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Screening and Rapid Identification of *Campylobacter Spp.* DNA by *Fla*A PCR Based Method on Chicken and Human Fecal Samples in Egypt

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Abstract: Due to culturability requirements encountered by the conventional isolation of *Campylobacter* spp., rapid molecular techniques for its direct identification from clinical samples are useful. In this study, *Campylobacter* spp. DNA from human stool and chicken fecal samples were detected by flagellin gene (*fla*A) PCR. A total of 297 samples consisting of 163 adult human stools (102 from diarrheic patients and 61 from healthy persons) and 134 chicken feces were subjected to *fla*A PCR. Ten reference strains of *Campylobacter* spp. were included in this study as positive controls. Thirteen stool samples (7.98%) from the human fecal samples and 39 chicken fecal samples (29.1%) yielded the genus specific 1.7 Kb amplicon of *Campylobacter* spp. Eight (7.84%) diarrheic human stool specimens out of 102 samples and 5 (8.2%) apparently healthy human stool specimens out of 61 samples were positive by *fla*A PCR assay. All the *Campyloacter* reference strains examined giving the specific amplicon of 1.7 Kb. The existence of *Campylobacter* spp. DNA detected by *flaA* PCR in poultry and human samples taken from locations of Egypt highlights the zoonotic potential of *Campylobacter*. To the best of our knowledge, this is the first report in Egypt that uses *flaA* PCR as a rapid screening method for the direct detection of *Campylobacter* spp. from human and chicken feces.

Key words: *Campylobacter*, human stool, poultry feces, *fla*A PCR

INTRODUCTION

Campylobacters are considered one of the most frequent causes of foodborne bacterial infections in developing as well as developed countries (Allos, 2001). Zoonotic infections with *Campylobacter* spp. (campylobacteriosis) are usually the result of the consumption of contaminated poultry meat that has not been properly prepared or cross contamination between raw poultry and hands of food preparers, kitchen utensils, other foods, cutting boards, etc. (Guyard-Nicodème *et al*., 2013).

The identification of *Campylobacter* in chicken feces is crucial especially before slaughtering since the bacterium is carried in the alimentary tract of live birds, and contaminated fecal material may contaminate broiler carcasses during processing (Oosterom *et al*., 1983). In the countries where slaughtering and evisceration processes of poultry were manual, the chance for presence of large numbers of *Campylobacter* spp. in poultry meat was high (Parkar *et al*., 2013).

The isolation and identification of thermophilic *Campylobacter* from clinical samples by conventional culture methods and biochemical identification tests are laborious, time consuming, and expensive (Endtz *et al*., 1991). Also, direct identification of pathogens by PCR in environmental samples such as feces, provides an improved method for the detection of this foodborne pathogen (Fode-Vaughan *et al*., 2001). The use of PCR-based methods for the rapid identification of *Campylobacter* may provide a more reliable method to detect broiler flocks contaminated with this pathogen before the birds enter processing facilities (Persson and Olsen, 2005).

Although there are many reports on the usage of *flaA* PCR as a valuable tool for the detection of *Campylobacter* spp. from human and chicken feces (Linton *et al*., 1997; Lawson *et al*., 1998; Fitzgerald *et al*., 2001; Al Amri *et al*., 2007), data on direct PCR identification of *Campylobacter* from fecal samples in many developing countries such as Egypt is still lacking. The overall aim of this study was to assess the applicability of *flaA* PCR for rapid detection of *Campylobacter* spp. directly from human and chicken feces and to investigate the role of chicken as a potential source for human infections with *Campylobacter* in the examined area.

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MATERIALS AND METHODS

Sample collection: A total of 297 samples consisting of 163 human fecal samples (102 from diarrheic patients and 61 from healthy persons) and 134 chicken fecal samples were included in this study. The diarrheic stool samples were taken from four private clinical laboratories in the district of Mansoura City, Egypt. All the diarrheic patients (62 male and 40 female) were between 18 and 50 years of age with foul-smelling diarrhea, fever and abdominal disturbances. Stool samples from healthy persons (36 female and 25 male) were collected from rural households that raised chickens on a small scale near Mansoura City, Egypt. A detailed questionnaire was taken from diarrheic patients and healthy persons with information about their health status, previous exposure to diarrhea and contact with live birds. Before stool sample collection, verbal consent was taken from patients in the private clinical laboratories that provided the diarrheic samples, and also from healthy persons.

Chicken fecal samples were randomly collected from birds in five broiler flocks located in the district of Mansoura City, Egypt. Broiler flocks were located within the same proximity from which human stool samples were collected and the five flocks were of 10,000-20,000 birds with an average age of 28-35 days at the time of sample collection.

Fecal samples were collected in sterile sample vials during the period from November 2013 to March 2014, transferred within 2 h from collection to the laboratory of Hygiene and Zoonoses Department, Faculty of Veterinary Medicine, Mansoura University and stored at -20°C until DNA extraction.

DNA extraction from clinical samples: DNA was extracted from clinical samples by suspending 2 g of human or chicken fecal sample in 3 ml of phosphate buffered saline (PBS) in a closed plastic tube and then mixing by vortexing for 1 min at room temperature. From this suspension, 200 µl was transferred to a 1.5 ml sterile eppendorf tube and DNA was extracted using the QIAamp DNA stool extraction kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

Reference strains: The following type strains were used as reference strains: *C. jejuni* ATCC 33560 (from bovine feces), *C. fetus* subsp. *fetus* ATCC 27374, *C. lari* ATCC 35221 (from Herring gull cloacal swab) and *C. coli* ATCC 86119 (from poultry). *C. jejuni* PPMQ2b and PPMQ3b were isolated from processed broiler carcasses (Hinton *et al*., 2004). All other isolates including *C. jejuni* 86605 (poultry), 48100 (poultry), 1997-8 (human) and 1999-1 (human) were provided by Dr. Richard Meinersmann of the Agricultural Research Service, Russell Research Center, Athens, GA.

Growth of reference strains and DNA extraction: Stock cultures of *Campylobacter* spp. reference strains were plated onto fresh blood agar plates (Remel®, Lenexa, KS) and incubated in a GasPak Jar with a CampyPak Plus Hydrogen and carbon dioxide with Integral Palladium Catalyst to set microaerophilic conditions for 48 h at 37°C (Hinton, 2006). After incubation, harvesting of cultures was done by pouring 9 ml of 0.1% Difco Bacto Peptone solution to the surface of the plates and using sterile bacterial cell spreaders to remove bacterial growth from the agar surface. The extraction of DNA from the bacterial culture of reference strains was done using PureLink Genomic DNA extraction Kit (Invitrogen Life Technologies, Carlsbad, CA).

PCR: The primer pairs used were *fla*A.F (5'-GGATTTCGTATTAACACAAATGGTGC-3') and *fla*A.R (5'-CTGTAGTAATCTTAAAACATTTTG-3'), which generate a 1.7 Kb amplicon (Nachamkin *et al*., 1993). PCR was performed in a final volume of 25 µl PCR mixture consisting of 12.5 µl of 2X PCR Master Mix (Promega, Madison, WI), 1 µM of each primer, and 2.5 µl template DNA. Following an initial denaturation for 5 minutes at 94°C, products were amplified by 30 cycles of denaturation for 30 sec at 94°C, annealing for 60 sec at 55°C, and elongation for 90 sec at 72°C with a final extension step of 7 minutes at 72°C. Positive DNA detection for each sample was identified by its specific bp DNA bands on 2% agarose gel, stained with ethidium bromide and evaluated under UV transilluminator. A positive control consisting of DNA extracted from *C. jejuni* 86605 and also a negative control (nuclease free water instead of DNA template) were included in each PCR run.

RESULTS AND DISCUSSION

The PCR based methods used for direct detection of *Campylobacter* isolates recovered from different sources are required for infection control and also provides a useful aid in the study of risk assessment of *Campylobacter* and its zoonotic potential. By using *fla*A PCR to screen human and chicken feces for the presence of *Campylobacter* spp. DNA, 7.98% of the human stool samples (13/163) yielded the 1.7 kb amplicon for *Campylobacter* genus identification (Fig. 1) where 8 samples originated from 102 diarrheic stool specimens and 5 were from 61 healthy specimens. The presence of *Campylobacter* in chicken feces was higher than that from human stool samples. Approximately 29% (39/134) of the extracted chicken fecal samples were positive for the 1.7 Kb fragment targeting *fla*A (Fig. 2) and all the reference strains including *C. jejuni*, *C. coli*, *C. fetus* and *C. lari* were positive by *fla*A PCR (Fig. 3).

Findings from this study confirmed that the genus specific 1.7 Kb fragment targeting *fla*A is well identified in samples containing this bacterium. These results are

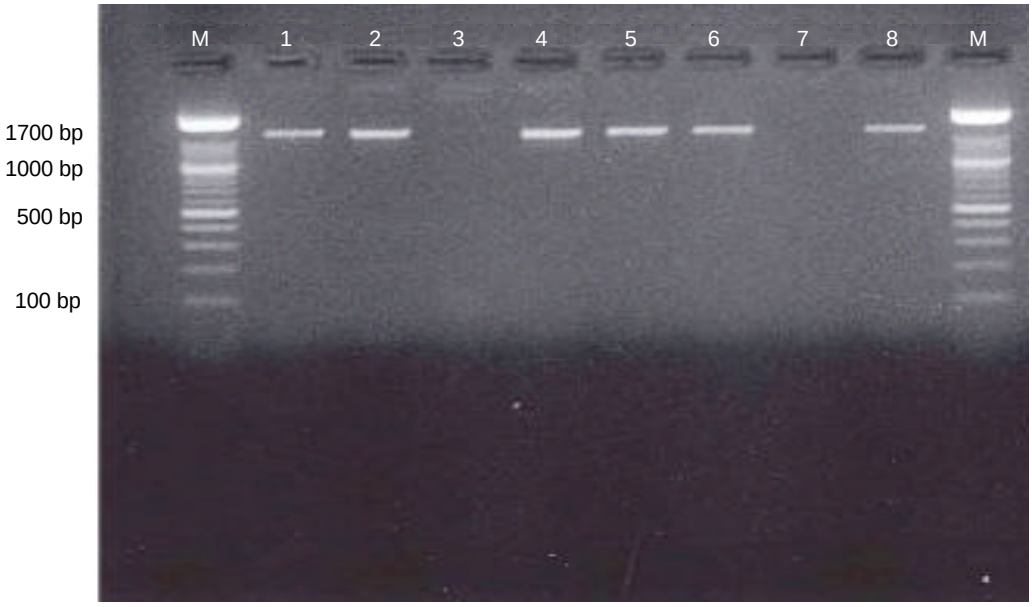

Fig. 1: PCR identification of *Campylobacter* spp. DNA from human stool samples. Lane M: 100 bp DNA ladder. Lane 1, 2, 3, 4 and 5: DNA extracts from diarrheic human stool samples. Lane 6, 7 and 8: DNA extracts from healthy persons stool samples.

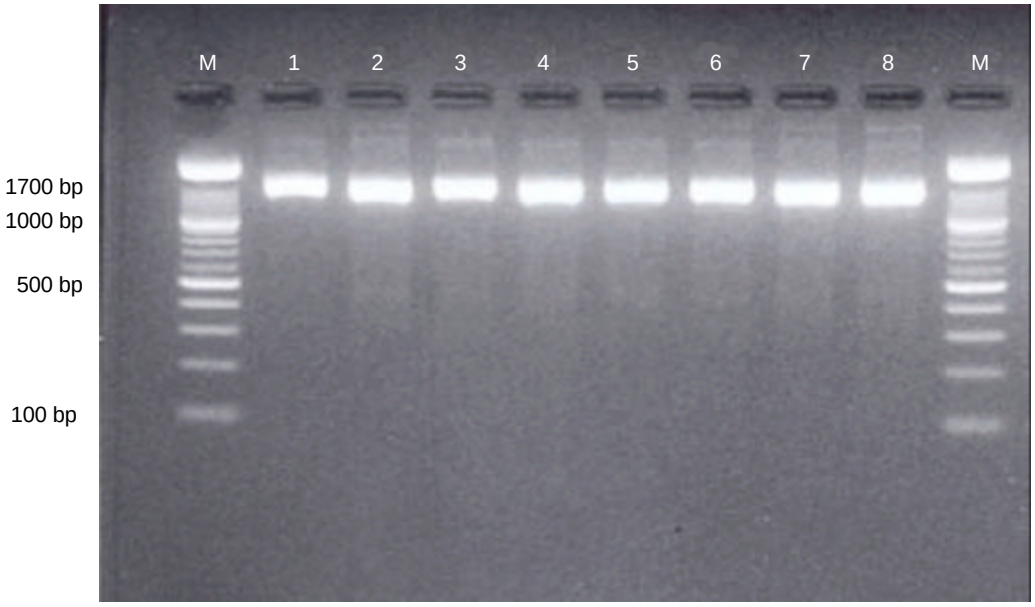

Fig. 2: PCR identification of *Campylobacter* from chicken fecal samples. Lane M: 100 bp DNA ladder. Lane 1, 2, 3, 4, 5, 6 and 7: DNA extracts from chicken fecal samples. Lane 8: *C. jejuni* 86605 (Control Positive).

consistent with many studies that detected *Campylobacter* spp. in feces by *fla*A PCR (Nachamkin *et al*., 1993; Linton *et al*., 1997; Lawson *et al*., 1998; Fitzgerald *et al*., 2001). It was also observed that the *fla*A PCR-based assay in this study could identify *Campylobacter* spp. DNA from human and chicken feces, although these clinical samples might contain PCR inhibitors such as DNases, polysaccharides, and proteases (Wilson, 1997). However, DNA extraction by the QIAamp DNA stool extraction kit provided an effective tool for destruction of PCR inhibitors that interfere with DNA amplification. The usefulness in the QIAamp DNA

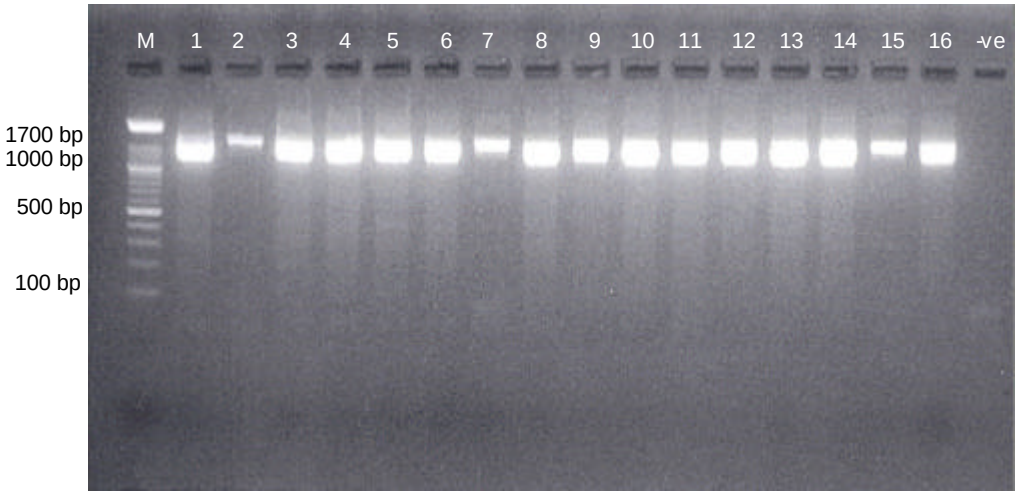

Fig. 3: PCR identification of *Campylobacter* from chicken fecal samples. Lane M: 100 bp DNA ladder. Lane 1, 2, 3, 7, 15 and 16: DNA extracts from chicken fecal samples. Lane 4: *C. coli* ATCC 86119. Lane 5: *C. jejuni* ATCC 33560. Lane 6: *C. jejuni* 86605. Lane 8: *C. jejuni* PPMQ2b. Lane 9: *C. jejuni* PPMQ3b. Lane 10: *C. jejuni* 48100. Lane 11: *C. fetus* subsp. *fetus* ATCC 27374. Lane 12: *C. lari* ATCC 35221. Lane 13: *C. jejuni* 1997-8. Lane 14: *C. jejuni* 1999-1. Lane -ve: Control Negative.

stool extraction kit has been previously determined by McOrist *et al*. (2002) in a study of a comparative assessment of DNA extraction kits from feces. This study confirmed that the QIAamp DNA stool extraction kit was the most effective extraction method among those tested for DNA extraction. Moreover, many researchers are obtaining the desired effect and satisfactory results by using QIAamp DNA stool extraction kit that sufficiently removed PCR inhibitors in fecal samples that interfered with PCR (Inglis and Kalischuk, 2003; LaGier *et al*., 2004).

It is not surprising that *Campylobacter* spp. was detected by *fla*A PCR from the stool of diarrheic patients; however, the identification of *Campylobacter* from stool of apparently healthy persons may be attributed to contact with infected animals, rearing of animals in personal homes and unhygienic conditions. Healthy carriers of *Campylobacter* was also observed in a study by Coker *et al*. (2002) who found that the recovery of *Campylobacter* organisms from apparently healthy children was common in developing countries. Also, the development of pre-immunity against *Campylobacter* spp. might lead to the occurrence of asymptomatic infection (Allos, 2001).

It was also clearly noticed that all the stool samples from healthy participants that were positive for *flaA* PCR for *Campylobacter* DNA were taken from those individuals living in rural areas who had direct or indirect contact with live birds. This finding strengthens the notion that poultry play an important role as a reservoir for human campylobacteriosis in Egypt. There is a close association between human and live birds in this area, and freshly slaughtered poultry comprises a significant portion of the diets of most Egyptians. The carcasses of these broilers may become contaminated by fecal bacteria during slaughter (USDA, 2001), and if the carcasses are not proper cleaned and sanitized, *Campylobacter* may survive for up to one week in humid, wet environments of the poultry carcasses (Corry and Al-Ataby, 2001).

In conclusion, the findings from this study indicate that screening of clinical samples from both human and chicken with a rapid and accurate molecular approaches such as *fla*A PCR is beneficial in implementation of appropriate control measures that will reduce the number of cases of human campylobacteriosis associated with the consumption of contaminated poultry and other food products. The presence of *Campylobacter* circulated in the examined area that was detected in human stool and chicken fecal samples indicates that further study to isolate and characterize the isolates by genotyping to identify which species circulated between poultry and human is needed. This information will aide in identifying potential source of campylobacteriois in human. The rapid detection of *Campylobacter* spp. within a few hours provided a valuable tool which can be used for epidemiological surveillance and prevention strategies especially in developing countries such as Egypt.

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Conflicts of interest

Authors declared no conflict of interest.

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