Prevalence of *Escherichia coli* O157 strains in cattle, pigs and humans in North West province, South Africa

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WE HAVE DETERMINED THE PREVALENCE of *Escherichia coli* O157 strains in cattle, pigs and humans. Eight hundred faecal samples were analysed, but only those isolates that satisfied all primary (oxidase and the triple sugar iron) and secondary identification criteria (API 20E) for *E. coli* are reported. A total of 294 *E. coli* isolates were further analysed for *E. coli* O157 characteristics by the slide agglutination test with *E. coli* O157-specific monovalent antiserum. Seventy-six *E. coli* O157 isolates were positively identified. In this group of isolates, the prevalence was higher in pigs (44–50%) than in cattle (5.4–20%) and humans (7.5%). Within the pig isolates, the prevalence was higher in the commercial (51%) than in the communal (44%) pigs. Similarly, the prevalence was higher in faeces from commercial (14–20%) than communal cattle (5.4%). This study highlights the need for correct hygiene, especially with commercial farming of cattle and pigs, and the processing of their products. Through this precaution, prevalence of *E. coli* O157 in farm animals can be minimized, preventing spread from animals to humans.

Introduction

Escherichia coli O157:H7 has been identified as an important pathogen that causes disease in humans in developing countries, particularly in the southern hemisphere, including South Africa.[1] A causative factor is the faecal contamination of food.[2] Undercooked or ready-to-eat food items have been identified as a reason for some outbreaks.[3] This pathogen can also be transmitted by person-to-person contact, and by drinking contaminated water.[4] The prevalence of *E. coli* O157 in humans depends on the frequency and severity of exposure to this pathogen.[5] *E. coli* can be the cause of diseases in humans that range from diarrhoea to the more complicated haemolytic uraemic syndrome (HUS) and haemorrhagic colitis (HC).[6] The disease mechanism is through the production of virulent gene products that include shiga toxins (stx_1, stx_2), enterohaemolysin (*hlyA*) and the attachment and effacing lesion gene (*eae*).[7,8] There is inadequate information published on the prevalence of *E. coli* O157 in faeces of animals, especially pigs,[1,9] in South Africa. While only three cases of human infection by this pathogen are recorded in South Africa, we ascribe this to the fact that patients rarely report their cases to a hospital.[1,10,11] *E. coli* O157 can contaminate meat as a result of poor animal management practices, transmitting the pathogen to humans. We report on the prevalence of *E. coli* O157 in human, cattle and pig faecal samples, and discuss the health risk that it may present to South African meat consumers.

Materials and methods

Sample collection and microbiological analysis

Analytical protocol was as previously reported[12] but with some modifications. In our study, we used MacConkey broth that is generally recommended for the selection and recovery of *Enterobacteriaceae.* Human stool samples were collected from a local provincial hospital in the North West province of South Africa. Pig faecal samples were collected from a communal and a commercial farm in Tlapeng and Mareetsane, respectively. Cattle faecal samples were obtained from a communal farm in Mogosane and two commercial farms in Lichtenburg and Rustenburg, respectively. Animal faecal samples were collected directly from the rectum of animals using sterile arm-length gloves and the material placed in sterile sample collection bottles. Samples were immediately transported on ice to the laboratory for analysis. A loopful of the faecal sample was inoculated into MacConkey broth medium (3 ml) [Biolab, Merck (South Africa)] and incubated at 37°C for 18 to 24 hours. After incubation, a tenfold serial dilution with sterile distilled water was performed, and aliquots (100 μl) of each dilution plated onto Sorbitol MacConkey agar (Biolab, Merck). Plates were incubated at 37°C for 18 to 24 hours. Potential *E. coli* isolates were subcultured onto Sorbitol MacConkey agar and these plates were incubated at 37°C for 18 to 24 hours. Isolates were Gram stained[13] and pure Gram-negative rods were selected for biochemical identification.

Primary and secondary identification tests are described in the Appendix.

Results

Experimental results are all recorded in Table 1. In total, 76 *E. coli* O157 samples were isolated during our study. These comprised 37 and 23 isolates obtained from commercial and communal pigs, respectively, and only three from human stool samples. Similarly, only five and four isolates were identified from the faecal samples of commercial cattle in Rustenburg and Lichtenburg, respectively. Furthermore, only four *E. coli* O157 samples were isolated from the faecies of cattle from the communal farm at Mogosane.

Discussion

Table 1 indicates that the overall prevalence of *E. coli* O157 in pig faeces (44–50%) is higher than that in cattle (5.4–20%) and human stool samples (7.5%). Interestingly, the prevalence of *E. coli* O157 is relatively higher in the faeces samples of animals that have been farmed commercially, rather than communally. The results of our study on the prevalence of *E. coli* O157 are higher than those previously reported.[1,9] In one of these studies, only 10 *E. coli* O157 isolates were from pigs with haemolytic colitis.[9] Similarly, despite the fact that the animals at the Mogosane communal farm were all suffering from diarrhoea, it was surprising that the prevalence of *E. coli* O157 was lower in these animals than in non-diarrhoea commercial animals (Table 1). Commercial farm animals are kept closer together,

Table 1. The prevalence of *Escherichia coli* O157:H7 in cattle, pig and human stool samples.

Sample source	Sample station	No. of *E. coli* O157 isolates	Prevalence (%)
Cattle	Lichtenburg	4	20
	Rustenburg	5	14
Pigs	Mogosane	4	5.4
	Tlapeng	23	44
	Mareetsane	37	51
Human	Mafikeng provincial hospital	3	7.5

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facilitating mutual faecal and other forms of contamination. Animals in the communal (free-range) environment can acquire pathogens through rainfall runoff, or soil contamination, such as by human excreta. The prevalence of *E. coli* O157 in pig faeces contradicts the view that pigs are not potential hosts to these pathogens.[16] The greater incidence of *E. coli* O157 in pig faeces over cattle faeces may be attributable to differences in the hygiene management practices of the respective animals. A high market demand for pork in South Africa amplifies the risk that diseased animals pose to human health.

Cattle, rather than pigs, have been identified as the main reservoir of *E. coli* O157 strains, due to the higher pH in the gastro-intestinal tract[9,17] of cattle, compared with pigs. Our results contradict these findings, as we found that the prevalence in cattle is lower than in pigs. Nonetheless, our results are in agreement with other published data of 7.5–8% in adult cows.[18,19] The higher prevalence of *E. coli* O157 in faecal samples from commercially farmed animals stresses the need for improved hygiene practices on farms. The faecal shedding rates of *E. coli* O157 by animals, especially cattle, change with season.[20] Our study does not take account of this variable.

The prevalence of *E. coli* O157 in humans can be linked to the degree of exposure to contaminated animal products. Poor animal hygiene has been identified by others as the contributor to human infections.[2,7] Our study shows that only three cases of *E. coli* O157 were diagnosed from isolated stool samples of patients who visited the hospital with diarrhoea. The reason we give is that few diarrhoea patients visit the hospital. Diagnosis of *E. coli* O157 diarrhoeal cases can also be hampered by the possible presence of other diarrhoea-causing pathogens.[21] *E. coli* O157 was found to be present in a small proportion (7.5%) of the human stool samples analysed and can be the causative agent of diarrhoea in these cases. Our study did not investigate the role that pigs and/or cattle might play in the transmission of *E. coli* O157 to humans in North West province. The results obtained in this study highlight the need for responsible hygienic practices to control the incidence of *E. coli* O157 in farm animals and prevent its spread from animals to humans. More research is needed that would involve sampling animal faecal samples concurrently with meat products, water samples and human stool samples, so as to identify clearly the direct roles that animals play in the transmission of these pathogens to humans. This may facilitate the identification of the source of contamination during outbreaks of infections.

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Appendix

Primary identification tests.

Oxidase test

This test was performed using the TestOxidase™ reagent (PL.390) from Mast Diagnostics (Neston, Wirral, U.K.) in accordance with the manufacturer's published protocol. A well-isolated pure colony was placed on a filter paper using a sterile wire loop. A drop of the TestOxidase™ reagent was added onto it and mixed. After 30 seconds, the filter paper was observed for colour change. Isolates that produced a purple colour were presumptively considered to be *E. coli.*

Triple sugar iron test

Triple sugar iron (TSI) agar obtained from Biolab, Merck (South Africa) was used to assay *E. coli* content,[14] with the substrates glucose, sucrose and lactose at sample concentrations of 0.1, 1.0 and 1.0%, respectively. Results are interpreted as before.[15]

Secondary identification tests.

API 20E test

The API 20E test was performed in accordance with the manufacturer's protocol (BioMérieux, Marcy l'Etoile, France).

Serotyping

Escherichia coli isolates were further analysed for characteristics of *E. coli* O157 by slide agglutination test with *E. coli* O157-specific monovalent antiserum (Mast Diagnostics, Neston, Wirral). *E. coli* O157:H7 (ATCC 43889 and ATCC 43888) strains were used as positive controls.

Haemolysis on blood agar

Haemolytic activity was determined by culturing on blood agar, supplemented with 5% (v/v) sheep blood.[8]