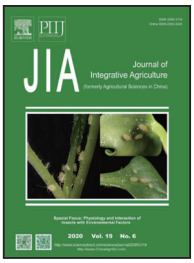
Journal of Integrative Agriculture 2020, 19(6): 1656–1670

Available online at www.sciencedirect.com

ScienceDirect



RESEARCH ARTICLE

Molecular detection of virulence genes in *Campylobacter* species isolated from livestock production systems in South Africa

Bongekile NGOBESE[1], Oliver Tendayi ZISHIRI[1], Mohamed Ezzat EL ZOWALATY[2, 3]

[1] *Discipline of Genetics, School of Life Sciences, University of KwaZulu-Natal, Durban 4000, South Africa*

[2] *Zoonosis Science Center, Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala SE-75185, Sweden*

[3] *Infectious Diseases and Anti-Infective Therapy Research Group, College of Pharmacy and Sharjah Medical Research Institute, University of Sharjah, Sharjah 27272, UAE*

Abstract

Campylobacter species are a major cause of foodborne bacterial infections in both developed and developing countries worldwide. *Campylobacter jejuni* is responsible for the majority of infections. This study was conducted to identify virulence-associated genes in *Campylobacter* species isolated from livestock production systems in South Africa. A total of 250 fecal samples consisting of cattle (*n*=50), chickens (*n*=50), goats (*n*=50), sheep (*n*=50) and pigs (*n*=50) were randomly collected from livestock in Eastern Cape and KwaZulu-Natal provinces of South Africa between April and October 2018. The samples were analyzed for the presence of virulence genes in *Campylobacter* species using molecular PCR-based methods. It was found that 77 and 23% of *Campylobacter jejuni* and *Campylobacter coli* respectively were isolated from all the livestock samples. There were positive significant (P<0.05) correlations amongst all the virulence genes that were investigated. Chi-square and Fisher's exact tests were implemented to test for the effect of livestock species on the presence or absence of virulence genes. The study demonstrated that most of livestock species can potentially cause zoonotic infections and food poisoning due to the high prevalence of *Campylobacter*. The high prevalence of virulence genes highlights the significance of *Campylobacter* in livestock production systems in South Africa. This requires the implementation of one-health approaches to reduce the impact of foodborne and zoonotic diseases for the welfare of human and animal health.

Keywords: *Campylobacter*, zoonotic, cytotoxicity, pathogenicity, virulence, campylobacteriosis, foodborne, livestock, PCR, one-health

1. Introduction

Campylobacter is a leading foodborne pathogen, with *C. jejuni* and *C. coli* being responsible for the vast majority of human clinical cases, among which 80–90% are due to *C. jejuni* and the remainder is caused by *C. coli* (CDC 2013; Epps *et al*. 2013; Ragimbeau *et al*. 2014; Cantero *et al*. 2018). *Campylobacter jejuni* and *C. coli* are the most prevalent isolates from human cases of campylobacteriosis

Received 29 April, 2019 Accepted 28 October, 2019
Correspondence Mohamed Ezzat EL ZOWALATY, E-mail: elzow005@gmail.com

doi: 10.1016/S2095-3119(19)62844-3

with *C. jejuni* being responsible for the majority of infections worldwide (Biswas *et al*. 2011). Other studies have also reported that *C. jejuni* and *C. coli* are responsible for diarrhea in an estimated 400–500 million people globally each year (Lapierre *et al*. 2016; Azrad *et al*. 2018; García-Sánchez *et al*. 2018; Wieczorek *et al*. 2018). There are other *Campylobacter* spp. that may also play an important role in enteritis such as *C. lari*, *C. upsaliensis*, *C. rectus*, and *C. concisus*, however, few data are available (Obeng *et al*. 2012; Casagrande Proietti *et al*. 2018). Campylobacteriosis is a chronic enteric infection mainly caused by cytotoxin-producing *Campylobacter* spp. that invade and colonize the gastrointestinal tract in humans and other mammals (Otigbu *et al*. 2018). It has also been reported that *Campylobacter* spp. colonize enteric tracts of birds, cattle, sheep, and pigs (Premarathne *et al*. 2017). *Campylobacter* spp. are motile, spiral, rod-shaped, or curved Gram-negative bacteria (Kaakoush *et al*. 2015; García-Sánchez *et al*. 2018). *Campylobacter* spp. are non-spore-forming, approximately 0.2 to 0.8 μm by 0.5 to 5 μm, in size and are chemoorganotrophs (Kaakoush *et al*. 2015).

Campylobacteriosis is a dominant bacterial cause of foodborne infections and is considered the main public health problem in Europe and several other countries worldwide (Iglesias-Torrens *et al*. 2018). Eating or handling raw or undercooked meat, especially poultry, is a major risk factor for human campylobacteriosis (Lapierre *et al*. 2016). Other sources of *Campylobacter* infections include contaminated drinking water and dairy products, for example, unpasteurized milk, swimming in natural water sources, contact with pets and other environmental sources (Kaakoush *et al*. 2015; Szczepanska *et al*. 2017; García-Sánchez *et al*. 2018). Recently, cattle, pigs, sheep, and goats have also been identified as a source of campylobacteriosis (Karikari *et al*. 2017). It has been reported by Wysok and Wojtacka (2018) that molecular genotyping and statistical modelling studies have demonstrated that pig and cattle also signify a vital source for human infections. In Finland and Europen Union, cattle were found to contribute equally to human infections when compared to chickens. Pork products has also been implicated in human *Campylobacter* infections (Wilson *et al*. 2008; Wysok and Wojtacka 2018). Foreign travel may also be a risk factor for *Campylobacter* infections, especially in children previously unexposed to exotic or antibiotic-resistant strains present in contaminated meat or water (Iovine 2013; Kaakoush *et al*. 2015). In general, the consumption of contaminated livestock products may lead to campylobacteriosis (CDC 2013).

Campylobacteriosis affects mainly children under the age of 5 years, elderly patients, pregnant women and patients suffering from the immunocompromised disease such as AIDS and cancer (Iglesias-Torrens *et al*. 2018). According to the research of Premarathne *et al*. (2017), a hospital-based study elucidated that *Campylobacter* species were reported at a 5% prevalence level and placed among the five causative agents associated with infant diarrhea. South Africa is under a major threat, since it has been reported to have extremely excessive records of individuals who are suffering from immunocompromised diseases such as AIDS (Kaakoush *et al*. 2015; Reddy and Zishiri 2018). Patients suffering from *C. jejuni* and *C. coli* infections usually experience symptoms such as fever, increasing bloody or watery diarrhoea, weight loss, abdominal pain and severe cramps that last longer than one week (Szczepanska *et al*. 2017; Iglesias-Torrens *et al*. 2018). After ingesting the bacterium, the symptoms usually begin within 24 to 72 h depending on the dosage of the microorganism present in the contaminated food, milk or water ingested (Zaidi *et al*. 2012). Moreover, *Campylobacter* spp. have also been most frequently associated with the development of immunoreactive complications such as Guillain-Barre Syndrome (Van den Berg *et al*. 2014) and Miller Fisher Syndrome (Lapierre *et al*. 2016). However, there are other immunoreactive complications which include brain abscess, meningitis, bacteremia, sepsis, endocarditis, myocarditis, reactive arthritis and clinical manifestations that results in complication in the reproductive tract (Biswas *et al*. 2011; Kaakoush *et al*. 2015; Iglesias-Torrens *et al*. 2018; Wieczorek *et al*. 2018). These complications are known to enhance the significance of *Campylobacter* infection (Biswas *et al*. 2011).

The pathogenesis of *Campylobacter* infection is very complicated and still poorly understood compared to other pathogens such as *Escherichia coli* and *Staphylococcus*, however, the molecular genetic background of this pathogen has been reasonably studied (Wieczorek *et al*. 2018). However, there are anecdotal reports that suggest that the presence of genes involved in motility, colonization, adherence, epithelial cell invasion, and toxin production play a significant role in the disease development (Thakur *et al*. 2010; Wieczorek and Osek 2013; Lapierre *et al*. 2016). Livestock fecal samples are considered to be the most appropriate type of sample for studying *Campylobacter* virulence factors. In South Africa limited data exist on the prevalence of *Campylobacter* in livestock production systems. Thus, monitoring of virulence genes in *Campylobacter* is highly relevant to public health. Against this background, the aim of this study was to determine the prevalence of virulence genes associated with motility, cytotoxicity, invasion, and adherence among *Campylobacter* spp. isolated from different livestock production systems in South Africa.

2. Materials and methods

2.1. Ethical considerations

The study was approved by the Animal Research Ethics Committee of the University of Kwa-Zulu Natal (Reference numbers AREC/051/017M, AREC 071/017, AREC 014/018). The field sampling protocols, samples collected from animals, and the research were conducted in full compliance with Section 20 of the Animal Diseases Act of 1984 (Act No. 35 of 1984) and were approved by the South African Department of Agriculture, Forestry and Fisheries DAFF (Section 20 approval reference number 12/11/1/5 granted to Prof. M. E. El Zowalaty).

2.2. Samples collection

A total of 250 fecal samples were randomly collected from cattle, chickens, goats, pigs and sheep in different small-scale farms, in two provinces in South Africa. The first batch of samples was collected from Eastern Cape Province in Flagstaff Area and the second batch was collected from Ladysmith Area in KwaZulu-Natal Province between May and October 2018 (Fig. 1). Fifty samples were randomly collected from each of the following livestock animals: cattle, chickens, goats, pigs and sheep. Freshly excreted faeces were collected using sterile swabs and the directly inoculated into charcoal broth (Fluka Analytical supplemented with CCDA Selective Supplement (Charcoal Cefoperazone Desoxycholate Agar Selective Supplement) (Sigma-Aldrich). All the collected samples were immediately transported to the University of KwaZulu-Natal Westville Genetics Laboratory for processing and incubation at 37°C for 48 h under microaerophilic conditions (5% O_2, 10% CO_2 and 85% N_2) created by CampGen (Oxoid, UK) gas generating packs in an anaerobic jar, within the same day of sample collection.

2.3. Samples processing

Following incubation, the faecal samples in charcoal broth (59751 Blood Free Campylobacter Broth; Blood-Free Campylobacter Broth; Modified CCDA-Preston Broth; Modified Charcoal Cefoperazone Desoxycholate Broth) (Fluka Analytical) were filtered through a 0.65-μm cellulose nitrate filter (Sartorius Stedim Biotech, Germany) onto modified charcoal cefoperazone deoxycholate (mCCDA)

Fig. 1 Map of South Africa showing the geographic locations of the farms where samples were collected for this study. The first location where the samples were collected is Ladysmith, which situated in Kliprivier (O), KwaZulu-Natal, South Africa, with the geographical coordinates are 28°33′0′′S and 29°47′0′′E. The second location where the samples were collected is Flagstaff, that is in Siphaqueni, Eastern Cape, South Africa, its geographical coordinates are 31°5′0′′S and 29°29′0′′E. The actual location where the samples were collected are in the square.

(blood-free agar) (Oxoid, England) (Kaakoush *et al*. 2015; Lapierre *et al*. 2016; Otigbu *et al*. 2018). Approximately 500 µL of the incubated charcoal broth was evenly distributed over the filter aseptically; once the liquid had been filtered through, forceps were used to aseptically remove the filter. The culture plates were then placed in an inverted position in an anaerobic jar containing an gas generation system (CampyGen sachet, Oxoid) and then incubated at 37°C for 48 h (Otigbu *et al*. 2018).

2.4. Microbiological isolation

The methods for microbiological isolation of *Campylobacter* spp. were performed as previously described (Reddy and Zishiri 2018). In a nutshell, subsequent to incubation, a loopful of the enriched sample was streaked in duplicates to achieve isolated colonies onto mCCDA agar (Oxoid, England) containing *Campylobacter*-selective supplement SR0155 (Oxoid, England), and the plates were incubated at 37°C for 48 h under microaerobic conditions created by CampyGen (Oxoid, UK) gas generating packs in an anaerobic jar. Following incubation, species identity was confirmed, following DNA extraction, by PCR targeting of the *hipO* gene specific for *C*. *jejuni* (Marinou *et al*. 2012) and *asp* gene specific for *C*. *coli* (Al Amri *et al*. 2013).

2.5. DNA extraction

DNA was extracted using the conventional boiling method as previously described (Reddy and Zishiri 2018). Pure single colonies of *Campylobacter* spp. were isolated from plates and suspended in 300 µL TE buffer then vortexed for homogenization of cells. The suspension was boiled at 100°C for 10 min and then immediately cooled on ice. After centrifugation at 14 000×g for 5 min, the supernatant was transferred to a new tube and stored at –20°C until use in PCR (Gibreel *et al*. 2004). Reference strain *C*. *jejuni* ATCC 29428 was used as positive control and DNA was similarly extracted from the reference strain. Thermo Scientific Nanodrop 2000, UV-Vis Spectrophotometer (Wilmington, Delaware, USA) was used to check the concentration and quality of the isolated DNA (Reddy and Zishiri 2018). Following analysis of the extracted DNA, results within the range of 1.8–1.9 at the ratio of 260/280 were regarded as pure DNA and used in PCR. Concentrations of the DNA were also adjusted accordingly with sterile water for subsequent PCR reactions.

2.6. Identification of virulence-associated genes using PCR

DNA from cultured *Campylobacter* isolates was amplified using PCR. In order to differentiate between the species responsible for infection in livestock, two species-specific genes were used. The *hipO* gene is the hippuricase gene specific for *C*. *jejuni* (Marinou *et al*. 2012) and *asp* gene is the aspartokinase gene specific for *C*. *coli* (Al Amri *et al*. 2013). The PCR was used to detect ten virulence genes in the total DNA of *Campylobacter* isolates and to examine the prevalence of these genes within the livestock: *cadF*, *hipO*, *asp*, *dnaJ*, *ciaB*, *cdtA*, *cdtB*, *cdtC*, *flaA*, and *pldA*. PCR primers were sourced from Inqaba Biotechnologies, South Africa. Forward and reverse primers specific for the virulence genes under investigation were designed based on the gene sequence information in the GenBank database and in previously published studies (Al Amri *et al*. 2007; Chansiripornchai and Sasipreeyajan 2009; Rizal *et al*. 2010). Target virulence genes, primer sequences, product sizes and annealing temperatures were shown in Table 1.

PCR was carried out using the T100™ Thermal Cycler (Bio-Rad, Singapore) for a 25-µL reaction system using Thermo Scientific DreamTaq Green PCR Master Mix (2×). A total of 12.5 µL DreamTaq Green PCR Master Mix was used with 1.5 µL of each primer of a 10 µmol L^{-1} primer concentration, 5 µL template DNA and 4.5 µL nuclease-free water making a total volume of 25 µL. The amplification conditions for *cadF*, *hipO*, *asp*, *dnaJ*, *ciaB*, *flaA*, and *pldA* consisted of an initial denaturalisation at 95°C for 3 min, 45 cycles at 94°C for 30 s, specific Tm for each primer for 30 s, and 72°C for 1 min, followed by a final extension at 72°C for 5 min. The *cdt* genes (*cdtA*, *cdtB* and *cdtC*) were run using different amplification conditions according to the research of Rizal *et al*. (2010). The conditions consisted of an initial denaturation at 94°C for 15 min, 45 cycles at 94°C for 1 min, specific Tm for each cdt primer for 1 min, and 72°C for 1 min, followed by a final extension at 72°C for 7 min. Amplicons were electrophoresed on a 1.5% agarose gel run at 70 V for 60 min, stained with ethidium bromide, and visualized using the ChemiDoc™ MP Imaging System (Bio-Rad).

2.7. Statistical analyses

The detected virulence associated genes in *C*. *jejuni* and *C*. *coli* were analyzed using IBM SPSS statistics (version 27.0.1). Pearson's correlation analyses were performed in order to establish the strength and direction of relationships between the specific virulence-associated genes in an effort to determine whether the presence of one virulence gene was associated to the presence of the other. Fisher's exact test, chi-square test and logistic regression analysis were performed to examine for significance of whether the presence of virulence associated genes detected using PCR was determined by whether the isolates originated from different livestock species currently studied. Statistically

significant was defined as $P<0.05$. In every model included, the dependent variable was whether a virulence gene present or absent (1=present, 2=absent) and explanatory variable were if the isolates originated from cattle, chickens, goats, sheep and pigs.

3. Results

3.1. Bacterial isolates

A total of 250 samples were collected and tested for the presence of *Campylobacter* spp. Out of the total samples, 104 samples were positive for *C. jejuni* and 31 were positive for *C. coli* using PCR methods. Data are summarized in Table 2 and Fig. 2, in which the number of tested samples, the isolate percentage for each source, and the prevalence of *C. jejuni* and *C. coli* are presented.

3.2. Molecular detection of virulence genes

The detected virulence genes are shown in Fig. 3 and species identification was confirmed by detection of *hipO* (735 bp) and *asp* gene (500 bp). *Campylobacter jejuni* is the only species which contain the hippuricase (*hipO*) gene because it has not been detected in any other *Campylobacter* species (Rizal *et al*. 2010). Additionally, the aspartokinase (*asp*) gene is specific to *C. coli* (Al Amri *et al*. 2007; Rizal *et al*. 2010). All isolates (100%), regardless of the species identification, were positive for genus-specific *cadF* gene (Shams *et al*. 2016) based on PCR detection of 400 bp amplicon (lane 3).

Lane 4 represented the *cdtA* gene (370 bp), lane 5 represented the *cdtB* gene (495 bp), and lane 6 represented the *cdtC* gene (182 bp), a tripartite toxin that is required for the toxin to be fully functionally active, where *cdtA* and *cdtC* genes are two heterodimeric subunits responsible for toxin binding to the cell membrane and for delivery of *cdtB*, which is the enzymatically active subunit into target cells (Asakura *et al*. 2008; Silva *et al*. 2011). Lane 7 represented the *flaA* gene (113 bp), for motility and is very essential for survival under different chemotactic conditions encountered in the gastrointestinal tract and for the colonization of the small

Table 1 Target virulence-associated genes, primer sequences, amplicon sizes and annealing temperatures

Target gene	Primer sequence (5′→3′)	Product size (bp)	Annealing temperature (°C)	Reference
asp	F-GGTATGATTTCTACAAAGCGAGA R-ATAAAAGACTATCGTCGCGTG	500	53	Al Amri *et al.* (2007)
hipO	F-GAAGAGGGTTTGGGTGGT R-AGCTAGCTTCGCATAATAACTTG	735	53	Al Amri *et al.* (2007)
cadF	F-TTGAAGGTAATTTAGATATG R-CTAATACCTAAAGTTGAAAC	400	43	Chansiripornchai and Sasipreeyajan (2009)
cdtA	F-CCTTGTGATGCAAGCAATC R-ACACTCCATTTGCTTTCTG	370	49	Rizal *et al.* (2010)
cdtB	F-GTTAAAATCCCCTGCTATCAACCA R-GTTGGCACTTGGAATTTGCAAGGC	495	51	Rizal *et al.* (2010)
cdtC	F-CGATGAGTTAAAACAAAAAGATA R-TTGGCATTATAGAAAATACAGTT	182	48	Rizal *et al.* (2010)
flaA	F-AATAAAAATGCTCATAAAAACAGGTG R-TACCGAACCAATGTCTGCTCTGATT	113	53	Chaisowwong *et al.* (2012)
ciaB	F-TGCGAGATTTTTCGAGAATG R-TGCCCGCCTTAGAACTTACA	527	54	Chansiripornchai and Sasipreeyajan (2009)
pldA	F-AAGAGTGAGGCGAAATTCCA R-GCAAGATGGCAGGATTATCA	385	46	Chansiripornchai and Sasipreeyajan (2009)
dnaJ	F-ATTGATTTTGCTGCGGGTAG R-ATCCGCAAAAGCTTCAAAAA	177	50	Chansiripornchai and Sasipreeyajan (2009)

Table 2 Sample origin, number, and percentage of positive isolates of *Campylobacter jejuni* and *Campylobacter coli* from five livestock animal species

Sample origin	No. of samples	Total *Campylobacter*	No. of *C. jejuni*	No. of *C. coli*
Cattle	50	25 (50%)	19 (76%)	6 (24%)
Chicken	50	40 (80%)	28 (70%)	12 (30%)
Goat	50	31 (62%)	25 (81%)	6 (19%)
Sheep	50	14 (28%)	14 (100%)	0 (0%)
Pig	50	25 (50%)	18 (72%)	7 (28%)
Total	250	135 (100%)	104 (77%)	31 (23%)

intestine (Asakura *et al*. 2008; Silva *et al*. 2011). Lane 8 represents the *pldA* virulence gene with an amplicon of 385 bp. Lane 9 represented the *ciaB* gene at 527 bp and the *dnaJ* gene represented in lane 10 at 177 bp. The number of isolates that were positive for each virulence gene was represented in Figs. 4 and 5. There were statistically significant differences (P<0.05) observed between the percentages of virulence genes found in all the livestock.

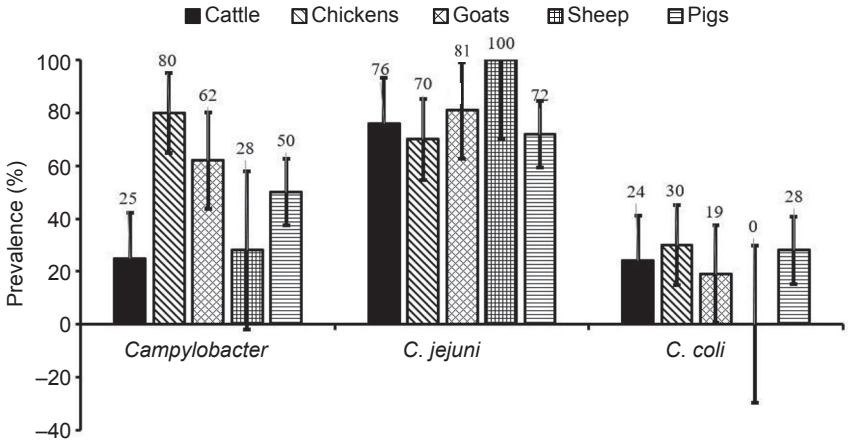


Fig. 2 Shows the prevalence of *Campylobacter jejuni* and *Campylobacter coli* in *Campylobacter* isolated from livestock. Data are the percentage prevalence±standard error.

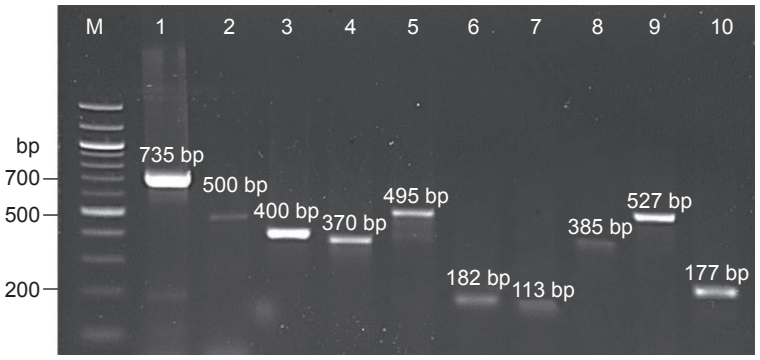


Fig. 3 PCR products for species identification and virulence-associated genes investigated in *Campylobacter* spp. Lane M, 100-bp marker; 1, *hipO*; 2, *asp*; 3, *cadF*; 4, *cdtA*; 5, *cdtB*; 6, *cdtC*; 7, *flaA*; 8, *pldA*; 9, *ciaB*; 10, *dnaJ*.

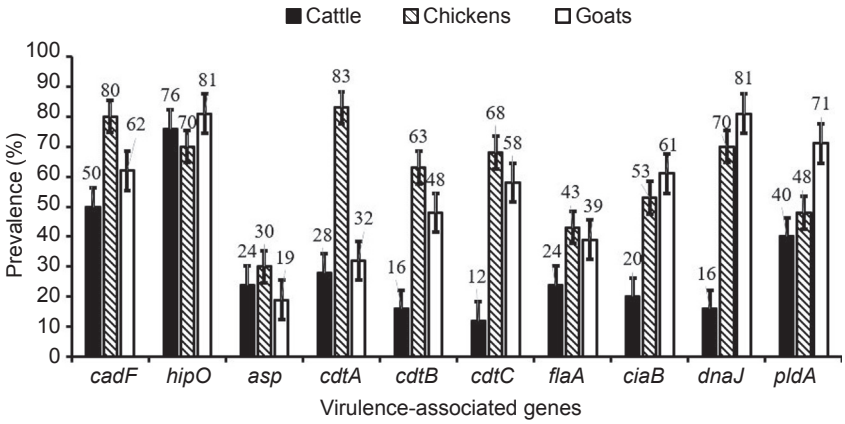


Fig. 4 Prevalence of virulence-associated genes in *Campylobacter* spp. in cattle, chickens, and goats feces. Data are the percentage prevalence±standard error.

Results in Fig. 6 showed that *C. jejuni* may be responsible for the majority of infections (76%) in cattle samples compared to the low incidence of *C. coli* found only in 24% of samples. Of the *C. jejuni* isolated from cattle, 37, 21, 16, 32, 26, 53 and 21% were positive for *cdtA*, *cdtB*, *cdtC*, *flaA*, *ciaB*, *dnaJ* and *pldA*, respectively, and *C. coli* revealed 50, 17, 33, 50, 50, 50 and 50%, respectively. Isolates from chickens (Fig. 7) showed a high prevalence of *C. jejuni* (70%) as compared to 30% of *C. coli*. In chicken, *C. jejuni* isolates showed 96, 86, 93, 61, 75, 64, and 100% prevalence

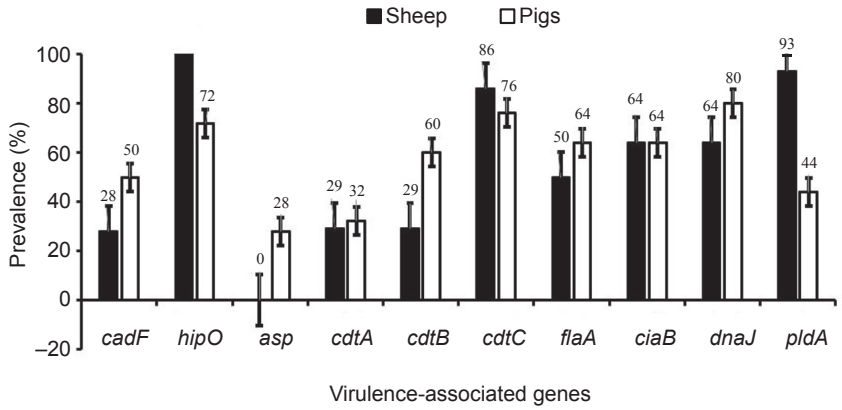


Fig. 5 Prevalence of virulence-associated genes in *Campylobacter* spp. in sheep and pigs feces. Data are the percentage prevalence±standard error.

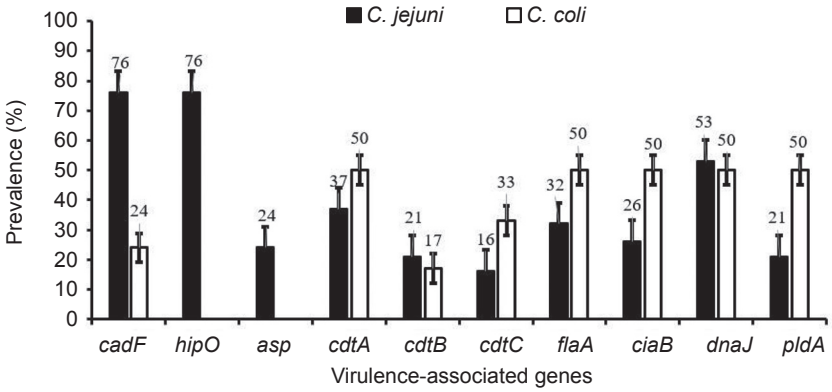


Fig. 6 Prevalence of virulence-associated genes in *Campylobacter jejuni* and *Campylobacter coli* isolated from cattle feces. Data are the percentage prevalence±standard error.

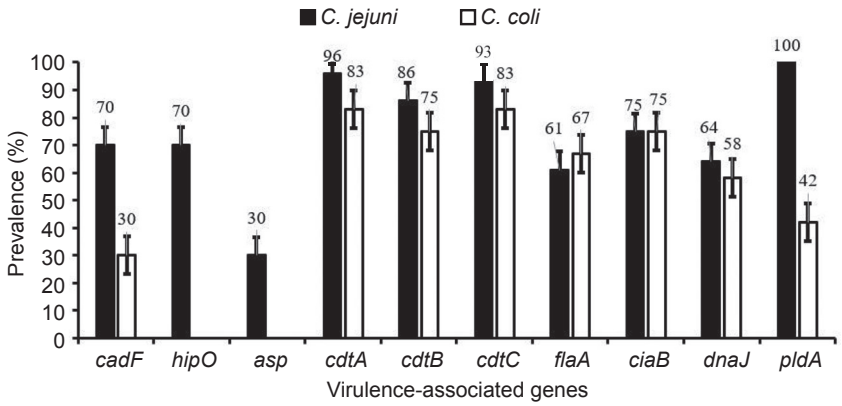


Fig. 7 Prevalence of virulence-associated genes in *Campylobacter jejuni* and *Campylobacter coli* isolated from chickens feces. Data are the percentage prevalence±standard error.

of *cdtA*, *cdtB*, *cdtC*, *flaA*, *ciaB*, *dnaJ*, and *pldA*, respectively, while *C. coli* isolates showed 83, 75, 83, 67, 75, 58, and 42% for the presence of *cdtA*, *cdtB*, *cdtC*, *flaA*, *ciaB*, *dnaJ*, and *pldA*, respectively. Isolates from goats (Fig. 8) showed a high prevalence of *C. jejuni* (81%) as compared to 19% of *C. coli*. In goats, *C. jejuni* demonstrated 40, 60, 60, 48, 76, 88, and 96% prevalence of *cdtA*, *cdtB*, *cdtC*, *flaA*, *ciaB*, *dnaJ*, and *pldA*, respectively, while *C. coli* isolates revealed 50, 100, 83, 33, 67, 50, and 100% for the presence of *cdtA*, *cdtB*, *cdtC*, *flaA*, *ciaB*, *dnaJ*, and *pldA*, respectively.

Isolates from sheep (Fig. 9) showed 100% of *C. jejuni*, hence none of the isolates were found to be *C. coli*. Sheep isolates positive for *C. jejuni* showed 29, 29, 85, 50, 75, 93, and 75% prevalence of *cdtA*, *cdtB*, *cdtC*, *flaA*, *ciaB*, *dnaJ*, and *pldA*, respectively. Results in Fig. 10 indicated that *C. jejuni* is responsible for the majority of infections (72%) in pigs compared to the low incidence of *C. coli* found only in 28% of samples. Of the *C. jejuni* isolated from pigs, 44, 72, 89, 89, 89, 61, and 100% were positive for *cdtA*, *cdtB*, *cdtC*, *flaA*, *ciaB*, *dnaJ*, and *pldA*, respectively and *C. coli* revealed 29, 57, 86, 57, 71, 43, and 100%, respectively.

3.3. Statistical analyses

Statistically significant (P<0.05) positive correlations between all the virulence genes examined in the current study are depicted in Table 3 of the Pearson correlations. The results observed indicated a very strong correlation between all the virulence genes, where all the P-values were less than 0.05 level of significance. The presence of all the genes were strongly correlated (P<0.05) with the presence of the species identification genes (*hipO* and *asp* genes) respectively indicating that if the species are confirmed as *C. jejuni* by the *hipO* gene or the *C. coli* by the *asp* gene, there is a high probability that the virulence genes will be present.

Results in Table 4 demonstrated that there is a significant relationship observed between *cdtA* and *cdtB* genes due

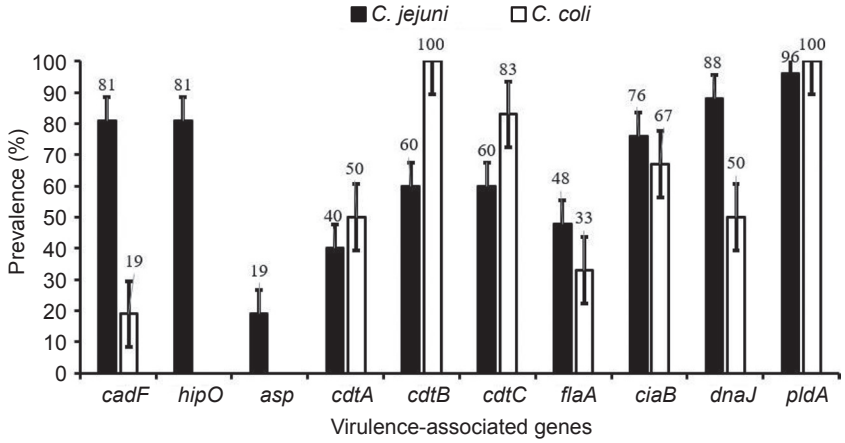


Fig. 8 Prevalence of virulence-associated genes in *Campylobacter jejuni* and *Campylobacter coli* isolated from goats feces. Data are the percentage prevalence±standard error.

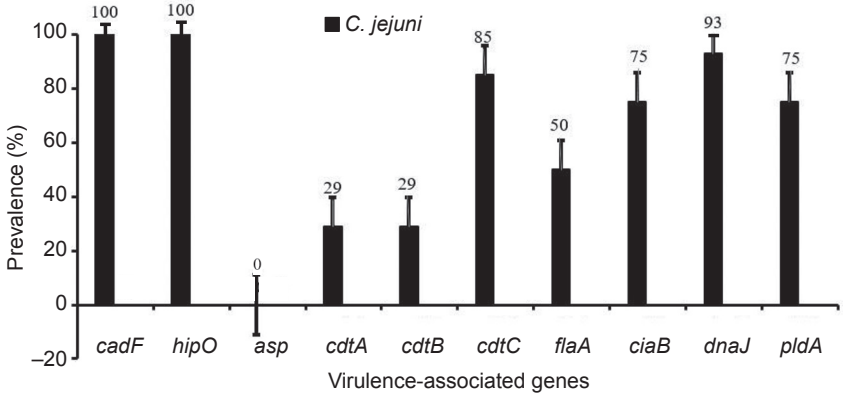


Fig. 9 Prevalence of virulence-associated genes in *Campylobacter jejuni* isolated from sheep feces. Data are the percentage prevalence±standard error.

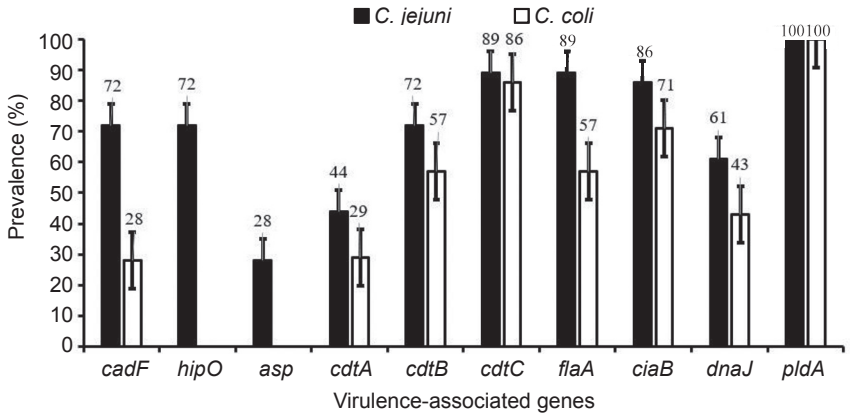


Fig. 10 Prevalence of virulence-associated genes in *Campylobacter jejuni* and *Campylobacter coli* isolated from pigs feces. Data are the percentage prevalence±standard error.

Table 3 Comparison of Pearson's correlations for virulence-associated genes detected in *Campylobacter* species from cattle, chickens, goats, sheep and pigs feces isolates

Gene	Statistical test	*cadF*	*hipO*	*asp*	*cdtA*	*cdtB*	*cdtC*	*flaA*	*ciaB*	*dnaJ*	*pldA*
cadF	Pearson correlation	1	1.000**	0.361**	0.497**	0.502**	0.532**	0.528**	0.599**	0.611**	0.666**
	Sig. (2-tailed)	–	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
hipO	Pearson correlation	1.000**	1	0.361**	0.497**	0.502**	0.532**	0.528**	0.599**	0.611**	0.666**
	Sig. (2-tailed)	0.000	–	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
asp	Pearson correlation	0.361**	0.361**	1	0.322**	0.341**	0.345**	0.282**	0.333**	0.177**	0.272**
	Sig. (2-tailed)	0.000	0.000	–	0.000	0.000	0.000	0.000	0.000	0.005	0.000
cdtA	Pearson correlation	0.497**	0.497**	0.322**	1	0.417**	0.502**	0.341**	0.434**	0.318**	0.308**
	Sig. (2-tailed)	0.000	0.000	0.000	–	0.000	0.000	0.000	0.000	0.000	0.000
cdtB	Pearson correlation	0.502**	0.502**	0.341**	0.417**	1	0.458**	.423**	0.397**	0.304**	0.465**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	–	0.000	0.000	0.000	0.000	0.000
cdtC	Pearson Correlation	0.532**	0.532**	0.345**	0.502**	0.458**	1	0.279**	0.477**	0.362**	0.354**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	–	0.000	0.000	0.000	0.000
flaA	Pearson Correlation	0.528**	0.528**	0.282**	0.341**	0.423**	0.279**	1	0.417**	0.323**	0.311**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000	0.000	0.000
ciaB	Pearson correlation	0.599**	0.599**	0.333**	0.434**	0.397**	0.477**	0.417**	1	0.505**	0.481**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000	0.000
dnaJ	Pearson correlation	0.611**	0.611**	0.177**	0.318**	0.304**	0.362**	0.323**	0.505**	1	0.495**
	Sig. (2-tailed)	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	–	0.000
pldA	Pearson correlation	0.666**	0.666**	0.272**	0.308**	0.465**	0.354**	0.311**	0.481**	0.495**	1
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–

**, correlation is significant at the 0.01 level (2-tailed).

Table 4 Chi-square test and Fisher's exact test for the effect of species location on the presence/absence of virulence-associated genes investigated

Statistical test	Asymptotic significance (2-tailed)									
	cadF	*hipO*	*asp*	*cdtA*	*cdtB*	*cdtC*	*flaA*	*ciaB*	*dnaJ*	*pldA*
Pearson's chi-square test	0.083	0.083	0.139	0.000	0.000	0.114	0.316	0.246	0.829	0.149
Fisher's exact test	0.091	0.091	0.196	0.000	0.003	0.129	0.319	0.271	0.878	0.178

to the presence of these genes in the livestock samples examined (P<0.05) for chi-square and Fisher's exact statistical test on the effect of species location when compared to other genes examined. Table 5 showed that all the genes are statistically significant from the results observed (P<0.05) for both the chi-square and Fisher's exact statistical test on the effect of sample species amongst the genes investigated.

The presence of the virulence genes was predicted in livestock isolates using the source of isolates and sample species as a predictor where a logistic regression analysis (Tables 6 and 7) was conducted. A test of the full model

against a constant only model for the effect of species location indicated that genes *cdtC*, *flaA*, *ciaB*, *dnaJ*, and *pldA* were not statistically significant because the *P*-value was greater than 0.05 level of significance (Table 6). While in Table 7 which represent the sample species indicated that *cdtB*, *cdtC*, *flaA*, *ciaB*, *dnaJ*, and *pldA*, were not statistically significant (*P*<0.05). The Wald criterion demonstrated that *cdtA* and *cdtB* virulence genes made a significant contribution to prediction of the presence of these genes in livestock isolates with *P*=0.000 and 0.002, in Table 6, respectively. The Wald criterion in Table 7 demonstrated that only *cdtA* gene made a significant contribution to prediction of the presence of these genes in livestock isolates with *P*=0.026.

4. Discussion

The present study reports on the analysis of the prevalence of virulence genes among livestock-associated *Campylobacter* isolates, where *C. jejuni* and *C. coli* were detected in cattle, chickens, goats, sheep, and pig fecal samples. The hippuricase (*hipO*) gene is specific for *C. jejuni* and it was not detected in any other *Campylobacter* species, while the aspartokinase (*asp*) gene is specific for *C. coli* (Rizal *et al*. 2010; Reddy and Zishiri 2018). Out of the isolates (*n*=135, out of 250) that tested positive for *Campylobacter*, the majority of them were identified as *C. jejuni* and the remaining were found to be *C. coli*. The prevalence of *C. jejuni* and *C. coli* in the current study are similar to previous studies in this area (Silva *et al*. 2011; Kaakoush *et al*. 2015; Lapierre *et al*. 2016; Cantero *et al*. 2018; Reddy and Zishiri 2018; Wieczorek *et al*. 2018). The correlation analysis indicated that there was a strongly significant (*P*<0.05) positive correlation (100%) between the *hipO* gene and *cadF* gene, hence using PCR in the current study, all the isolates positive for the *hipO* genes were also positive

Table 5 Chi-square test and Fisher's exact test for the effect of sample species on the presence/absence of virulence-associated genes investigated

Statistical test	Asymptotic significance (2-tailed)									
	cadF	*hipO*	*asp*	*cdtA*	*cdtB*	*cdtC*	*flaA*	*ciaB*	*dnaJ*	*pldA*
Pearson's chi-square test	0.000	0.000	0.009	0.000	0.000	0.000	0.023	0.001	0.033	0.000
Fisher's exact test	0.000	0.000	0.001	0.001	0.000	0.000	0.019	0.001	0.034	0.000

Table 6 Logistic regression analysis results indicating the significance effect of species location of the virulence-associated genes found in cattle, chickens, goats, sheep and pigs feces[1)]

Gene	–2logLikelihood	B	SE	Wald *P*-value	OR	95% CI
cdtA	246.962	–1.420	0.321	0.000	0.242	0.129–0.454
cdtB	282.261	–0.951	0.309	0.002	0.386	0.211–0.708
cdtC	311.909	–0.467	0.296	0.115	0.627	0.351–1.121
flaA	270.841	–0.325	0.325	0.317	0.723	0.383–1.365
ciaB	296.477	–0.355	0.307	0.247	0.701	0.385–1.279
dnaJ	305.432	0.067	0.311	0.829	1.069	0.582–1.966
pldA	319.173	–0.422	0.293	0.150	0.656	0.369–1.165

[1)] B, coefficient for the constant (also called the 'intercept') in the null model; SE, standard error around the coefficient for the constant; *P*, probability significance value; OR, odds ratio for the independent variable XI and it gives the relative amount by which the odds of the outcome increase (OR greater than 1) or decrease (OR less than 1) when the value of the independent variable is increased by one unit; 95% CI, 95% confidence interval.

Table 7 Logistic regression analysis results demonstrating the significance effect of sample species of the virulence-associated genes found in five different livestock feces[1)]

Gene	–2logLikelihood	B	SE	Wald *P*-value	OR	95% CI
cdtA	265.962	0.246	0.110	0.026	1.279	1.030–1.589
cdtB	282.261	–0.011	0.103	0.918	0.989	0.809–1.211
cdtC	311.909	–0.158	0.097	0.103	0.853	0.705–1.033
flaA	269.716	–0.113	0.107	0.290	0.893	0.725–1.101
ciaB	296.477	–0.100	0.100	0.320	0.905	0.744–1.101
dnaJ	305.432	0.038	0.098	0.696	1.039	0.858–1.258
pldA	319.173	–0.081	0.095	0.395	0.922	0.766–1.111

[1)] B, coefficient for the constant (also called the 'intercept') in the null model; SE, standard error around the coefficient for the constant; *P*, probability significance value; OR, odds ratio for the independent variable XI and it gives the relative amount by which the odds of the outcome increase (OR greater than 1) or decrease (OR less than 1) when the value of the independent variable is increased by one unit; 95% CI, 95% confidence interval.

for the *cadF* gene (100%). This is due to that *cadF* gene is also classified as specific-genus for *Campylobacter* spp. The high prevalence of the *cadF* gene also indicates that isolates from livestock samples have pathogenic potential properties to humans (Khoshbakht *et al*. 2013). Khoshbakht *et al*. (2013) further assumed that *cadF* gene are probably conserved among *Campylobacter* isolates. This gene encodes *Campylobacter* species adhesion to fibronectin that is an important virulence factor for colonization of the epithelial cells (Wysok and Wojtacka 2018; Farfan *et al*. 2019). Furthermore, the correlation analysis demonstrated that the presence of the *asp* gene was positively correlated only in 36% to the presence of the *hipO* gene (P<0.05). Our results are in compliance with other studies as *C. coli* is not prevalent as *C. jejuni* as the cause of infection in livestock and humans.

Regardless of species identification, all the isolates were positive for *cadF* (*Campylobacter* adhesion to fibronectin) gene which facilitates adherence to fibronectin in the gastrointestinal epithelial cells of the animals and humans (Bolton 2015). Additionally, the *cadF* gene also plays a major important role in the invasion of the epithelial cells (Bolton 2015). Our results are in agreement with studies with regards to the high prevalence of the *cadF* gene in *Campylobacter* isolated from livestock production systems (Konkel *et al*. 1999; Biswas *et al*. 2011; Khoshbakht *et al*. 2013; Wieczorek and Osek 2013; Sen *et al*. 2018). This gene is mediated by a 37-kDa fibronectin-binding out membrane protein and is crucial for *Campylobacter* adherence to and colonization of the host cell surface (Bolton 2015). The current study showed a high prevalence (100%) of the *cadF* gene, which demonstrates that the majority isolates originating from the studied livestock fecal samples have the high potential of pathogenicity properties in *Campylobacter* spp. of the livestock production systems. Furthermore, the high prevalence of *cadF* gene indicated that this gene is probably highly conserved among *Campylobacter* isolates. Hence, the *cadF* gene is also known to have an ability for colonization of the chicken guts (Khoshbakht *et al*. 2013). High prevalence (100%) of *cadF* gene in cattle and chicken isolates was previously reported (Datta *et al*. 2003). Similarly, Acik *et al*. (2013) reported the high prevalence of this gene in 97% of cattle origin isolates. Furthermore, Wysok and Wojtacka (2018) detected 100% of pig isolates and 76.6% of cattle isolates had *cadF* gene. The high prevalence of *cadF* gene is due to the fact that this gene promotes bacteria-host cells interaction and it has been described as a conserved and genus-specific gene (Shams *et al*. 2016). The differences in the prevalence of this gene may be due to genetic diversity, isolation methods, and transport conditions in the isolates from different geographical areas.

The putative virulence genes include cytolethal distending toxin (CDT), as well as *cdtA*, *cdtB*, and *cdtC*, toxin genes encoding for *Campylobacter* cytotoxins. Cytotoxin produced by *Campylobacter* spp. causes DNA lesions, chromatin fragmentation, cytoplasm distension and cell cycle arrest in the G2/M transition phase, leading to progressive cellular distension and ultimately, cell death (Silva *et al*. 2011; Bolton 2015; Lluque and Riveros 2017; Ghorbanalizadgan *et al*. 2018). The virulence of *Campylobacter* spp. is associated with the production of cytotoxins, where, in the current study all the investigated isolates harbored the cytotoxicity genes *cdtA*, *cdtB*, and *cdtC*. Herein, the low prevalence of *cdtA*, *cdtB*, and *cdtC* genes in cattle isolates was observed. Whereas, in the study that was conducted by Lapierre *et al*. (2016), a high prevalence of these genes from cattle fecal isolates was reported, the differences may be due to genetic factors, seasonal factors, types and number of samples, isolation methods and transport conditions in the isolates from different geographical areas. On the other hand, Findik *et al*. (2011) reported high prevalence of cytotoxicity (*cdts*) genes in cattle isolates. In the study performed by Gonzalez-Hein *et al*. (2013), *cdtB* was found in all the cattle isolates examined, the catalytic subunit *cdtB* is encoded by the *cdtB* gene and it has a DNaseI-like activity which is responsible for DNA double strand breaks and the *cdtB* gene is delivered into target cells by *cdtA* and *cdtC* which are binding proteins. Furthermore, *cdtB* gene is translocated into the host cell membrane and causes cell cycle arrest in the G2M phase resulting in cell death (García-Sánchez *et al*. 2018).

The chicken feces isolates in our study demonstrated a very high prevalence for these genes. Furthermore, Lapierre *et al*. (2016) also found high prevalence of *cdtA* and *cdtB* from chicken fecal samples. On the other hand, Wieczorek *et al*. (2018) found high prevalence of *cdtA*, *cdtB*, and *cdtC* genes in chicken feces isolates. However, Khoshbakht *et al*. (2013) found 100% in all the *cdts* genes in the chicken isolates, this findings further confirmed that all the three genes products are required for the toxin to be fully functionally active (Bolton 2015). Nevertheless, Rizal *et al*. (2010) reported the low prevalence rate of these cytotoxicity (*cdts*) genes in chicken feces isolates. In the current study, the prevalence of *cdtA* and *cdtB* was found to be very low, while that of *cdtC* was high in sheep feces isolates. From our understanding, the low prevalence in sheep feces isolates may be due to that sheep feces get dry easily as soon as they leave the caecum, and *Campylobacter* are thermophilic bacteria and are able to grow at high temperatures between 37 and 42 degrees Celsius. On the other hand, in the study performed by Acik *et al*. (2013), the prevalence of cytotoxicity (*cdts*) genes were found to be high in *Camplyobacter* sheep isolates. However, Findik *et al*. (2011) reported very high prevalence of the cytotoxicity (*cdts*) genes in sheep feces

isolates. The differences may be due to genetic factors, feed and environmental conditions, primers, method of detection and geographical areas. The prevalence of *cdtA* gene was found to be low in pig feces isolates, however, the prevalence rates of *cdtB* and *cdtC* were high. This may also be due to genetic factors, season of samples collection, primers and different geographical areas. In goat feces isolates the prevalence of *cdtA* and *cdtB* genes were low, whereas that of *cdtC* was much higher, and in sheep feces isolates the prevalence of the *cdtA* and *cdtB* genes were found to be very low, but then *cdtC* gene was very higher. The detection of three toxin genes (*cdtA*, *cdtB* and *cdtC*) demonstrates the necessity of these genes to activate CDT synthesis (Bolton 2015; Silva *et al*. 2011). Hence, all three genes are required for the toxin to be functionally active (Bolton 2015; Silva *et al*. 2011). It is indeed generally accepted that the *cdt* genes are widespread amongst cattle, chicken and pig isolates (Silva *et al*. 2011).

Another most common putative virulence gene is the the *flaA* gene which encodes for flagellin (Guerry 2007). The *flaA* gene is essential for the motility and colonization of the bacteria in small intestines (Bolton 2015; Wysok and Wojtacka 2018). Other studies have reported that the *flaA* gene is responsible for the expression of adherence, colonization of the gastrointestinal tract and invasion of the host cells, consequently arresting the immune response (Silva *et al*. 2011; Cantero *et al*. 2018; García-Sánchez *et al*. 2018; Farfan *et al*. 2019). Pig and sheep feces isolates demonstrated a high prevalence of the *flaA* gene in the current study, respectively. The high prevalence of this gene among the isolates indicated the importance of this virulence marker in *Campylobacter* spp. and this is due to the fact that this gene is highly conserved among *Campylobacter*. However, the prevalence of this gene in chicken, goats, and cattle was found to be low. On the other hand, a high prevalence of *flaA* gene in chicken feces isolates was reported (Wieczorek *et al*. (2018). Similarly, Farfan *et al*. (2019) reported that *flaA* (100%) was the most prevalent gene in chicken and cattle feces isolates. The difference in the prevalence of this gene from isolates in our study was probably due to differences in the origins of these species and the primers used. Herein, the correlation analysis of the *flaA* gene (53%) was observed to be high when compared with that of *cdtA* and *cdtB* genes $P<0.05$.

The *ciaB* (*Campylobacter* invasive antigen B) gene is known to play a very important role both in the invasiveness and in the colonization of the epithelial cells in animals (Wieczorek *et al*. 2018). This gene is also reported to be crucial in the initial stages of colonization (Guerry 2007), the high prevalence of this gene in *Campylobacter*, currently tested especially among the strains isolated from chicken, goat, sheep, and pig fecal samples may suggest that these bacteria were able to overcome stressful conditions during the passage through the gastrointestinal tract and induce the disease (Guerry 2007). Furthermore, *ciaB* gene had a high significant positive correlation (60%) $P<0.05$ compared to *cdtA*, *cdtB*, *cdtC* and *flaA*. The study conducted by Wieczorek *et al*. (2018) is in concordance with our study, they also reported a high prevalence of *ciaB* gene (98.4%) the differences may be due to genetic variation in the origin of the isolates from different geographical areas. With regards to *dnaJ* gene, which is responsible for adherence and enable *Campylobacter* species to cope with diverse physiological stress (Chansiripornchai and Sasipreeyajan 2009), there was a significant positive correlation when there was a presence of *asp* and *ciaB* genes present in samples ($P<0.05$).

The *pldA* gene encodes an outer-membrane phospholipase A, that is involved in the invasion of the host cells and the phospholipase is associated with the hemolytic activity (Hamidian *et al*. 2011; Ghorbanalizadgan *et al*. 2014; Tabatabaei *et al*. 2014). In *Campylobacter* spp., this gene is also known as a virulence gene that is responsible for expression of invasion and colonization in the small intestine of the animals (Biswas *et al*. 2011). The prevalence of the *pldA* gene in the current study was a very high prevalence in goats and sheep feces isolates, our results are in agreement with the study reported by Khoshbakht *et al*. (2013) where they found the proportion of the *pldA* gene to be very high in *Campylobacter* isolates. High prevalence of this gene, means that it is activated within the animals and it does not cause any infections in the animal because it lives in commensal with an animal, however, if humans eat/consume meat/any product from that particular animal they get infected because the gene will be activated and cause food poisoning. Nevertheless, the proportion of *Campylobacter* isolates in cattle, chickens, and pigs was found to be low in our study. Farfan *et al*. (2019) also reported very low prevalent of *pldA* gene for cattle and chicken *C. jejuni* isolates. Our current study is also in agreement with the study reported by Rizal *et al*. (2010) that the prevalence of *pldA* gene was very low in chicken *Campylobacter* isolates. The reasons for low prevalence of *pldA* gene in the present study may be due to genetic variation, primers differences, different *Campylobacter* isolates, seasonal conditions during sampling in the isolates from different geographical areas. In the current study, there was a very high positive correlation of the *pldA* (67%) gene compared to *ciaB* ($P<0.05$) which is also a gene responsible for colonization and invasion of *Campylobacter* spp.

The findings of the present study demonstrated that isolates of *C. jejuni* and *C. coli* from cattle, chickens, goats, sheep and pigs possess a variation of different virulence factors associated with the processes such

as motility, adherence, invasion and cytotoxicity from the genes investigated, which gave an understanding of molecular epidemiology and the pathogenicity of *Campyloobacter* spp. in livestock production systems in South Africa. It was indicated that the fecal isolates with crucial pathogenic factors responsible for *Campylobacter* motility (*flaA*), adherence and colonization (*cadF*, *pldA* and *dnaJ*), cytotoxicity production (*cdtA*, *cdtB* and *cdtC*) and invasiveness (*ciaB*) were highly conserved among isolates of different origins, which indicated a pathogenic potential to humans. The *cadF* (*Campylobacter* adhesion to fibronectin) gene was present at 100% in all the livestock isolates investigated, which indicated that this gene is specific-genus for *Campylobacter*. The prevalence of *flaA* gene in cattle and chickens was relatively low and therefore, the role in the potential pathogenicity of this gene should be further evaluated.

In contrast, the prevalence of *cdtA* and *cdtB* gene in sheep isolates was low, hence none of the sheep isolates were positive for *C. coli*. This may be explained due to that sheep fecal matter was too dry and the environment is not favorable for *Campylobacter* to thrive, therefore, it may be difficult to rescue and isolate the bacteria because they die as soon as they leave the caecum. Future studies are required in this region using samples from the caecum of the animals.

5. Conclusion

Nonetheless, the high prevalence of virulence factors observed in the current study highlight the need for continued public health monitoring and surveillance of *Campylobacter* virulence genes in different environment from animals and food, to help early detection of virulence gene especially in animal husbandry and to evaluate the impact of strategies designed to reduce the prevalence of virulence genes in livestock since it causes food poisoning to humans. The implementation of one-health approaches is important to monitor and reduce the impacts of health threats across humans, animals, agricultural and environmental interfaces.

Acknowledgements

We would like to acknowledge the four anonymous farmers who allowed us to collect samples for this research from their farms. We would like to thank the South African National Research Foundation for supporting this research through the Thuthuka Funding Instrument (TTK170411226583). We would also like to thank the College of Agriculture, Engineering and Science as well as the School of Life Sciences at University of KwaZulu-Natal (Westville Campus), South Africa for their support during the execution of this research. Authors would like to thank the anonymous reviewers for their valuable comments that significantly improved the manuscript.

References

Acik M N, Karahan M, Ongor H, Cetinkaya B. 2013. Investigation of virulence and cytolethal distending toxin genes in *Campylobacter* spp. isolated from sheep in Turkey. *Foodborne Pathogens and Disease*, **10**, 589–594.

Al Amri A, Senok A C, Ismaee A Y, Al-Mahmeed A E, Botta G A. 2007. Multiple PCR for direct identification of *Campylobacter* spp. in human and chicken stools. *Journal of Medical Microbiology*, **56**, 1350–1355.

Al Amri A, Senok A C, Ismaeel A Y, Al-Mahmeed A E, Botta G A, Lake R. 2013. Human and animal *Campylobacteriosis* in Tanzania: A review. *BMC Microbiology*, **95**, 169–174.

Asakura M, Samosornsuk W, Hinenoya A, Misawa N, Nishimura K, Matsuhisa A, Yamasaki S. 2008. Development of a cytolethal distending toxin (*cdt*) gene-based species-specific multiplex PCR assay for the detection and identification of *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter fetus*. *FEMS Immunology & Medical Microbiology*, **52**, 260–266.

Azrad M, Tkhawkho L, Isakovich N, Nitzan O, Peretz A. 2018. Antimicrobial susceptibility of *Campylobacter jejuni* and *Campylobacter coli*: Comparison between Etest and a broth dilution method. *Annals of Clinical Microbiology and Antimicrobials*, **17**, 23.

Biswas D, Hannon S J, Townsend H G, Potter A, Allan B J. 2011. Genes coding for virulence determinants of *Campylobacter jejuni* in human clinical and cattle isolates from Alberta, Canada, and their potential role in colonization of poultry. *International Microbiology*, **14**, 25–32.

Bolton D J. 2015. *Campylobacter* virulence and survival factors. *Food Microbiology*, **48**, 99–108.

Cantero G, Correa-fiz F, Ronco T, Strube M, Cerda-Cuellar M, Pedersen K. 2018. Characterization of *Campylobacter jejuni* and *Campylobacter coli* broiler isolates by whole-genome sequencing. *Foodborne Pathogens and Disease*, **15**, 145–152.

Casagrande Proietti P, Pergola S, Bellucci S, Menchetti L, Miraglia D, Franciosini M P. 2018. Occurrence and antimicrobial susceptibility of *Campylobacter* spp. on fresh and refrigerated chicken meat products in Central Italy. *Poultry Science*, **97**, 2895–2901.

CDC (Centre for Disease Control). 2013. Vital signs: Incidence and trends of infection with pathogens transmitted commonly through food-foodborne diseases active surveillance network, 10 U.S. sites, 1996–2010. *MMWR Morbidity and Mortality Weekly Reports*, **60**, 749–755.

Chaisowwong W, Kusumoto A, Hashimoto M, Harada T, Maklon K, Kawamoto K. 2012. Physiological characterization of *Campylobacter jejuni* under cold stresses conditions: Its potential for public threat. *Journal of Veterinary Medical*

Science, **74**, 43–50.

Chansiripornchai N, Sasipreeyajan J. 2009. PCR detection of four virulence-associated genes of *Campylobacter jejuni* isolates from Thai broilers and their abilities of adhesion to and invasion of INT-407 cells. *Journal of Veterinary Medical Science*, **71**, 839–844.

Datta S, Niwa H, Itoh K. 2003. Prevalence of 11 pathogenic genes of *Campylobacter jejuni* by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. *Journal of Medical Microbioliology*, **52**, 345–348.

Epps S V, Harvey R B, Hume M E, Phillips T D, Anderson R C, Nisbet D J. 2013. Foodborne *Campylobacter*: Infections, metabolism, pathogenesis and reservoirs. *International Journal of Environmental Research and Public Health*, **10**, 6292–6304.

Farfan M, Lartiga N, Benavides M B, Alegria-moran R, Saenz L, Salcedo C, Lapierre L. 2019. Capacity to adhere to and invade human epithelial cells, as related to the presence of virulence genes in, motility of, and biofilm formation of *Campylobacter jejuni* strains isolated from chicken and cattle. *Canadian Journal of Microbiology*, **65**, 126–134.

Findik A, Ica T, Onuk E E, Percin D, Kevenk T O, Ciftci A. 2011. Molecular typing and *cdt* genes prevalence of *Campylobacter jejuni* isolates from various sources. *Tropical Animal Health and Production*, **43**, 711–719.

García-sánchez L, Melero B, Rovira J. 2018. *Campylobacter* in the food chain. *Advances in Food and Nutrition Research*, **86**, 215–252.

Ghorbanalizadgan M, Bakhshi B, Kazemnejad lili A, Najar-peerayeh S, Nikmanesh B. 2014. A molecular survey of *Campylobacter jejuni* and *Campylobacter coli* virulence and diversity. *Iranian Biomedical Journal*, **18**, 158–164.

Ghorbanalizadgan M, Bakhshi B, Najar-Peerayeh S. 2018. Heterogeneity of cytolethal distending toxin sequence types of *Campylobacter jejuni* and correlation to invasion/cytotoxicity potential: The first molecular survey from Iran. *Microbial Pathogenesis*, **114**, 213–218.

Guerry P. 2007. *Campylobacter flagella*: Not just for motility. *Trends of Microbiology*, **15**, 456–461.

Gibreel A, Tracz D M, Nonaka L, Ngo T M, Connell S R, Taylor D E. 2004. Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from 1999 to 2002, with special reference to *tet*(O)-mediated tetracycline resistance. *Antimicrobial Agents and Chemotherapy*, **48**, 3442–3450.

Gonzalez-hein G, Huaracan B, Garcia P, Figueroa G. 2013. Prevalence of virulence genes in strains of *Campylobacter jejuni* isolated from human, bovine and broiler. *Brazilian Journal of Microbiology*, **44**, 1223–1229.

Hamidian M, Sanaei M, Bolfion M, Dabiri H, Zali M R, Walther-Rasmussen J. 2011. Prevalence of putative virulence markers in *Campylobacter jejuni* and *Campylobacter coli* isolated from hospitalized children, raw chicken, and raw beef in Tehran, Iran. *Canadian Jourrnal of Microbiology*, **57**, 143–148.

Iglesias-torrens Y, Miro E, Guirado P, Llovet T, Munoz C, Cerda-Cuellar M, Madrid C, Balsalobre C, Navarro F. 2018. Population structure, antimicrobial resistance, and virulence-associated genes in *Campylobacter jejuni* isolated from three ecological niches: Gastroenteritis patients, broilers, and wild birds. *Frontiers in Microbiology*, **9**, 1676.

Iovine N M. 2013. Resistance mechanisms in *Campylobacter jejuni*. *Virulence*, **4**, 230–240.

Kaakoush N O, Castano-Rodriguez N, Mitchell H M, Man S M. 2015. Global epidemiology of *Campylobacter* infection. *Manual of Cinical Microbiology*, **28**, 687–720.

Karikari A B, Obiri-danso K, Frimpong E H, Krogfelt K A. 2017. Antibiotic resistance of *Campylobacter* recovered from faeces and carcasses of healthy livestock. *Biomed Research International*, **2017**, 4091856.

Khoshbakht R, Tabatabaei M, Hosseinzadeh S, Shekarforoush S S, Aski H S. 2013. Distribution of nine virulence-associated genes in *Campylobacter jejuni* and *Campylobactercoli* isolated from broiler feces in Shiraz, Southern Iran. *Foodborne Pathogens and Disease*, **10**, 764–770.

Konkel M E, Gray S A, Kim B J, Garvis S G, Yoon J. 1999. Identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* based on the cadF virulence gene and its product. *Journal of Clinical Microbiology*, **37**, 510–517.

Lapierre L, Gatica M A, Riquelme V, Vergara C, Yanez J M, San martin B, Saenz L, Vidal M, Martinez M C, Araya P, Flores R, Duery O, Vidal R. 2016. Characterization of antimicrobial susceptibility and its association with virulence genes related to adherence, invasion, and cytotoxicity in *Campylobacter jejuni* and *Campylobacter coli* Isolates from animals, meat, and humans. *Microbial Drug Resistance*, **22**, 432–244.

Lluque A, Riveros M. 2017. Virulence and antimicrobial resistance in *Campylobacter* spp. from a peruvian pediatric cohort. *Scientifica*, **2017**, 2–7.

Marinou I, Bersimis S, Ioannidis A, Nicolaou C, Mitroussia-Ziouva A, Legakis N J, Chatzipanagiotou S. 2012. Identification and antimicrobial resistance of *Campylobacter* spp. isolated from animal sources. *Frontiers in Microbiology*, **3**, 58.

Obeng A S, Rickard H, Sexton M, Pang Y, Peng H, Barton M. 2012. Antimicrobial susceptibilities and resistance genes in *Campylobacter* strains isolated from poultry and pigs in Australia. *Journal of Applied Microbiology*, **113**, 294–307.

Otigbu A C, Clarke A M, Fri J, Akanbi E O, Njom H A. 2018. Antibiotic sensitivity profiling and virulence potential of *Campylobacter jejuni* isolates from estuarine water in the eastern Cape province, South Africa. *International Journal of Environmental Research and Public Health*, **15**, 5–15.

Premarathne J, Anuar A S, Thung T Y, Satharasinghe D A, Jambari N N, Abdul-mutalib N A, Huat J T Y, Basri D F, Rukayadi Y, Nakaguchi Y, Nishibuchi M, Radu S. 2017. Prevalence and antibiotic resistance against tetracycline in *Campylobacter jejuni* and *Campylobacter coli* in cattle and beef meat from Selangor, Malaysia. *Frontiers in Microbiology*, **8**, 2254.

Ragimbeau C, Colin S, Devaux A, Decruyenaere F, Cauchie H

M, Losch S, Penny C, Mossong J. 2014. Investigating the host specificity of *Campylobacter jejuni* and *Campylobacter coli* by sequencing gyrase subunit A. *BMC Microbiology*, **14**, 2–12.

Reddy S, Zishiri O T. 2018. Genetic characterisation of virulence genes associated with adherence, invasion and cytotoxicity in *Campylobacter* spp. isolated from commercial chickens and human clinical cases. *Onderstepoort Journal of Veterinary Research*, **85**, 1–9.

Rizal A, Kumar A, Vidyarthi A. 2010. Prevalence of pathogenic genetics in *Campylobacter jejuni*. *Journal of Food Safety*, **12**, 29–34.

Sen K, Lu J, Mukherjee P, Berglund T, Varughese E, Mukhopadhyay A K. 2018. *Campylobacter jejuni* colonization in the crow gut involves many deletions within the cytolethal distending toxin gene cluster. *Applied and Environmental Microbiology*, **84**, 2–8.

Shams S, Bakhshi B, Tohidi Moghadam T. 2016. In silico analysis of the *cadF* gene and development of a duplex polymerase chain reaction for species-specific identification of *Campylobacter jejuni* and *Campylobacter coli*. *Jundishapur Journal of Microbiology*, **9**, e29645.

Silva J, Leite D, Fernandes M, Mena C, Gibbs P A, Teixeira P. 2011. *Campylobacter* spp. as a foodborne pathogen: A review. *Frontiers in Microbiology*, **2**, 200.

Szczepanska B, Andrzejewska M, Spica D, Klawe J J. 2017. Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from children and environmental sources in urban and suburban areas. *BMC Microbiology*, **17**, 80.

Tabatabaei M, Aski H S, Shayegh H, Khoshbakht R. 2014. Occurrence of six virulence-associated genes in *Arcobacter* species isolated from various sources in Shiraz, Southern Iran. *Microbial Pathogenesis*, **66**, 1–4.

Thakur S, Zhao S, Mcdermott P F, Harbottle H, Abbott J, English L, Gebreyes W A, White D G. 2010. Antimicrobial resistance, virulence, and genotypic profile comparison of *Campylobacter jejuni* and *Campylobacter coli* isolated from humans and retail meats. *Foodborne Pathogens of Disease*, **7**, 835–844.

Van den Berg B, Walgaard C, Drenthen J, Fokke C, Jacobs B C, Van Doorn P A. 2014. Guillain-Barré syndrome: Pathogenesis, diagnosis, treatment and prognosis. *Nature Reviews Neurology*, **10**, 469–482.

Wieczorek K, Osek J. 2013. Antimicrobial resistance mechanisms among *Campylobacter*. *Biomed Research International*, **2013**, 2–10.

Wieczorek K, Wolkowicz T, Osek J. 2018. Antimicrobial resistance and virulence-associated traits of *Campylobacter jejuni* isolated from poultry food chain and humans with diarrhea. *Frontiers in Microbiology*, **9**, 1508.

Wilson D J, Gabriel E, Leatherbarrow A J, Cheesbrough J, Gee S, Bolton E, Fox A, Fearnhead P, Hart C A, Diggle P J. 2008. Tracing the source of campylobacteriosis. *PLoS Genetics*, **4**, e1000203.

Wysok B, Wojtacka J. 2018. Detection of virulence genes determining the ability to adhere and invade in *Campylobacter* spp. from cattle and swine in Poland. *Microbial Pathogenesis*, **115**, 257–263.

Zaidi M B, Campos F D, Estrada-Garcia T, Gutierrez F, Leon M, Chim R, Calva J J. 2012. Burden and transmission of zoonotic foodborne disease in a rural community in Mexico. *Clinical Infectious Diseases*, **55**, 51–60.

Executive Editor-in-Chief CHEN Hua-lan
Managing editor ZHANG Juan