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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 MgCl₂, 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), cefoxime (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**

χ² 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
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

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**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 diarrheic and non-diarrheic faeces samples.
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 10 resistant strains had zones of <20 mm and 8 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.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>C. jejuni</th>
<th>C. coli</th>
<th>Combined resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>73S (≥ 28.3 mm)</td>
<td>17R (≤ 15.2 mm)</td>
<td>66S (≥ 28)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>72S (≥ 29 mm)</td>
<td>18R (≤ 17 mm)</td>
<td>24R (≤ 16.2)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>78S (≥ 20 mm)</td>
<td>12R (&lt; 20 mm)</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>37S (≥ 27 mm)</td>
<td>53 (≤ 15.2 mm)</td>
<td></td>
</tr>
<tr>
<td>Gentamycin</td>
<td>66S (≥ 28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>69S (≥ 28 mm)</td>
<td>21R (≤ 16.2 mm)</td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>C. jejuni</th>
<th>C. coli</th>
<th>Combined resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>41</td>
<td>7</td>
<td>20.4</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>41</td>
<td>8</td>
<td>20.4</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>41</td>
<td>7</td>
<td>22.5</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>41</td>
<td>13</td>
<td>26.7</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>41</td>
<td>25</td>
<td>58.9</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>41</td>
<td>36</td>
<td>71.1</td>
</tr>
<tr>
<td>Imipenem</td>
<td>41</td>
<td>10</td>
<td>22.2</td>
</tr>
<tr>
<td>Cefoxime</td>
<td>41</td>
<td>36</td>
<td>85.6</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>41</td>
<td>33</td>
<td>77.8</td>
</tr>
<tr>
<td>Methicillin</td>
<td>41</td>
<td>41</td>
<td>100</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>41</td>
<td>41</td>
<td>100</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>41</td>
<td>11</td>
<td>23.3</td>
</tr>
</tbody>
</table>

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 Swift’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, cefxime, 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


1992; Bottom of Form, 73: 279-285.


