Molecular characterization of shiga toxin producing *Escherichia coli* isolated from calves

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figures **Title** **page**

Summary

Shiga toxin producing *Escherichia coli* is a contaminant of food and water that in humans causes a diarrheal syndrome followed by more severe disease of the kidneys and an array of symptoms of the central nervous system. The isolation of Shiga toxin producing *Escherichia coli* (STEC) from diarrheic and apparently healthy calves is problematic due to the lack of differential phenotypic characteristics from non pathogenic *Escherichia coli*. The development of molecular reagents capable of identifying both toxin and serogroup specific genetic determinants holds promise for a more comprehensive characterization of stool samples and isolation of STEC strains. In this study, 300 stool samples from diarrheic and apparently healthy calves were screened for STEC using PCR targeting Shiga toxin determinants. In addition, routine culture methods for isolating O157 and non O157 STEC were also performed. The screening assays of serotyping isolates revealed detection of 7 (4.1%) of O157H7, 156 (92.8%) of non O157 and 5 (3.1%) for Un typable strains. These included serotypes of O157H7 and O26 from diarrheic sample, while detected O78, O55 and O126 from apparently healthy calves.

17 isolates of *Escherichia coli* were tested by PCR for detection of 4 genes included shiga toxin genes (***eae*A**,***stx*1**,***stx*2** and ***hly***), results showed detection of 8 genes of ***eae*A** with percentage of (47.1%), 5 genes of ***stx*1** with percentage of (29.4%) while 6 genes of ***hly*** with percentage of (35.3%) and ***stx*2** gene could not be detected.

Multiple drug resistance patterns among the 5 STEC (***stx*1**) for detected ***Aada*1**, ***TeT*(A)** , ***sul*1** and ***dfr*A** genes by PCR showed in the present study in Table (25) that ***Aada*1** gene was detected in all 5 STEC (***stx*1**) strains with percentage of (100%) which highly resistance to gentamycine and streptomycine while detected ***TeT*(A)** gene in 3 STEC (***stx*1**) strains with percentage of (60%) which resistance to Tatracycline and dected ***sul*1** gene in 1 STEC (***stx*1**) strains with percentage of (20%) , which resistance to slupha and detected ***dfr*A** gene in 1 STEC (***stx*1**)

strains with percentage of (20%) which Resistance to Trimethoprime from tested samples.
The high rate of STEC isolation and the diversity of STEC serogroups described here in point to calves as important reservoirs of STEC in our setting. A prevalence of STEC in both diarrheic and apparently healthy calves.

Escherichia coli serogroups associated with human disease may indicate a source of potential public health risk in our community. The presence of virulence associated traits in these isolates is currently being investigated.
The partial coding of gene sequencing of shiga toxin gene in the five yielded PCR positive shiga toxin producing *E. coli* strains done in elim biopharmaceuticals (California,USA)and deposited in Gene Bank under the following numbers :

1- M K 950774 *E. coli* O157H7 (AM1) Egypt.
2- M K 950775 *E. coli* O157H7 (AM2) Egypt.
3- MK 950776 *E. coli* O55 (AM3)Egypt.
4- M K 950777 *E. coli* O126(AM4)Egypt.
5- MK 950778 un typable *E. coli* (AM5)Egypt.

Nucleotide identity percentage was 100%between *E. coli* O 157H7(AM1)(Accession no. MK 950774)and the un typable *E. coli* strain(AM5)(Accession no. MK 950778).
Also , nucleotide identity percentage was 100%between *E. coli* O55(AM3)(Accession no. MK950776)and *E. coli* O126(AM4)(Accession no. 950777).
And the nucleotide identity percentage was 99.8%between *E. coli* O157H7 (AM1)(Accession no. MK 950774), un typable *E. coli* strain(AM5)(Accession no. MK 950778), *E. coli* O55(AM3)(Accession no. MK950776) and *E. coli* O126(AM4)(Accession no. 950777).

Conclusions:

It is clear that STEC are highly pathogenic and that virulence is not dependent on a single gene or gene product but is a multi factorial process.

The organism can survive in water and a wide range of foods including acidic products and then remain viable after transit through the acidic environment of the stomach. Colonization of the bowel, mediated by one or more of a range of potential adhesions

In conclusion, the high rate of STEC isolation and the diversity of STEC sero groups described here in point to calves as important reservoirs of STEC in our setting. A prevalence of STEC in both diarrheic and apparently health calves revealing its important role of these animals as areservoir of potentially pathogenic *E. coli* in humans. *Escherichia coli* sero groups associated with human disease may indicate a source of potential public health risk in our community. The presence of virulence associated traits in these isolates is currently being investigated.

Knowledge of the prevalence of non O157 STEC in various sources is essential to design effective intervention strategies to prevent food borne illness outbreaks in humans. There are several studies reporting the prevalence of non O157 *E. coli* serogroups in cattle feces.