspensions were prepared in a sterile normal saline and the suspensions were adjusted to a turbidity equivalent to a 0.5 McFarland standard. Sterile cotton-tipped swabs were used to transfer the inoculum onto Mueller-Hinton plates to produce a confluent lawn of bacterial growth. After the inoculum on the plates was dried, antibiotic discs were distributed over the inoculated plates using a BBL Sensi-disc dispenser (BBL Becton Dickinson Microbiology Systems, Cockeysville, MD). These plates were then incubated at 42°C for 48 hours under microaerobic conditions. The following antimicrobials were tested: streptomycin (10 μg), amoxicillin (10 μg), ampicillin (10 μg), ciprofloxacin (5 μg), cefuroxime sodium (30 μg), gentamicin (10 μg), cloxacillin (5 μg), tetracycline (30 μg), nitrofurantoin (30 μg), amikacin (30 μg), erythromycin (15 μg) and norfloxacin (10 μg) (Span Diagnostic, Surat India). After 48 hours of incubation, the diameters of inhibition zones were measured with slipping calipers. For test and interpretation of the results we followed the general guidelines of NCCLS (2002) and Gaudreau and Gibert (1997).

Ethical considerations

The permission to carry out this study was granted by the Morogoro municipal livestock officer and ethical approval for the study was given by the Ethical Committees of Sokoine University of Agriculture (Morogoro, Tanzania). Verbal consent was obtained from each of the heads of households after explaining the purpose and importance of the study prior to purchase of ducks.

Results

Prevalence and species distribution of *Campylobacter* isolates

Thermophilic *Campylobacter* isolation frequencies in different suburbs are summarized in Table 1. The overall prevalence of thermophilic *Campylobacter* in ducks was 80% (95% confidence interval (CI)=70.2–87.7; n=72). Higher infection rate ($p<0.05$) was recorded in adult ducks (91.3%; n=42) than ducklings (68.2%; n=30). The isolation rate of *C. jejuni* was significantly ($P<0.001$) higher (81.9%; n=59) than that of *C. coli* (18.1%; n=13).

Table 1 Thermophilic *Campylobacter* isolation frequency in different suburbs of Morogoro municipality

Suburbs	Number of *Campylobacter* isolates (%)	Species	
		Number of *C. jejuni* (%)	Number of *C. coli* (%)
Chamwino (n=17)	12 (70.5)	8 (66.7)	4 (33.3)
Kihonda (n=5)	4 (80.0)	4 (100.0)	0 (0.0)
Kilakala (n=6)	5 (83.3)	3 (60.0)	2 (40.0)
K'ndege (n=8)	7 (87.5)	6 (85.7)	1 (14.3)
Konga (n=19)	14 (73.7)	10 (71.4)	4 (26.6)
Mafiga (n=5)	4 (80.0)	3 (75.0)	1 (25.0)
Mtoni (n=5)	5 (100.0)	4 (80.0)	1 (20.0)
Mwanza (n=5)	2 (40.0)	2 (100.0)	0 (0.0)
Nanenane (n=10)	10 (100.0)	10 (100.0)	0 (0.0)
Vibandani (n=10)	9 (90.0)	9 (100.0)	0(0.0)
Total (90)	72 (80.0)	59 (81.9)	13 (18.1)

Table 2 Prevalence (%) of antimicrobial resistance among the 50 isolates of *C. jejuni* duck isolates

Antibiotic used	Number of resistant isolates (%)		
	Adult ducks	Ducklings	Total
Streptomycin	0 (0.0)	0 (0.0)	0 (0.0)
Nitrofurantoin	0 (0.0)	0 (0.0)	0 (0.0)
Amikacin	0 (0.0)	0 (0.0)	0 (0.0)
Norfloxacin	4 (8.0)	1 (2.0)	5 (10.0)
Ciprofloxacin	4 (8.0)	1 (2.0)	5 (10.0)
Amoxicillin	7 (14.0)	3 (6.0)	10 (20.0)
Cloxacillin	7 (14.0)	4 (8.0)	11 (22.0)
Gentamicin	9 (18.0)	3 (6.0)	12 (24.0)
Erythromycin	14 (28.0)	7 (14.0)	21 (42.0)
Cefuroxime sodium	16 (32.0)	8 (16.0)	24 (48.0)
Tetracycline	24 (48.0)	13 (26.0)	37 (74.0)
Ampicillin	29 (58.0)	12 (24.0)	41 (82.0)

Antimicrobial resistance results

Table 2 shows *C. jejuni* prevalence of antimicrobial resistance to 12 antibiotics. All *C. jejuni* isolates from ducks were susceptible to streptomycin, nitrofurantoin and amikacin. Forty eight percent, 74% and 82% of the isolates were resistant to cefuroxime sodium, tetracycline and ampicillin respectively. Between 20–50% of *C. jejuni* isolates were resistant to several antibiotics which included erythromycin, gentamicin, cloxacillin and amoxicillin. It was observed that *C. jejuni* isolates from adult ducks showed significantly higher rates of resistance ($p<0.05$) to most antibiotics than did duckling isolates.

Discussion

This study has demonstrated the significance of ducks as reservoirs in the dissemination of thermophilic *Campylobacter* in particular *C. jejuni* in Morogoro municipality. To the best of our Knowledge this is the first report of the occurrence of thermophillic *Campylobacter* in ducks in Tanzania. Although higher prevalence of *Campylobacter* recorded in this study concurred with findings by Luechtefeld et al. (1980); Prescott and Bruin-Mosch (1981); Kasrazadeh and Genigeorgis (1987); it far exceeds that in local scavenging ducks in Ghana where Abrahams et al. (1990) reported a prevalence of 43.5%.

Contrary to the present findings; Tsai and Hsiang (2005) established a prevalence of 43.5% in intensively kept ducks. Variations in isolation rates may be due to several reasons, such as differences in local prevalence of *Campylobacter* in the specific region and management of ducks where the study was conducted.

The observed prevalence of *C. jejuni* in ducks (81.9%) in comparison with other thermophilic *Campylobacter* appears to be comparable to findings of Kasrazadeh and Genigeorgis (1987); Bryan and Doyle (1995) and Whyte et al. (2004). However, other investigators reported higher prevalance of *C. coli* than other thermophilic *Campylobacter* in ducks (Ridsdale et al. 1998) (Pezzotti et al. 2002; Machado et al. 1994). Studies by Madden et al. (1998) and Aquino et al. (2002) showed no difference between prevalence of *C. coli* and *C. jejuni* in ducks. The difference in species isolations may depend on common *Campylobacter* spp. circulating in the local environment, sampling techniques employed, seasonality and laboratory methodologies employed. In the present study, *C. jejuni* was isolated from adult ducks and ducklings of either sex sampled from different locations within Morogoro municipality. Previous similar studies in Morogoro also found a higher prevalence of *C. jejuni* in chickens, humans and craws than *C. coli* (Jiwa et al. 1994; Mdegela et al. 2006). This shows that *C. jejuni* is the common species that may be circulating from either ducks to chickens, humans and other animals and vice versa.

Relationship of age and carrier rate of *Campylobacter* has been explained before in other species of domestic animals. The present findings which are inline with those of Kazwala et al. (1992) and Mccrea et al. (2006) who pointed out that the carrier rate of thermophillic *Campylobacter* in adult chickens is higher than that of ducklings. Studies in chickens by Kazwala et al. (1992) found that *Campylobacter* infection rates in poultry increase with age. Chicks may become colonized with *Campylobacter* within seven days of life but the bacterial burden may reach up to 1.2×10^{7}CFU/g as the age increases (Saleha et al. 1998). However, the results of our study are contrary to those of Kasrazadeh and Genigeorgis (1987) who found 100% prevalence in eight days old ducklings. These findings underline that association of age and carriage of *Campylobacter* requires further study. It can be speculated that high infection rates in adult ducks is due to longevity and feeding behaviour on wet feeds which increase chances of getting repeated infections (Adekeye et al. 1989; Saleha et al. 1998). Indeed, ducks prefer wet feed of which under free scavenging environments the exposure rate to infection is high.

Comparison of antibiotic resistance patterns revealed that all tested isolates were sensitive to streptomycin, nitrofurantoin, and amikacin. The findings are inline with studies by Aydin et al. (2001) and Sâenz et al. (2000). Streptomycin, nitrofurantoin, and amikacin are not among the commonly used drugs in veterinary practice in Tanzania and this could be the reason for none of duck *C. jejuni* strains appeared to be resistant. Differently, *C. jejuni* resistance to ampicillin was the highest among all antibiotics tested which was earlier correlated with ß-lactamase (Taylor and Courvalin 1988). Resistance to ampicillin has previously been reported by other researchers elsewhere (Quinn et al. 1998; Aydin et al. 2001). It is possible that production of ß-lactamase by *Campylobacter* isolates in this study is responsible for high frequency of ampicillin resistant strains.

Due to inherent susceptibility of *C. jejuni* to erythromycin, it has been used as first line drug in treatment of *Campylobacter* human enteritis (Engberg et al. 2001; Gibreel and Taylor 2006). However, the present findings do not fully support this assertion. The increased rate of resistance to erythromycin in developing countries has also been demonstrated in Nigeria where in two studies 10 years apart; sensitivity to erythromycin dropped from 82% of the tested strains to 20.8% (Coker and Adefeso 1994).

Studies by Hoge et al. (1998), Prats et al. (2000) and Senok et al. (2007) reported a high level of ciprofloxacin resistance (>80%) contrary to the present study where resistance was 16%. The present study is inline with the findings of 6-10% resistance reported by Bae et al. (2004) and Hassan et al. (2007). Hakanen et al. (2003) reported a resistance rate of 68% in Finland while Kurincic et al. (2005) reported a resistance of 58.2% in Slovenia. The low resistance rate observed in this study may be due to the fact that fluoroquinolones have not been extensively used in veterinary practice in Tanzania. Reports from developed countries show a marked resistance to quinolones which have been associated with topoisomerases mutations in quinolone resistance determining region of *gyrA* gene (Nachamkin et al. 2001, Backmann et al. 2004).

Furthermore, the study has recorded *C. jejuni* resistance rate of 74% to tetracyclines which is the most extensively used drug in veterinary medicine in

Tanzania. High resistance of *Campylobacter* strains to tetracyclines has previously been reported by various researchers (Prats et al. 2000; Beilei et al. 2003; Bae et al. 2004). This higher level of *C. jejuni* resistance to tetracycline may be probably due to its overuse in animals which might have caused the bacteria to acquire a new resistance gene which is plasmid-mediated (Roberts 1996). Hence, there is possibility of gene transfer among *Campylobacter* and other bacteria in the animals. Relatively high resistance of the isolated strains has also been shown to gentamycin despite its recommended use. These data emphasize the need of antibiotic resistance surveillance among the isolates of veterinary and medical importance in Tanzania.

The findings further demonstrated that *C. jejuni* isolates from adult ducks showed significantly higher rates of resistance to most antibiotics than did duckling isolates. This may be due to the longer raising period of adult ducks (six months and above). Therefore the birds get exposed to different types of antibiotics for a longer period and this may account for the higher rate of resistant strains of *Campylobacter*.

Generally antimicrobial resistance described in this paper could be due to multifactorial determinants. It is accepted now that food animals are primary source of antibiotic resistance genes present in human food-borne pathogens, and imprudent use of antibiotics in humans also significantly lead to development and selection of resistant strains (Bae et al. 2004; Nachamkin et al. 2001). Thus the observed multidrug resistance in this study poses a threat to humans by limiting therapeutic choice of antibiotics. In conclusion, findings from this study have established a high prevalence of thermophilic *Campylobacter* in particular *C. jejuni* in ducks. High rate of antimicrobial resistance recorded may result from indiscriminate use of antibiotics in animals and may pose serious danger to the public health.

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