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Full Length Research Paper

Campylobacteriosis in sheep in farm settlements in the Vhembe district of South Africa

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A total of 300 freshly voided sheep faeces were collected and screened for the presence of *Campylobacter* spp. using standard microbiological techniques. The samples were obtained randomly from 3 farm settlements in the Venda Region, South Africa in 2008 and 2009. The recovery rate was 30.0% (90 of 300) for all faeces. Of these, 65 (72.2%) were from diarrheic and 25 (27.8%) were from non-diarrheic faeces. Out of the 90 *Campylobacter* spp. isolated, 41(45.6%) were *Campylobacter jejuni* and 49 (54.4%) were *C. coli*. Sixty-three (70%) of the isolates were β-haemolytic, while 17 (18.9%) were α-haemolytic and 10 (11.1%) were non-haemolytic on 5% sheep red blood cells. The antibiotic resistance patterns of the 90 *Campylobacter* isolates were examined by the disc diffusion method. All *Campylobacter* isolates from the farms were resistant to at least one of the 12 antibiotics tested. The prevalence rate of *C. coli* resistance to ciprofloxacin was 20.4% compared with *C. jejuni,* 17.1%. Similar rates were noted for tetracycline for the two species. *C. jejuni* showed a higher rate of resistance to erythromycin (22.0%) compared with *Campylobacter coli* (10.2%). Significantly higher frequency of kanamycin resistance was recorded for *C. jejuni* compared to *C. coli* ($p < 0.005$). However, for ciprofloxacin, tetracycline, erythromycin, imipenem, gentamycin and ampicillin comparable resistant profiles were recorded for *C. jejuni* and *C. coli* isolates from the farms. The high prevalence of *Campylobacter* spp. in sheep is of public significance in the Venda Region. The observed multi-drug resistance and especially resistance to macrolides and fluoroquinolones in this study pose a threat of transfer of antibiotic resistance to human pathogens because of the close contact between sheep and humans.

Key words: Antibiotics, *Campylobacter*, haemolytic, macrolides, pathogens, resistance.

INTRODUCTION

The incidence of campylobacteriosis has increased during the last decade, and it is the leading cause of bacterial enteritis in developing and developed countries (Allos, 2001; Coker, 2002; Wingstand et al., 2006; EFSA, 2007).

Several epidemiological studies in different countries have identified sources of *Campylobacter* enteritis in man to include animals, food, water, and milk products (Oporto et al., 2007; Esteban et al., 2008). Reports of *Campylobacter* enteritis in developing countries (Padungton et al., 2005; Uaboi-Egbenni et al., 2008), point to an urgent need to explore prevalence rates, antibiograms and haemolytic activities in animals because of the zoonotic nature of infections and for proper planning of effective prevention and control measures (Raji et al., 1997; Oporto et al., 2009).

Studies on campylobacteriosis in sheep are scanty (Koides, 1991; Stanley et al., 2003; Oporto et al., 2009) despite the array of clinical manifestations. In sheep, campylobacteriosis is characterized by abortion, still births, and birth of weak lambs during late pregnancy (Kimberly, 1988; Dennis, 1990; Raji et al., 2000). *Campylobacter jejuni* and *Campylobacter fetus* have been identified as the most common causative agents of this disease. The infection is highly contagious and may cause up to 79% of ewes to abort when the organisms

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are newly introduced into the flock (Kimberly, 1990; Raji et al., 2000). Few reports suggest that sheep shed *Campylobacter* organisms but at a lesser rate than other animals (Stanley and Jones, 2003). Susceptible ewes may acquire infection through ingestion of contaminated *Campylobacter* organisms with fecal material or uterine discharge. Other sources of infection may include faeces of carrier sheep and other mammals (Raji et al., 2000).

Antimicrobial treatment is indicated for systemic *Campylobacter* infections in immune-suppressed patients and for severe or long-lasting infections (Allos, 2001). Erythromycin is considered the drug of choice for treating *Campylobacter* gastroenteritis, and ciprofloxacin and tetracycline are used as alternative drugs (Nachamkin et al., 2000) but several species are resistant to these antibiotics (Unicomb et al., 2008; Pezzotti et al., 2003; Oporto et al., 2009).

Although, the majority of *Campylobacter* infections are self-limiting, complicated cases may warrant antimicrobial therapy. Antimicrobial susceptibility data show an increase in the number of fluoroquinolone-resistant and, to a lesser extent, macrolide-resistant *Campylobacter* strains causing human infections (Pezzotti et al., 2003; Gibreel and Taylor, 2006; Anderson et al., 2006). Antibiotic resistance in human medicine is mainly linked to human misuse of antimicrobial agents, but there is accumulating evidence that antimicrobial resistance originating from the use of antimicrobials in food animals might complicate therapy of human infections (Oporto et al., 2009). Antimicrobials used therapeutically or prophylactically in animals husbandry can also exert selective pressure on bacteria that infect food animals and reach humans via food products (van den Bogaard et al., 2000; Oporto et al., 2007; Krutkiewicz et al., 2009). The extensive development of resistance to tetracycline and ciprofloxacin in various countries has led to a decrease in their clinical use (Trieber and Taylor, 2000). In addition, the increasing emergence of erythromycin resistance among isolates of *C. jejuni* and *C. coli* has prompted a search among newer macrolide derivatives for those useful against *Campylobacter* isolates. Local and international committees have highlighted the need for better control of antibiotics usage in human medicine and veterinary husbandry (EFSA, 2007).

In this sense, systematic monitoring of the occurrence of antimicrobial resistance in *C. jejuni* and *C. coli* originating from animals can serve as an indicator of the selective pressure these bacteria are undergoing and could assist in monitoring development of resistance. Isolates of *C. jejuni* and *C. coli* with resistance to various antimicrobial agents have been reported in both developed and developing countries (Hart and Kariuki, 1998; Valenza et al., 2010). Since the 1990s, a significant increase in the prevalence of resistance to macrolides among *Campylobacter* spp. has been reported, and this is recognized as an emerging public health problem (Engberg et al., 2001). It has been suggested by some investigators that resistance to macrolides is mainly found in isolates of animal origin, especially *C. coli* from pigs and also *C. jejuni* from chickens (Van Looveren et al., 2001).

A prevalence study recently carried out in the Basque Country (Northern Spain) identified 28.3% (34/120) of ovine and 18.0% (37/206) of bovine farms positive for *C. jejuni* (Oporto et al., 2007), and even higher values (38.2%, 13/34) in free-range poultry farms (Esteban et al., 2008). Bacterial antibiotic resistance genes are commonly carried on plasmids and could also be chromosomally borne as well as on extrusion factors (Anderson et al., 2006; Moore et al., 2005; Oporto et al., 2009; Chaban et al., 2010).

The application of PCR may provide a more accurate description of the prevalence of *Campylobacter* spp. associated with livestock. Marshall et al. (1999) and Samie et al. (2006) used the primer sequences designed to amplify a 1004 bp fragment within the coding region of the 16S rRNA gene in *Campylobacter* species. The design was based on an alignment of the full 16S rRNA sequences of *Campylobacter* species, which demonstrated common conserved regions that served as targets for the primers.

The common association between *Campylobacter* spp., animals and human diseases make them a potential source of antibiotic resistance genes, although information on the susceptibility of *Campylobacter* spp. from animals is scanty. The most important aspects of this study are the novel findings of campylobacteriosis among sheep in farm settlements in local communities, their multiple antibiotic resistant profiles and haemolytic activities and the untoward public health significance.

MATERIALS AND METHODS

Collection of faeces

Samples were collected from three farms (designated A, B and C for confidentiality). Briefly, one hundred (100) freshly voided faeces were collected at random from apparently healthy sheep from each of the farms with the aid of oven-sterilized spatula in sterile 50 ml plastic containers and transported to the laboratory within 2 h for initial processing. A total of 300 samples consisting of diarrheic and non-diarrheic faeces were collected. Once in the laboratory, the faeces were immediately processed. About 2 gm of each sample was transferred to 6 ml of sterile brain heart infusion broth and left to emulsify at room temperature for 10 to 20 min to release the bacteria. The suspension was used directly for detection of *Campylobacter*.

Isolation and identification of *Campylobacter* by conventional culture methods

Twenty microlitres of faecal suspension was spread on the surface of charcoal cefoperazone deoxycholate agar plates (CM 739 [Oxoid] with cefoperazone supplement SR 155E). The plates were incubated in a 2 L anaerobic jar under microaerophilic conditions employing the Campygen gas generating kit (Oxoid CM025) at 42°C for 48 h. Colonies suspected to be *Campylobacter* were further purified on blood agar plates (Blood Agar Base No.2 (Oxoid)

supplemented with 5% sterile laked horse blood). All isolates were characterized by their catalase, oxidase reactions, and susceptibility to nalidixic acid by standard procedures (Baker et al., 2008; Chaban et al., 2010). The resulting isolates were subsequently stored at -80°C in brain heart infusion broth with 15% glycerol until further investigation.

Confirmation of positive *Campylobacter* isolates

Identification of *Campylobacter* isolates was done using Dryspot *Campylobacter* test kit (Oxoid Basingstoke, Hampshire, England). The test is specific for pathogenic *Campylobacter* strains belonging to *C. jejuni, C. coli, C. upsaliensis* and *C. lari*. The Oxoid agglutination test was done according to the manufacturer's instruction. Agglutination under normal lighting conditions indicated that the test organism was *Campylobacter* and belonged to any of the four species mentioned earlier (Baker et al., 2008; Chaban et al., 2010).

Discrimination of *Campylobacter* species

The dryspot-positive campylobacters were further subjected to Mast diagnostic *Campylobacter* kits consisting of urease, indoxyl acetate and hippurate test (ampoules) and/or indoxyl acetate, urease and hippurate strips. Briefly, 24 h cultures of the *Campylobacter* were inoculated into the urease, indoxyl acetate and hippurate test solutions according to the manufacturer's instructions. These were then incubated for 4 h for colour development. For urease, development of pink colour was indicative of urease enzyme production (*C. lari*), development of pink colour in hippurate solution indicated production of hippuricase enzyme (*C. jejuni*). In the case of indoxyl acetate solution, change of colour from colourless to blue/purple was indicative of the presence of *C. jejuni/C. coli*. A reaction positive for Indoxyl acetate reaction but negative for hippurate test solution, confirmed C*. coli*. A positive for both reactions was indicative of *C. jejuni* (Baker et al., 2008; Chaban et al., 2010).

Alternatively, the indoxyl acetate strips and hippurate strips were impregnated with wet cultures and allowed to stay for 3 to 5 min. Development of blue/purple colour in the case of indoxyl acetate strips and development of pink colour in the case of hippurate strips within this period was indicative of positive reaction (*C. jejuni* and *C. coli*) and *C. jejuni* respectively.

Isolation of bacterial genomic DNA

Genomic DNA was obtained by the whole-cell lysate method as described by Marshall *et al*. (1999). Briefly, cells from a 24 to 48 h culture grown on Columbia blood agar were re-suspended in sterile distilled water to an equivalent of 2.5 McFarland value. The suspensions were boiled to 100°C for 20 min in an Eppendorff tube. The resulting templates were either used immediately for PCR or were kept at 4°C for up to 1 month.

PCR Confirmation of *Campylobacter*

In order to ascertain if the dryspot/Mast diagnostic kit-positive *Campylobacter* isolates were actually campylobacters, they were subjected to PCR identification using the general primers for the identification of campylobacteria. These primers are also specific for other members of the Campylobacteriaceae (*Helicobacter* and *Arcobacter*).

However, *Arcobacter* and *Helicobacter* spp. show negative reaction to the *Campylobacter* dryspot kit. Hence, any amplification of the primer sequences at the 1,004-bp fragment within the coding region of 16S rRNA confirms that such isolates are *Campylobacter* spp. and not *Helicobacter* or *Arcobacter* spp. The PCR-RFLP method used in this study was as previously described by Marshall et al. (1999). Briefly, amplification was done in 50 µl reaction volume containing 5 µl of whole-cell lysate, 1µl each primer, 10x buffer (invitrogen), 1.5 mM $MgCl_2$, 200 µM each deoxynucleotide (invitrogen) and 5U Taq DNA polymerase (invitrogen). The PCR amplification was performed with a - thermocycler (ESCO Swift Mini Thermal Cycler Version 1.0, ESCO Technologies, Philadelphia U.S.A). The samples were subjected to an initial denaturation for 2 min at 95°C, followed by 30 amplification cycles, each consisting of 94°C for 30 s, 52°C for 30 s, and 72°C for 90s. A final primer extension at 72°C for 10min. was included. Oligonucleotide primers employed in this study are CAH16S 1a (5' – AAT ACA TCA AAG TCG AAC GA – 3') and CAH16S 1b (TTA ACC CAA CAT CTG ACG AC – 3'), respectively. The oligonucleotides used in this study were synthesized by Inqaba Biotechnologies (Pretoria, South Africa).

Blood haemolysis

To ascertain the pathogenic status of the isolates, the *Campylobacter* spp were subjected to haemolytic test according to the procedure of Samie et al. (2006). Briefly, a 24 h broth culture of *Campylobacter* spp. were placed onto Columbia agar base supplemented with sheep blood. Plates were incubated at 35°C for 24 h. Thereafter, plates were observed for complete, partial and no haemolysis.

Antimicrobial agents

The antibiotics tested in this study were: Trimethoprim (2.5 µg), Nalidixic acid (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), tetracycline (30 µg), ampicillin (10 µg), erythromycin (15 µg), streptomycin (10 µg), methicillin (µg), cefexime (30 µg), imipenem (µg), kanamycin (30 µg) and vancomycin (30 µg) (Oxoid, Unipath Ltd, Basingstoke, England).

Antimicrobial susceptibility testing

The method of Gaudreau and Gilbert (1997) was used. Briefly, the confirmed *Campylobacter* isolates were inoculated onto Mueller-Hinton agar plates carrying a maximum of six (6) discs. All plates were incubated at 35°C under a microaerophilic atmosphere obtained with a Campy gas generator envelope (Oxoid), for 24 h. The resulting zone diameters were measured with a graduated metre rule. Analysis of diameter was done according to the procedures of NCCLS (2002) now known as ICLS for enterobacteriaceae.

Statistical analysis

χ^2 test was used to evaluate the results, using the SPSS version 17.1. The results obtained were used to compare the antimicrobial resistance amongst *C. jejuni* and *C. coli* isolates as well as comparison of the prevalence of resistance to the various antibiotics used in human campylobacteriosis.

RESULTS

Three hundred freshly voided sheep faeces were

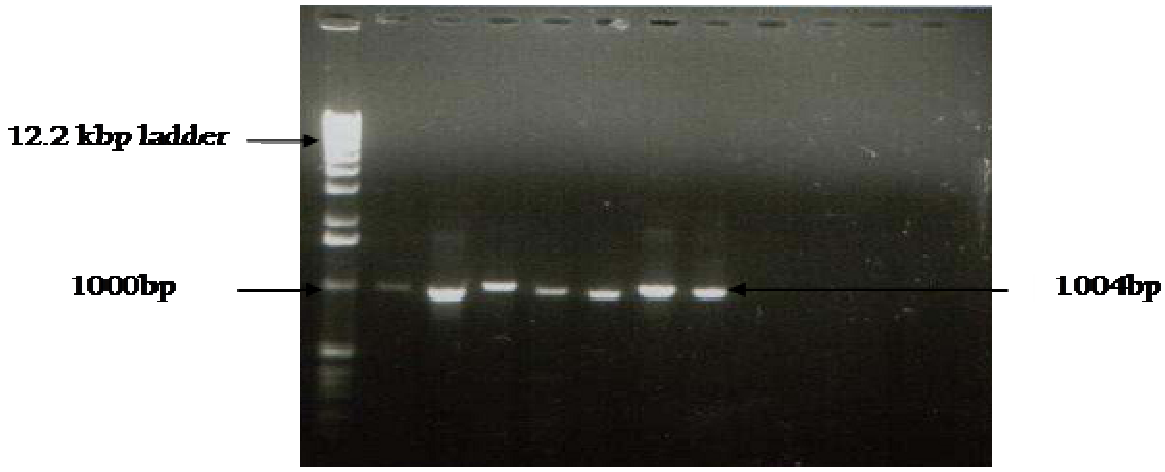

Figure 1. PCR products of amplified DNA from sheep *Campylobacter* isolates aligning at the 1004bp of a 12.2kbp ladder Lane 1= 1.9kb ladder; lane 2,3,4,5,6,7, 8 amplified bands of DNA from sheep *Campylobacter* strains.

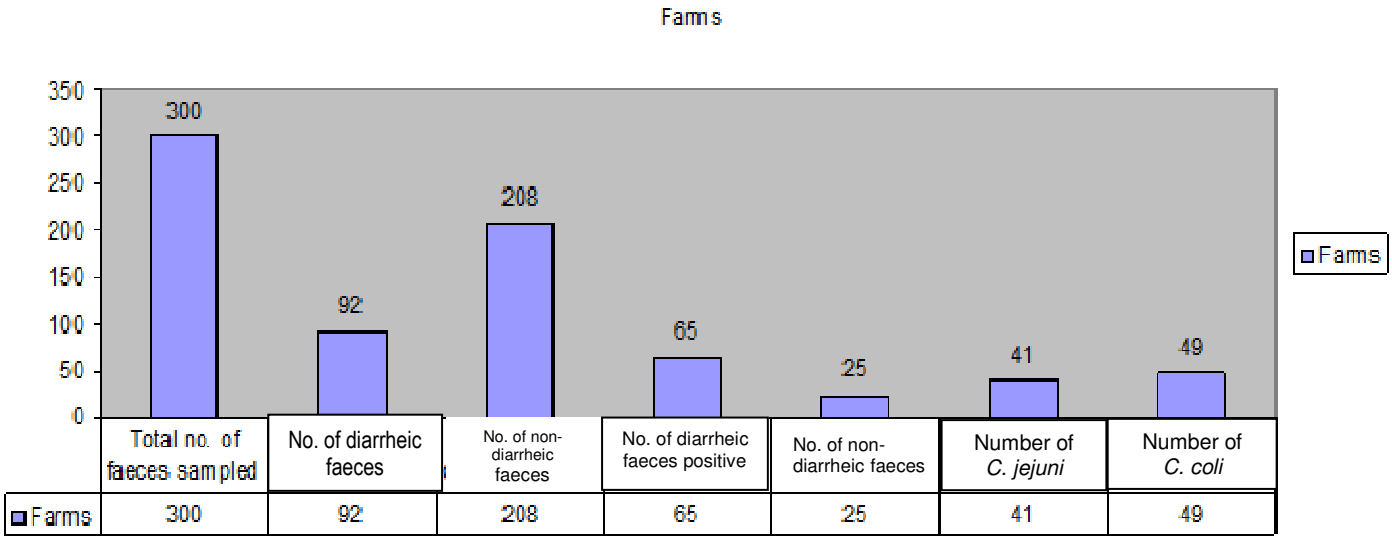

Figure 2. Distribution of *C. jejuni* and *C. coli* in diarreic and non-diarrheic faeces samples.

analyzed in the study. The recovery rate was 30.0% (90 of 300) for all cases. Ninety faecal samples were positive for *Campylobacter*. Of these, 65 (72.2%) were from diarrheic and 25(27.8%) were from non-diarrheic samples. Of the ninety (90) *Campylobacter* isolates obtained from the faeces, 41(45.6%) were *C. jejuni* while 49(54.4%) were *C. coli* (Figure 1). Of the 65 diarrheic positive faeces, 38 were *C. jejuni* and 27 *C. coli* and of the 25 non-diarrheic positive faeces, 3 were *C. jejuni* and 22 were *C. coli*. There was a higher prevalence rate of *C. jejuni* in diarrheic faeces than *C. coli* and vice versa. The high rate of incidence of *C. jejuni* in diarrheic faeces is an indication that the campylobacteriosis witnessed among sheep in these farms has its origin from *C. jejuni* infection.

Haemolysis of sheep red cells

Of the 90 *Campylobacter* strains isolated from the farms 63 (70%) were β-haemolytic, while 17(18.9%) were α-haemolytic and 10(11.1%) were non-haemolytic (Figure 2).

PCR study of *Campylobacter* isolates

The PCR micrographs of the DNA from *Campylobacter* strains from sheep are as indicated in Figure 3. The purified DNA from the *Campylobacter* strains amplified at the 1004 bp, which is the specific region for the conserved 16S rRNA for members of the genus

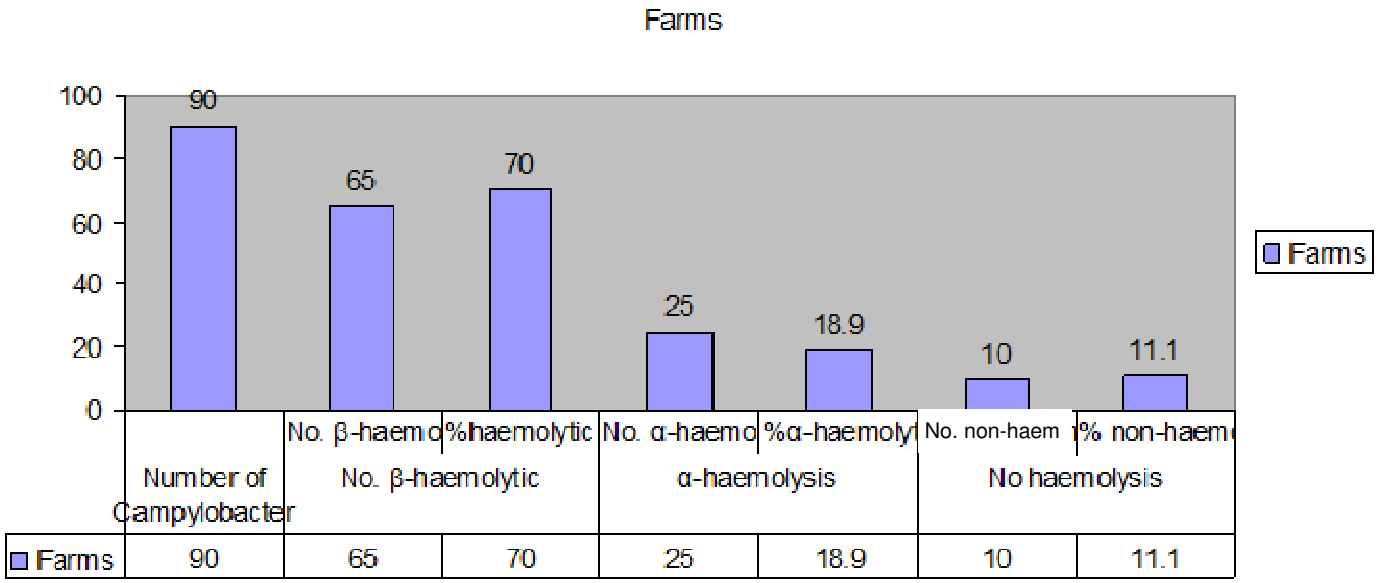

Figure 3. Haemolytic profile of campylobacters isolated from sheep faeces in South Africa

Campylobacter. The bands formed were confirmed as those for *Campylobacter* since the Oxoid *Campylobacter* dryspot agglutination test kit is specific for thermotolerant pathogenic members of the genus *Campylobacter* (*C. jejuni, C. coli and C. lari*). Furthermore, biochemical reactions involving catalase, cytochrome oxidase reaction, nalidixic acid sensitivity as well as motility test aided in the appropriate identification of the *Campylobacter* strains. However, since culture environments were done under 42°C, which is specific for the thermotolerant and pathogenic species, these *Campylobacter* strains could belong to any of *C. jejuni*, *C. coli* and *C. lari.* Specific identification by the Mast diagnostic kits differentiated the isolates into *C. jejuni* and *C. coli.*

Antimicrobial susceptibility

Ninety *Campylobacter* strains were exposed to 12 antibiotics. Of these, 73 were susceptible to ciprofloxacin with zone of inhibition diameters ≥28.3 mm, while 17 had zones ≤15.2 mm and were regarded as resistant. Seventy-two *Campylobacter* strains were susceptible to tetracycline with zone diameters ≥28.2 mm, while 10 resistant strains had zones of ≤17 mm and 8 had no zones around the disc. Seventy-eight erythromycin-susceptible strains had zone diameters ≥20 mm, while 8 resistant strains had zones of <20 mm and 5 had no zones around the disc. The 37 ampicillin-susceptible strains of *Campylobacter* spp. had zones of ≥27 mm, while the 40 ampicillin-resistant strains had zones of ≤15.2 mm, while 13 had no zones around the disc. The sixty-six gentamycin-susceptible and 24 gentamycin-resistant *Campylobacter* spp. had zone diameter of ≥28 and ≤16 mm (Table 1). *C. coli* had a higher rate of resistance to ciprofloxacin (20.4%) than *C. jejuni* (17.1%). The same trend was noticed for tetracycline with values of 20.4% *C. coli* and 19.5%, *C. jejuni* (Table 2). However, rate of resistance of *C. jejuni* to erythromycin (22.0%) was higher than *C. coli* (10.2%). The rest analyses were as shown in Figure 2.

A comparison of the occurrence of antimicrobial resistance among *C. jejuni* strains and *C. coli* in all farms is presented in Table 2. A significantly higher frequency of kanamycin resistance was recorded among *C. jejuni* and *C. coli* isolates from the farms ($p < 0.005$). However, for ciprofloxacin, tetracycline, erythromycin, imipenem, gentamycin and ampicillin comparable occurrences of resistance were recorded among *C. jejuni* and *C. coli* isolates from the farms.

DISCUSSION

Overall, in this study we found a high frequency of *Campylobacter* spp. in sheep (30%). The prevalence of species distribution of campylobacters in this study was *C. jejuni* 41 (45.6%), *C. coli* 49 (54.5%). The previously reported prevalence of campylobacters among sheep in Africa (Ethiopia) were C. *coli* (40.7%) and *C. jejuni* (59.3%) (Kassa et al., 2005). In their study they did not isolate *C. lari* from sheep. Our findings on the prevalence of campylobacters in sheep are in line with their report. There was a high prevalence of *Campylobacter* isolates in diarrheic (72.2%) than non-diarrheic faeces (27.8%) and the difference was of statistical significance ($p < 0.005$). Padungton and Kareene (2003) reported that

Table 1. Susceptibility profiles of 90 strains of *C. jejuni* and *C. coli* by disc diffusion using six (6) popularly employed antibiotics for the treatment of campylobacteriosis in humans.

Ciprofloxacin	Tetracycline	Erythromycin	Ampicillin	Gentamycin
73S S(≥ 28.3 mm)	72S(≥ 29mm)	78S(≥ 20mm)	37S(≥ 27mm)	66S (≥28)
17R (≤15.2 mm)	18R (≤ 17mm)	12R (<20mm)	53 (≤15.2mm)	24R (≤16.2)
Nalidixic acid				
69S (≥28 mm)				
21R (≤16.2 mm)				

S = Susceptible; R = Resistance.

Table 2. Antibiotic sensitivity patterns of *C. jejuni* and *C. coli* isolated from sheep in 3 farms in Venda Region, South.

Antibiotic	*C. jejuni*			*C. coli*			Combined resistance	
	No. of isolates	No. of resistant	% resistance	No. Exposed	No. Resistant	% resistance	Combined % resistance	P value
Ciprofloxacin	41	7	17.1	49	10	20.4	18.8	NS
Tetracycline	41	8	19.5	49	10	20.4	20.0	NS
Erythromycin	41	7	22.0	49	5	10.2	16.7	NS
Gentamycin	41	13	31.7	49	11	22.5	26.7	NS
Ampicillin	41	25	70.0	49	28	57.1	58.9	NS
Kanamycin	41	36	87.8	49	28	57.1	71.1	0.005
Imipenem	41	10	24.4	49	10	20.4	22.2	NS
Cefexime	41	36	87.8	49	41	83.7	85.6	NS
Vancomycin	41	33	80.5	49	37	75.5	77.8	NS
Methicillin	41	41	100	49	49	100	100	
Trimethoprim	41	41	100	49	49	100	100	
Nalidixic acid	41	11	26.8	49	10	20.4	23.3	NS

NS = Not statistically significant.

both *C. jejuni* and *C. coli* could cause a mild self-limiting enteritis and bacteraemia when inoculated orally into newborn calves. The frequency of *Campylobacter* spp. isolation (30%) in sheep in this study was higher than those reported in studies conducted in Portugal (15%) (Cabrita et al., 1992), in Norway (8.1%) (Rosef et al., 1983) and Brazil (20%) (Aquino et al., 2002).

The main species of *Campylobacter* isolated from sheep faeces in this study were *C. coli* (54.5%) and *C. jejuni* (45.5%). This pattern of shedding is similar to the report from Preston, Lancashire in the United Kingdom by Padungton and Kaneene (2003). However, in terms of species distribution we observed lower prevalence of *C. jejuni* and higher prevalence of *C. coli* compared with the findings of Kassa et al. (2005). The contamination of sheep carcasses during slaughter processes could also represent a potential source of human infection. Reports have shown that the shedding of campylobacters in faeces by sheep varies considerably with the time of the year (Stanley et al., 1998). The shedding of campylobacters by sheep has the potential to contaminate pastures and surface waters. In this wise, contamination of surface and sub-surface waters may transmit *Campylobacter* spp. within herds and between farms and other livestock groups (Jones et al., 1999). This may have been responsible for the high campylobacteriosis observed in the farms investigated. Our results are in line with the report of Stanley and Jones (2003) who observed campylobacteriosis and heavy shedding of *C. jejuni* and *C. coli* in cattle and sheep farms.

Few studies have reported campylobacteriosis resulting in abortion among sheep but the actual rates of prevalence were not ascertained (Dennis, 1990; Koides, 1991; Kimberly, 1988; Raji et al., 2000). The high occurrences of campylobacters in diarrheic faeces obtained from the farms were indicative of campylobacteriosis, which could result in economic loss of infected sheep in the flock. Kimberly (1988) reported Jenson and Swiff's diseases of sheep in Philadelphia, LA, USA, resulting from campylobacteriosis (enteritis and/stillbirth as well as abortion) caused by *C. jejuni* and its pathogenic allies.

Of the 90 *Campylobacter* isolates, 65 (72.2%) were

β-haemolytic, while 25 (18.9%) were α-haemolytic. These findings are consistent with the report of Samie et al. (2006) that most thermophilic *Campylobacter* isolates were β-haemolytic. The occurrence of non-haemolytic pathogenic thermophilic *Campylobacter* spp. as observed in this study may have presented a new idea that these *Campylobacter* pathogens could also lose their pathogenic attributes.

Resistance to fluoroquinolones has increased over the past years (Saenz et al., 1997; Prats et al., 2000; Reina et al., 1994). Results from recent susceptibility studies of *C. jejuni* and *C. coli* from poultry meat performed in different countries indicated substantial variation between countries. Relatively high resistance rates in *C. jejuni* strains isolated from chicken meat were reported from Belgium (Van Looveren *et al*., 2001), the United States (Ge et al., 2003), and Italy (Pezzotti et al., 2003), moderate rates were reported from Switzerland (Ledergerber et al., 2003) and Northern Ireland (Wilson, 2003), whereas limited occurrence of antimicrobial resistance among *C. jejuni* was reported from Sweden (Lindmark et al., 2004). A possible explanation for these differences might be that occurrences of antimicrobial resistance are reflecting the different national and regional policies in relation to the use of antimicrobial agents for food animals.

In the present study, there was a significant difference in ciprofloxacin and tetracycline resistance among isolates from sheep. This observation fits the finding of Oporto et al. (2009) who observed a high rate of resistance among *Campylobacter* isolates to these antibiotics. Our data (Table 2) also indicated that 19.5 and 20.5% of *C. jejuni* and *C. coli* isolates respectively from sheep faeces were resistant to tetracycline. Gibreel and Taylor (2006) in their review reported high rate of resistance to macrolides of clinical isolates of *C. jejuni* and *C. coli*. Most of the *Campylobacter* isolates were resistant to ampicillin (58.9%). This resistant rate of ampicillin is higher than that reported by Oporto et al. (2009) (26.4%) in their study of resistance profiles of *Campylobacter* spp. in Spain.

It is therefore possible that production of β-lactamase by *Campylobacter* isolates could have been responsible for the high frequency of ampicillin-resistant campylobacters (Baserisalehi et al., 2005). The *Campylobacter* isolates were also resistant to ciprofloxacin at 18.8% rate. However, 22.0% of *C. jejuni* and 10.2% of *C. coli* were resistant to erythromycin. These results are in contrast to reports by Isenberger et al. (2002) that most of the *Campylobacter* isolates in Vietnam and Thailand were resistant to nalidixic acid (73 vs. 7%, respectively, $p < 0.05$) and ciprofloxacin (77 vs, 7% respectively, $p < 0.05$). The observation was also contrary to studies of Oporto et al. (2009) where all their isolates were susceptible to erythromycin. Most isolates in this study (≥75%) were resistant to vancomycin, cefexime, methicillin and trimethoprim (Table 2). The combined rate of resistance of the *Campylobacter* spp. from the farms to gentamycin was 26.7%. Individually, resistance of *Campylobacter* to this antibiotic was *C. jejuni* (31.7%) and *C. coli* (22.5%). This observation harmonizes with the report of Nonga and Muhairwa (2010) in Tanzania who reported between 20 to 50% resistances, which contrasted with the observation of Oporto et al. (2009) who reported total susceptibility of *Campylobacter* isolates to gentamycin.

In addition, the report of Hong *et al*. (2007) and Nonga and Muhairwa (2010) were in contrast to our findings as these workers observed resistant rates as high as ≥94% of *Campylobacter* spp. to ciprofloxacin (95.9%), tetracycline (94.6) and nalidixic acid (94.6%). However, their reports on the resistant rate of campylobacters to erythromycin were in line with our findings.

Resistance to fluoroquinolones has increased over the past years in many parts of the world (Allos, 2001; EFSA, 2007; Engberg et al., 2001). In Spain, fluoroquinolone-resistant *C. jejuni* isolated from humans was first reported in 1988 (Reina et al., 1989) and resistance increased to high levels (Saenz et al., 2000; Prats et al., 2000). The activity of erythromycin against *C. jejuni* human isolates, the antibiotic of choice for the treatment of diarrhea caused by *Campylobacter* strains (especially in infants), seems to remain stable at rates below 5% (Saenz et al., 2000; Prats et al., 2000). The report of these workers is in contrast to our observation among animal isolates as we noted a higher rate of resistance to the macrolide (erythromycin) (16.6%). However, this is the most stable antibiotic to campylobacters isolated from sheep compared with other tested antibiotics. This susceptibility may be reassuring, but active and more extensive antimicrobial surveillance in campylobacters from animals is needed to allow future informed decisions about how macrolide antibiotics could be used in food animals while still safeguarding human health. With the emergence of resistant strains of campylobacters isolated from sheep as observed in this study, increasing passage of diarrheal faeces and with the way sheep are reared in Africa, there is every likelihood that an epidemic situation may arise through the dissemination of highly pathogenic antibiotic-resistant strains into the environment. Contamination of other farm animals co-reared with sheep and flock members as well as pollution of surface water through run-offs is of public health significance. To this end, it is important that the veterinary section of the department of health, South Africa investigate the cause of campylobacteriosis among sheep in these farms in order to prevent an epidemic situation that could in little time spread not only to other animals but also to humans.

Conclusion

This study has shown a high prevalence of campylobacteriosis in sheep in farm settlements in the

Vhembe district of the Limpopo Province, South Africa. In addition, we have also noted a high rate of β-haemolytic activities, which may indicate the ability of these isolates to cause pathological effects or death of cells. The high rate of isolation and multiple antibiotic resistances of *Campylobacter* species from diarrheic faeces of sheep calls for more elaborate studies to unravel the cause, origin and extent of campylobacteriosis not only among sheep but among other food animals, including their phylogenetic relatedness and the various genes coding for resistance and virulence in the Venda Region of South Africa.

REFERENCES

Alfredson DA, Akhurst RJ, Korolik V (2003). Antimicrobial resistance and genomic screening of clinical isolates of thermophilic *Campylobacter* spp. from South-West Queensland, Australia. J. Appl. Microbiol., 94: 494-500.

Allos BM (2001). *Campylobacter jejuni* infections: update on emerging issues and trends. Clin. Infect. Dis., 32: 1201-1206.

Anderson SR, Saadbye P, Shukri NM, Rosenquist H, Nielson NL, Boel J (2006). Antimicrobial resistance among *Campylobacter jejuni* isolated from raw poultry meat at retail level in Denmark. Int. J. Food Microbiol., 107: 250-255.

Aquino MH, Pacheco AP, Ferreira MC, Tibana A (2002). Frequency of isolation and identification of thermophilic campylobacters from animals in Brazil. Vet. J., 164: 159-161.

Baserisalehi MBN, Augustine EK, Al-Mahdi AY, Kapadnis BP (2004). Enhanced recovery and isolation of *Campylobacter* spp. from water using a novel devise. J. Appl. Microbiol, 96: 664-670.

Cabrita J, Rodrigues J, Braganca F, Morgado C, Pires I, Goncalves AP (1992). Prevalence, biotypes, plasmid profile and antimicrobial resistance of *Campylobacter* isolated from wild and domestic animals from northeast. Portugal. J Appl. Bacteriol., 73: 279-285.

Chaban B, Ngeleka M, Hill JE (2010). Detection and quantification of 14 *Campylobacter* species in Pet dogs reveals an increase in species richness in faeces of diarrheic animals. BMC Microbiology, 10, 1 – 7. 1992; Bottom of Form, 73: 279-285.

Dennis SM (1990). *Campylobacter* abortion in Sheep. In: Kirkbridge. CA (Ed.), Laboratory Diagnosis of livestock abortion. 3rd Edition. Ames Iowa, Iowa University Press, pp. 82-85.

EFSA (2007). "Report of the Task Force of Zoonoses Data Collection including a proposal for a harmonized monitoring scheme of antimicrobial resistance in *Salmonella* in fowl (Gallus gallus), turkeys, and pigs and *Campylobacter jejuni* and *C. coli* in broilers," The EFSA J., 96: 1-46.

Esteban JI, Oporto B, Aduriz G, Juste RA, Hurtado A (2008). "A survey of food-borne pathogens in free-range poultry farms," Int. J. Food Microbiol., 123(1 – 2): 177-182.

Gaudreau C, Gilbert H (1997). Comparison of disc diffusion and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* subsp. jejuni and Campylobacter coli. J. Antimicrob. Chemother., 39: 707-712.

Ge B, White DG, McDermott PF, Girard W, Zhao S, Hubert S, Meng J (2003). Antimicrobial –resistant *Campylobacter* species from retail raw meats. Appl. Environ. Microbiol., 69: 3005-3007.

Gibreel A, Taylor DE (2006). Macrolide resistance in Campylobacter jejuni and Campylobacter coli, J. Antimicrob. Chemother., 58: 243-255.

Hart CA, Kariuki S (1998). Antimicrobial resistance in developing countries. BMJ 317: 647-650.

Hong J, Man KJ, Kyung JW, Hyun KS, Wonki B, Cheong KH, Jereoyng G, Maeum K, Junghee S, Ho PY (2007). Prevalence and antibiotic resistance of *Campylobacter* spp. isolated from chicken meat, pork, and beef in Korea, from 2001 to 2006. J. of Food Protect., 70(4): 860-866.

Isenberger DW, Hoge CW, Srijan A, Pitarangsi C, Vithayasai N, Bodindatta L, Hickey KW, Cam PD (2002) Comparative antibiotic resistance of diarrheal pathogens from Vietnam and Thailand, 1996 – 1999. Emerg. Infect. Dis., 8: 175-180.

Jones K, Howard S, Wallace JS (1999). Intermittent shedding of thermophilic campylobacters by sheep at pasture. J. Appl. Microbiol., 86: 531-536.

Kassa T, Gebre-selassie S, Asrat D (2005). The prevalence of thermotolerant *Campylobacter* species in food animals in Jimma Zone, southwest Ethiopia. *Ethiop*. J. Health Dev., 19(3): 225-229.

Kimberling CV (1988). Diseases causing abortion. In: Kimberling CV. (Ed.). Jensen and Swiff's Diseases of Sheep. 3rd Edition, Philadelphia LA and Fabiger. pp. 57-63.

Koides P (1991). Incidence of *Campylobacter jejuni/coli* in carcasses of cattle, sheep and swine. Bulletin of The Hellenic Vet. Med. Soc., 42(4): 241-244.

Lederberger U, Regula G, Stephan R, Danuser J, Bissig B, Stark KDC (2003). Risk factors for antibiotic resistance in *Campylobacter* spp. isolated from raw poultry meat in Switzerland. PMC Public Health 3(39) (http: //www. biomedcentral.com/1471-2458/3/39).

Lindmark H, Harbom B, Thebo L, Andersson L, Hedin G, Osterman B, Lingber T, Andersson Y, Westoo A, Olsson EE (2004). Genetic characterization and antibiotic resistance of *Campylobacter jejuni* isolated from meats, water, and humans in Sweden. J. Clin. Microbiol., 42: 700-706.

Moore JE, Corocoran D, Dooley JSG, Fnning S, Brigid L, Matsuda M, McDowell DA, Megraud F, Millar BC, O'Mahony R, O'Riordan L, O'Rourke M, Rao JR, Rooney PJ, Sails A, Whyte P (2005). *Campylobacter*. Vet. Res. 36: 351-382.

Marshall SM, Melito PL, Woodward DL, Johnson WM, Rodger, FG, Mulvey MR (1999) Rapid Identification of *Campyloabcter, Arcobacter,* and *Helicobacter* Isolates by PCR-Restriction Fragment Length Polymorphism Analysis of the 16S rRNA Gene. J. Clin. Microbiol., 37(12): 4158-4160.

Nachamkin I, Engberg J, Aarestrup FM (2000) Diagnosis and antimicrobial susceptibility of *Campylobacter* spp. In: Nachamkin I. Blaser MJ, eds. *Campylobacter*, 2nd edn. Washington. Am. Soc. Microbiol., pp. 45-66.

National Committee for Clinical Laboratory Standards (2002). Performance Standards for Antimicrobial Disk Susceptibility Tests, 7th ed. Approved standard M2–A7. National Committee for Clinical Laboratory Standards, Wayne, Pa USA.

Nonga HE, Muhairwa AP (2010). Prevalence and antibiotic susceptibility of thermophilic *Campylobacter* isolates from free range domestic duck (*Cairina moschata*) in Morogoro municipality, Tanzania. Trop. Anim. Health Prod., 42(2): 165-172.

Oporto B, Ramon AJ, Hurtado A (2009). Phenotypic and Genotypic Antimicrobial Resistance Profile of *Campylobacter jejuni* Isolated from Cattle, Sheep, and Free-Range Poultry Faeces. Int. J. Microbiol.,

Oporto B, Esteban JI, Aduriz G, Juste RA, Hurtado A (2007). "Prevalence and strain diversity of thermophilic campylobacters in cattle, sheep and swine farms," J. Appl. Microbiol., 103(4): 977-984.

Padungton P, Kaneene JB (2005) *Campylobacter* in Food Animals and Humans in Northern Thailand. J. Food Protectn., 68(12): 2519-2526.

Padungton P, Kaneene JB (2003). *Campylobacter* spp. in human, chickens, pigs and their antimicrobial resistance. J. Vet. Med. Sci., 65: 161-170.

Pezzotti G, Serafin A, Luzzi I, Mioni R, Milan, M, Perin R (2003). Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in northeastern Italy. Int. J. Food Microbiol., 82: 281-287.

Prats G, Mirelis B, Llovet T, Munoz C, Miro E, Navarro B (2000). "Antibiotic resistance trends in enteropathogenic bacteria isolated in 1985 – 1987 and 1995 – 1998 in Barcelona," Antimicrob. Agents and Chemother., 44(5): 1140-1145.

Raji MA, Adekeye JO, Kwaga JKP, Bale JOO (2000). Bioserogroups of *Campylobacter* species isolated from sheep in Kaduna State, Nigeria. Small Rumin. Res., 37: 215-221.

Raji MA, Kawaga JKP, Bale JOO, Adekeye JO (1997). Campylobacteriosis in Nigeria – a review. Nig. Vet. J., 18: 84-89.

Reina J, Blanco I, Catala A, Alomar P (1989). "Aislaminento de

Campylobacter jejuni resistente al acido nalidixico," Enfermedades Infecciossy Microbiol. Clin., 7(5): 288-289.
Reina J, Ros MJ, Serra A, (1994). "Susceptibilities to 10 antimicrobial agents of 1,220 *Campylobacter* strains isolated from 1987 to 1993 from faeces of pediatric patients," Antimicrobial. Agents Chemother., 38(12): 2917-2920.
Rosef O, Gondrosen B, Kapperud G, Underdal B (1983). Isolation and characterization of *Campylobacter jejuni* and *Campylobacter coli* from domestic and wild mammals in Norway. Appl. Environ. Microbiol., 46: 855-859.
Saenz Y, Zarazaga M, Lantero M, Gastanares MJ, Baquero F, Torres C (2000). "Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997-1998", Antimicrob. Agents Chemother., 44(2): 267-271.
Samie A, Obi CL, Barrett LJ, Powell SM, Guerrant RL (2007). Prevalence of *Campylobacter* species, *Helicobacter pylori* and *Arcobacter* species in stool samples from the Venda region, Limpopo, South Africa: Studies using molecular diagnostic methods. J. Infect., 54(6): 558-566.
Stanley KN, Wallace JS, Currie JE, Diggle PJ, Jones K (1998). Seasonal variation of thermophilic campylobacters in lambs at slaughter. J. Appl. Microbiol., 84: 1111-1116.
Stanley K, Jones K (2003). Cattle and sheep farms as reservoirs of *Campylobacter*. J. Appl. Microbiol., 94 Suppl: 104S-113S.
Taylor DE, Courvalin P (1988). Mechanism of antibiotic resistance in *Campylobacter* spp. Antimicrob. Agents Chemother., 32(8): 1107-1112.
Trieber CA, Taylor DE (2000). Mechanisms of antibiotic resistance in *Campylobacter. In*: Nachamkin I, Blaser MJ (eds) *Campylobacter, 2nd edn*. Washington: Am. Soc. Microbiol., pp. 441-454.
Uaboi-Egbenni PO, Okolie, PN, Adesanya OD, Omonigbehin E, Sobande AO (2008). Epidemiological studies of the incidence of pathogenic *Campylobacter* spp. amongst animals in Lagos metropolis. Afr. J. Biotechnol., 7(16): 2852-2956.
Van Looveren M, Daube G, De Zutter L, Dumont JM, Lammens, C, Wijdooghe M, Vandamme P, Jouret M, Cornelis M, Goosens H (2001). Antimicrobial susceptibilities of *Campylobacter* strains isolated from food animals in Belgium. J. Antimicrob. Chemother., 48: 235-240.
Wagner J, Jabbusch M, Eisenblatter M (2003). Susceptibilities of *Campylobacter jejuni* isolates from Germany to ciprofloxacin, moxifloxacin, erythromycin, clindamycin, and tetracycline. Antimicrob. Agents Chemother., 47: 2358-2361.
Wilson IG (2003). Antibiotic resistance of *Campylobacter* in raw retail chickens and imported chicken portions. Epidemiol. Infect., 131: 1181-1186.
Wingstrand A, Neimann J, Engberg J, Nielsen EM, Gerner-Smidt P, Wegener HC, Molbak K (2006). Fresh chicken as main risk factor for campylobacteriosis, Denmark. Emerg. Infect. Dis., 12: 280-285.