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Isolation of Campylobacter species from the large intestines of domestic Pekin ducks obtained from a Wet Market in Penang, Malaysia

Frederick Adzitey1, 2*, Nurul Huda1, Rusul Gulam1

1Food Technology Programme, School of Industrial Technology, Universiti Sains Malaysia, Minden, 11800, Pulau Pinang, Malaysia
2University for Development Studies, Animal Science Department, Box TL 1882, Tamale, Ghana

Abstract

The occurrence of Campylobacter species in the large intestines of domestic Pekin ducks collected from a Wet Market in Penang, Malaysia is reported. Large intestinal samples were examined by direct swabbing of its contents on modified ceforazone charcoal deoxcholate agar (mCCDA) supplemented with mCCDA selective supplement and mCCDA supplemented with campylobacter growth supplement. A total of forty (40) duck intestinal contents were tested, of which 27 (67.5%) were positives for Campylobacter species. The study confirms that Campylobacter species can be isolated by direct swabbing on Campylobacter selective agar plates and that domestic Pekin ducks raised in Malaysia are potential sources and reservoirs for Campylobacter species.

Key words: Campylobacter species, isolation, large intestine, Pekin duck

Introduction

The food-borne pathogen, Campylobacter is a Gram negative, curved spiral or rod shaped, catalase positive and oxidase positive bacterium that is microphilic in nature (Corry et al. 2003; EFSA 2005). Campylobacters are very important cause of food-borne illnesses worldwide. They are known to be one of the main causative agents of gastroenteritis and septicaemia (Zhao et al. 2001). Campylobacter infections especially those caused by C. jejuni can also lead to systematic and chronic sequelae infections like Guillain-Barré syndrome, reactive arthritis, and septic abortion (ESFA 2005) which are much more detrimental and fatal. Because of the health implication posed by Campylobacter species, efficient methods for the isolation and identification of these pathogens are important for clinical, treatment and reporting purposes (Adzitey and Corry 2011; Adzitey and Nurul 2011).

There have been a number of studies on enumeration, isolation and identification of Campylobacters in foods of animal and plant origins, environmental samples and other specimens. Isolation or enumeration of Campylobacter species involve the use of liquid enrichment with selective agents and plating media with selective agents and/or indicator system(s) followed by confirmation of typical colonies either by oxidase, Grain stain and/or latex agglutination test (Corry et al. 1995; Corry et al. 2003; Adzitey and Nurul 2011). In recent times, emphasis is being laid on molecular identification and characterization of Campylobacter species, although the conventional method for identifying Campylobacters continues to be the most reliable method for obtaining viable isolates that can be further be characterized and studied (Engberg et al. 2000). Molecular identification and characterisation of Campylobacters have been said to be more rapid, reliable and specific compared to the convention method (Keramas

*Corresponding author. Mailing address: School of Industrial Technology, University science of Malaysia, Minden, 11800, Pulau Pinang, Malaysia.Tel: +604-6532112, Fax: +604-6573678,
E-mail: adzitey@yahoo.co.uk

et al. 2003). As such various rapid methods for identifying and characterizing Campylobacters species have been used (Van Doorn et al. 1999; Ertas et al. 2002; Aydin et al. 2007; Adzitey et al. 2011).

Duck production for meat, egg, feathers and other purposes has been practice over centuries. Despite this little attention has been given to the association between ducks and food-borne pathogens (Adzitey 2011; Adzitey et al. inpress), and in Malaysia such data is limited although Malaysia is the third leading producer of duck meat worldwide after China and France (FAO 2009). This also suggests that Malaysia makes significant contribution to the total duck production and consumption globally (Adzitey et al. inpress). This work was therefore carried out to isolate Campylobacter species from the large intestines of Pekin ducks using direct swabbing on two modified ceforazone charcoal deoxcholate agars (mCCDA's) which differed from each other by the type of supplement used.

Material and Methods

Isolation, confirmation and identification of Campylobacter species. The large intestines of Pekin ducks were swabbed using sterile disposable cotton swabs (Copan) and streaked unto 1) modified ceforazone charcoal deoxcholate agar (mCCDA) (Merck, Darmstadt, Cat. No. 1.00070.0500) supplemented with mCCDA selective supplement (Merck, Darmstadt, Cat. No. 1.00071.0001) and 2) mCCDA supplemented with Campylobacter growth supplement (FBP) (Oxoid, UK, SR0155E). Modified ceforazone charcoal deoxcholate agar plates were incubated at 42 ℃ for 48 hours under microaerobic atmosphere (created in BD GasPak™ EZ Systems) using a gas mixture of 10% CO2, 5% O2 and 85% N2. Presumptive Campylobacter colonies appeared small, gray and drop-like, small and shiny/slimy. Presumptive colonies were purified on 7% blood agar and confirmed using gram staining, catalase, oxidase, glucose utilization and growth tests. Additionally, Dryspot Campylobacter Test Kit (Oxoid, UK) was used to confirm the Campylobacter isolates, while species identification was achieved by hippurate hydrolysis (using remel ninhydrin reagent R21238 and remel hippurate disk R21085 test, Lenexa KS USA) and susceptibility of the isolates to nalidixic and cephalothin antibiotics.

Table 1. Prevalence of *Campylobacter* species in the large intestines of Pekin ducks

Duck Intestinal content	No. tested	No. positives	% prevalence	*Campylobacter* species	
				C. coli	*C. jejuni*
mCCDA + mCCDA supplement	40	27	67.5	12	15
mCCDA + FBP	40	18	45	12	6
Overall	40	27	67.5	24	21

Key: mCCDA = modified ceforazone charcoal deoxcholate agar

FBP= *Campylobacter* growth supplement (Sodium pyruvate, Ferrous sulphhate and sodium metabisulphite)

Results

Table 1 gives a breakdown of the results obtained by direct streaking of Pekin duck large intestinal contents using swabs on modified ceforazone charcoal deoxcholate agar supplemented with mCCDA selective supplement respectively. Twelve (12) C. coli and 15 C. jejuni were isolated on mCCDA + mCCDA growth supplement, while 12 C. coli and 6 C. jejuni were found on mCCDA + Campylobacter growth supplement (FBP). Isolation of Campylobacter species on mCCDA + mCCDA supplement was relatively better than mCCDA + FBP (27 against 18). (mCCDA + mCCDA supplement) and mCCDA supplemented with Campylobacter growth supplement (mCCDA + FBP). The overall isolation rate for the Campylobacter species was 67.5% (27 positives against 40 samples tested). Among the samples tested the frequency of occurrence C. jejuni and C. coli was 55.6% and 44.4%,

Discussion

Our result indicates that Campylobacters are present in the large intestines of Pekin ducks raised in Penang, Malaysia. It also confirms the isolation of Campylobacter species

from samples by direct plating without enrichment. Isolation of Campylobacter species by direct swabbing on Campylobacter selective agars (Skirrow, Preston selective agar and modified ceforazone charcoal deoxcholate agar plates) have been reported by other workers including Yildirim et al. (2005) and Savasan et al. (2004). The presence of Campylobacter species in the large intestine of Pekin ducks also suggest that, Campylobacters can be shed during defaecation. Under poor farming and environmental conditions, they will spread among farm equipments and subsequent flocks. In addition, Campylobacters may continue to be shed by defaecation during transportation and under faulty processing conditions during carcass dressing in slaughtering plants. Subsequently these may end up in the dressed duck meat, cross contaminate other foodstuffs and possibly cause human food-borne illness through the consumption of contaminated duck or other products. The health implication of Campylobacter infection has been mentioned previously (Zhao et al. 2001; ESFA 2005).

Campylobacter species in ducks have also been reported by few workers (Boonmar et al. 2007; Nonga and Muhairwa 2010). Boonmar et al. (2007), analyzed 140 samples of duck meat and duck intestines from slaughterhouses in Nakhon Pathom Province, Thailand and found 28 samples (20%) to be positive for Campylobacter species using the standard culture method (21 C. jejuni and 7 C. coli). In Morogoro Municipality-Tanzania, Nonga and Muhairwa (2010) reported an overall prevalence of thermophilic Campylobacter species from duck intestinal contents to be 80%, and the isolation rate of C. jejuni (81.9%) was also higher than C. coli (18.1%). In a previous study we analyzed 75 duck intestinal contents by enrichment, followed by plating and found 6 (8%) positive for Campylobacter species compared to this current study where we found 27 (67.5%) positives out of 40 duck intestinal contents analysed (Adzitey et al. 2010). Therefore we suggest that isolation of Campylobacter species from duck intestinal contents by direct swabbing on mCCDA agar was better for isolating Campylobacters than enrichment in Bolton broth followed by streaking unto mCCDA plate supplemented with mCCDA supplement. Sometimes Bolton broth gets overgrown with extended spectrum beta-lactamase (beta-lactams are penicillins and second generation penicillins - including cefaperazone, which is used in mCCDA), E. coli and other Enterobacteriaceae which can grow on mCCDA (personal communication with Dr. Janet Corry). This perhaps might have accounted for the low recovery of Campylobacter species from previous study involving duck samples as the mCCDA plates were overgrown by other bacteria some of which were E. coli when presumptive colonies were transferred unto eosin methylene blue agar and confirmed biochemically. Other workers in Malaysia have isolated Campylobacters from samples other than from ducks such as in broiler chickens, 46-93% (Saleha 2002) and in salad vegetables, 29-68% (Chai et al. 2007).

Conclusion

This study confirms the isolation of Campylobacter species from the large intestines of Pekin ducks raised in Penang-Malaysia. The isolation of Campylobacters on mCCDA + mCCDA supplement (27) was higher than on mCCDA + FBP (18). The overall occurrence of Campylobacter species in this study was 67.5%. Similar number of C. coli (24) and C. jejuni (21) were isolated although C. jejuni dominates in most human infections. The study also confirms that healthy ducks carry Campylobacter species in their intestines, the public health implication of which has to be considered.

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