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The Prevalence of Verocytotoxin-Producing *Escherichia coli* O157 (VTEC) in Dairy Cattle in Tripoli Area, Libya

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ABSTRACT

Infection with verocytotoxin-producing Escherichia coli O157 in humans can lead to mild or bloody diarrhoea with the haemolytic uremic syndrome (HUS) as possible complication. Cattle appear to be important reservoir for VTEC O157. Epidemiologic studies on the prevalence of VTEC O157 in dairy cattle in Libya have never been conducted. This information is important to develop a quantitative risk assessment model for the consumption of dairy products and meat. Hence the main objective described in this paper is to investigate the prevalence associated with VTEC O157 on dairy farms in Tripoli region. Faecal samples from 200 apparently healthy cows were collected once from 15 randomly selected dairy farms. All fecal samples were examined for VTECO157 by conventional plating using Sorbitol-MacConkey agar (SMAC). Isolated of E. coli were subjected to latex agglutination test. A structured questionnaire was used to collect information on the animals and farms. The results pointed out that, prevalence within-herd (individual) and among herds were 9% and 60% respectively. The highest prevalence of VTEC was in Janzour area (12.5%). and the lowest was in Ein Zarrah area (6.5%). Aiming at reducing risks for human by intervention at farm-level, it is of importance to reduce the number of positive animals for this, more research is needed to devise mitigation strategies that reduce farm contamination of VTEC.

Keywords: ***VTEC, Libya, E.coli.***

INTRODUCTION

Escherichia coli are normal inhabitants of the gastrointestinal tract of all warm-blooded animals, but variants of this species are among the important etiological agents of enteritis and several extra-intestinal diseases. The *E. coli* strains that cause diarrheal illness are categorized into pathogenicity groups based on virulence properties, mechanisms of pathogenicity, clinical symptoms and serology. The six main categories include enterotoxinogenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAggEC), enteroinvasive *E. coli*, (EIEC), diffusely adhered E.coli (DAEC), and Enterohaemorrhagic *E.coli* or Shiga (Vero) toxin-producing *E. coli* (STEC/VTEC). From a zoonotic point of view, STEC is the only *E. coli* pathogenicity group of major interest, as the shiga toxin-producing strains are able to cause severe disease in humans when being transmitted through the food chain from their animal reservoirs.

Epidemiological investigations revealed that cattle frequently harbour VTEC in their faeces and thus may represent an important source of infection. Numerous studies [1-5], have indicated that the organism is common in both dairy and beef herds, with a prevalence of up to 75% in dairy herds [6] and 63% in beef herds [7]. The prevalence for individual animals within herds in different parts of the world is estimated at 1.8 to 16%, with levels as high as 36% being reported [8-10]. Vero-toxin-producing Escherichia coli (VTEC) are emerging as a significant source of food-borne infectious disease all over the world. Illness caused by VTEC can range from self-limited, watery diarrhea to life-threatening manifestations such as hemorrhagic colitis, hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP) and death.

VTEC can potentially enter the human food chain from a number of animal sources, most commonly by contamination of meat with feces or intestinal contents

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after slaughter or cross-contamination of unpasteurized milk and milk products. Consumption of raw milk and ground beef has been linked epidemiologically with several outbreaks of disease caused by E. coli O157:H7. Such risks emphasize the importance and the need to develop long-term strategies to assure safety of foods from dairy cattle. Knowledge of the prevalence of VTEC in the faeces of cattle and the associated risk factors are important, because it may lead to intervention strategies before slaughter and during processing that will reduce the risk of bacterial contamination of the carcass. To our knowledge, epidemiological survey on the prevalence of VTEC O157 in dairy cattle farms in Libya had never been undertaken. Therefore, as a contribution to the understanding of the epidemiology of VTEC infection in livestock in Libya, we focused our work on cattle as a possible reservoir of these strains.

In this study, fecal samples were collected from dairy cattle from 4 areas in Tripoli, in an attempt to estimate the frequency of VTEC carriage in apparently healthy cattle herds in the city. The aim of study present in this paper is to assess the role of dairy cattle in human infection with Vero-toxin producing *Escherichia coli* (VTEC) by assembling database on the prevalence of VTEC in dairy herds. These data will be used as a part of our effort in quantitatively assesses the health risks to humans attributed to the consumption of dairy products which might be contaminated with VTEC.

METHODOLOGY

Study Area

Tripoli city is located in the north-western part of Libya, in an area along the seashore. Geographical coordinates of the location are latitude 32o: 56' North and longitude 13o: 10' East (Report of Engineering Consulting Office for Utilities/ Tripoli, 2000). Its climate is influenced by the Mediterranean Sea and characterized by relatively high rainfalls. In the autumn-winter season, between October and April, the rainfalls, on the average, amounts to 379 mm, whereas the remaining months are almost rainless. (Report of Engineering Consulting Office for Utilities/ Tripoli, 2000).

Study Design

A cross-sectional study was conducted in selected areas of Tripoli to determine the prevalence of VTEC O157 in dairy cattle farms .The study was carried out through the period from June to September 2010.

Sample Size and Sampling Methodology

The individual and herd sample sizes were calculated based on the estimated prevalence which previously published in different epidemiologic studies worldwide. For individual animals, the sample size was estimated by using 95% confidence interval level with an expected prevalence 15% VTEC O157 and with desired absolute precision of 5%. For herd, the sample size to be sampled from the target population was estimated with an expected herd prevalence of 75% and specified precision of 20% of the true prevalence with 95% certainty. Because of unavailability of cattle census (sampling frame) in the study area, a convenience sampling was used to select herds from the different regions. Then, a cluster sampling procedure as described by (Thrusfield, 1995) was applied by selecting all animals within the selected herds. Accordingly, a total of 204 animals should be sampled from 18 herds. For technical reasons a total of 200 cows from only 15 herds in 4 areas in Tripoli were examined (Table1)

Farm Visits and Sample Collection

Two-hundred fecal samples were collected from 15 selected dairy farms in Tripoli area. Animals were restrained in self-locking stanchions and approximately 100 g of fecal materials were rectally obtained by the use of sterile gloves changed for each animal to avoid cross-contamination. Fecal samples were collected in sterile plastic bags, stored in a cool box, and transported to the laboratory and analyzed within 4-6h after collection. In Laboratory, cross-contamination was minimized by washing (with water at approximately 50°C) the processing bench.

Table1: Regions, herd size and number of animal tested

Region	No. of herds	No. of animal tested
Engeala & Alsayied	6	38
Janzour	2	8
Khallet Alfergan	5	108
Ain Zarrah	2	46
Total	**15**	**200**

Epidemiologic Data collection (Questionnaire design)

A pre-tested questionnaire was developed to gather information on individual animal variables (e.g., age, sex, signs of diarrhoea…etc.) and herd level data (herd size, husbandry practices. etc.). All data were acquired from animal owners. A limited number of questionnaires were used as pilot-forms to examine its functionality before the start of the real study. Adjustments were made and the questionnaire was therefore used routinely.

Microbiology analysis

Selective enrichment and isolation of VTEC O157.

On arrival at the laboratory, each fecal sample was mixed, and 10 g of faeces was removed and put into 90 ml of Buffer Peptone Water (PBW) universal pre-enrichment broth (Difco, Inc., Detroit, Mich.) and incubated at 37°C for 18 to 24 h. Then, the tube was vortexed, and a swab sample was plated onto sorbitol-MacConkey agar (SMAC; Oxoid Ltd., Basingstoke, England), plate was streaked for isolation and incubated at 37°C for 18-20 h.

Biochemical confirmation

Isolates of O157 were confirmed by inoculated into an API20E strip (bioMerieux, Lyon France) for identification according to the manufacturer's specifications.

If the API strip identified the isolate as *E. coli*, then the isolate was inoculated onto semisolid nutrient agar and incubated at 37°C for 18 to 24 h. The isolate was inoculated onto Protect beads (Key Scientific Products, Round Rock, Tex.) according to the manufacturer's specifications and frozen at -70°C for long-term storage and for more confirmation.

Identification by Latex agglutination is by the combination of bacterial culture and latex agglutination (C/LA) was performed as follows. After incubation, sorbitol-nonfermenting colonies (up to 5 suspected colonies per sample, colorless (D-sorbitol-negative) colonies.) were picked and inoculated into nutrient agar and incubated at 37°C for 18 to 24 h for confirmation. After incubation, isolated colonies were tested for agglutination with an *E.coli* O157 latex test kit (bioMerieux, Lyon, France) see (Figure 1).

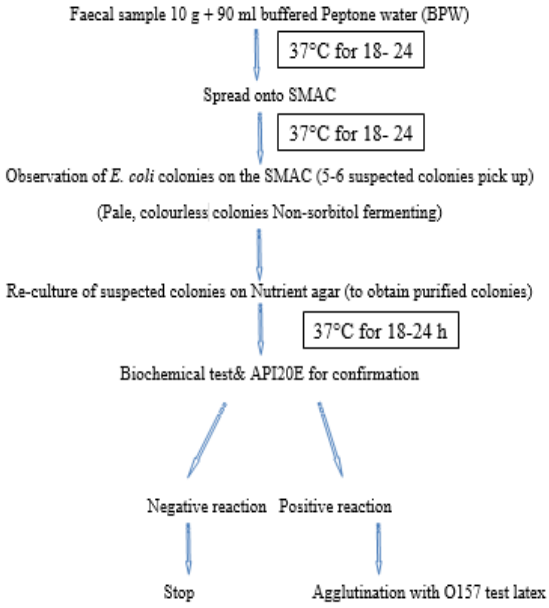

Figure 1: Selective enrichment and isolation of VTEC O157

Statistical Analysis

The data were analysed by chi-square (X2 –test) and Fisher's exact test using the statistical software package SPSS (version 17.0) (SPSS Inc., Chicago, IL, USA). The individual animal prevalence (within-herd prevalence) was calculated by dividing the number VTEC O157 positive animal by the total number of faecal samples tested. herd prevalence was calculated by dividing the O157 herd positive (herds were classified as VETC O157 positive if at least a single positive animal within the herd was detected) by the total number of herds tested.

RESULTS AND DISCUSSION

Herd and within-herd prevalence

A total of 15 dairy farms were visited once during the study period (Table 4) compromising 200 animals. Most of animals observed were apparently healthy. Among the studied dairy cattle, 60% of herds (intra-herd) were positive for VTEC O157 (one or more animals positive/herd) and 9% of individual animal (inter-herd) were positive for VTEC O157 (Table2) (Table 3) (Table4) (Figure 2, 3&4).

Table 2: Herd and within-herd prevalence of VTEC O157 in faeces of dairy cattle in Tripoli area

Prevalence	Positive O157 VTEC/n (%)	95% CI
individual(inter-herd)	18/200 (9)	(1.5% to 18.8)
Herds (intra-herd)	9/15 (60)	(40.5 to 82.6)

Table 3: Prevalence of VTEC O157 in feces of dairy cattle in 4 regions, Tripoli, Libya

Region	Herd tested	Animal tested	Animal positive (%)	95%CI	X^2	P-value
Engeala &Elsayied	6	38	4 (10.5)	2.6-27.5		
Janzour	2	8	1 (12.5)	1.6-38.5		
Khallat Elferjaan	5	108	10 (9.2)	2.0-20.8	0.6	0.4
Ain Zaara	2	46	3 (6.5)	3.0-24.0		
Total	**15**	**200**	**18 (9)**	**2.5-21.7**		

Table 4: Prevalence of VTEC O157 in faeces of dairy cattle and sampling time.

Farm	Sampling time	No. of animal tested	No. positive (%)
1	July 2010	6	1(16.6)
2	July 2010	4	0(0)
3	July 2010	6	1(16.6)
4	July 2010	3	0(0)
5	August 2010	7	0(0)
6	August 2010	5	1(20)
7	August 2010	10	6(60)
8	August 2010	66	3(4.5)
9	August 2010	5	2(40)
10	September 2010	10	0(0)
11	September 2010	16	0(0)
12	September 2010	10	0(0)
13	September 2010	40	2(5)
14	September 2010	6	1(16)
15	September 2010	6	1(16.6)
Total		200	18 (9)

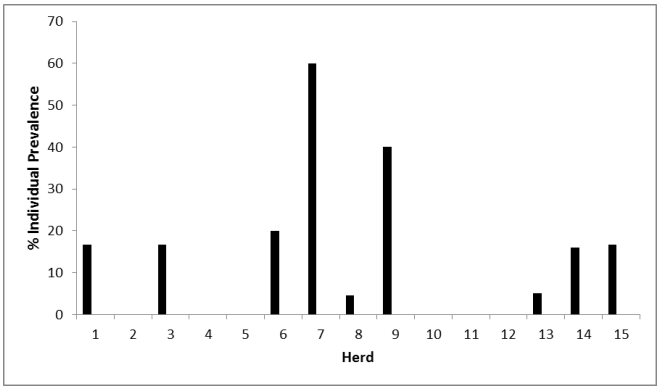

Figure 2: Individual and herd prevalence of VTEC O157 of 15 dairy cattle herds, Tripoli, Libya

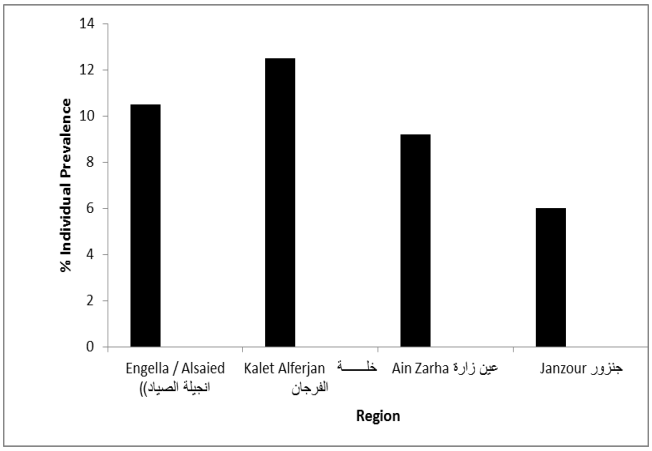

Figure 3. Individual prevalence of VTEC O157 in feces of dairy cattle in 4 regions, Tripoli, Libya.

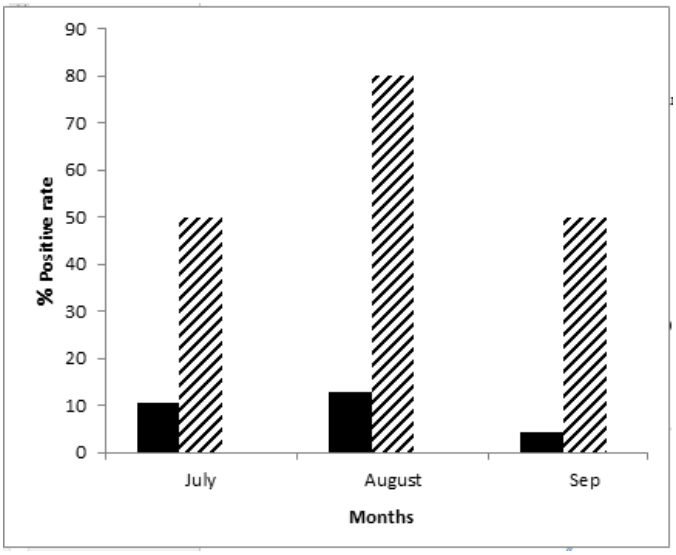

Figure 4: Individual and herd prevalence of VTEC O157 in dairy by month

The study was designed with the estimation of the prevalence of VTEC O157 in dairy cattle as a central goal. Two hundred individual fecal samples were collected from 15 dairy herds located in 4 regions of Tripoli area, Libya; in order to provide a reasonably precise and unbiased estimate of individual and herd prevalence of VTEC O157 in dairy cattle. To our knowledge, this is the first epidemiological survey on the occurrence of these pathogenic strains in apparently healthy dairy cattle in Libya. The study showed that VTEC O157 is present in all study areas with an individual and herd prevalence of 9% and 60%, respectively. This prevalence of VTEC in apparently healthy cattle is in the range of what has been reported in other countries where studies have been performed on fecal samples from cattle farms [11-13]. However, the ability to compare published prevalence studies is difficult due to variations in study population, sampling, and culture technique. Zhao et al. (1995) in china reported the finding of VTEC on 50% of dairy cattle farms, in 5.3% of cattle aged two to four months and in 2.9% of animals under the age of two months [14]. In Australia [15], detected O157 in 10% of fecal samples from grazing cattle and in 15% of fecal samples from feedlot cattle. In The Netherlands, 0–61% of dairy cattle carried O157 VTEC. The highest shedding prevalence was found among cattle aged three to 12 months [16]. In Scotland, 6.5% of cattle aged 12 to 30 months had O157 in feces, and 22.7% of farms were positive for O157 [17]. In Italy, VTEC O157 was detected in feces from 42% [18] and 24% of slaughtered cattle [19]. Oporto et al. in Spain detected VTEC O157:H7 and non-O157 in 3.8% and 35.9% of cattle herds, respectively [20]. Data from Norway showed the finding of VTEC O157 in up to 75% of all animals examined [21]. Cizek et al. isolated VTEC O157 from 2% of milk filters on 192 dairy farms in the Czech Republic [22].

Previous researches have suggested that almost all dairy farms will have cattle testing positive for VTEC O157 if screened often enough [7]. In the present study, we found a wide range in the prevalence of dairy herds shedding VTEC O157 (0–60%). Previous research has similarly shown a high degree of variation in fecal shedding of VETC O157 in dairy cattle, with reports ranging from 0.9 to 75% [23, 6]. Sampling techniques and culture methods might be contributed to the observed differences.It is not clear why dairy herds would be more likely than beef herds to harbor the organism, although epidemiologic evidence to date suggests that dairy animals may be the primary reservoir. The relatively high prevalence of VTEC strains found in cattle investigated in this study poses the question of whether humans are at risk of acquiring these infections. In the present study, using identical sampling and culture technique on multiple farms, we demonstrated differences that may occur in the fecal shedding of VTEC O157, not only between farms but also within a single dairy farm. Reasons for the high degree of variation in pathogen shedding from farm to farm are unknown, but may be related to numerous factors involving farm management, genetics, and nutrition. Management factors (feed types, housing) and seasonal effects associated with pathogen shedding in dairy cattle have been previously examined [24] and shown to have limited effects on pathogen shedding. However, it is important to note that all four regions sampled in this study were located within an approximately 15 Km-radius of one another and cows were housed and fed under similar conditions.

The dairy industry in Libya has been changed substantially within the past 15-20 years. Most dairy farms used to be governmental with a large farm size and large animal concentrations. Nowadays these dairy farms are become private ones, with a relatively small herd size which creating health, environmental and herd management concerns. Furthermore, dairy cattle

contribute a very high percentage of all non-fed beef in Libya, thus are an important vehicle for transmission of foodborne pathogens to humans.

In the present study certain limitations were observed which might affect or bias the apparent prevalence of VTEC O157. One limitation of this study is that the prevalence estimates may have been slightly biased, because herds were chosen conveniently, rather than randomly. This sampling method gives a larger probability of missing an infected animal (especially in low-prevalence herds). Another potential limitation of this study is that each farm was only visited once for collecting fecal samples which might indicating that strong fluctuations in apparent prevalence can occur. This single-time point sampling, such as the one used in our study, provide only limited information regarding the shedding patterns of VTEC O157. Through this misclassification bias, prevalence might be underestimated. Based on scientific evidence, VTEC O157 are shed intermittently [7], and sequential sampling with an interval (days, weeks, months) to deal with intermittent shedding was not within our study scope but should be advised to do so.

Based on the observations in this study, it can be concluded that VTEC O157 is prevalent in the dairy cattle population in Tripoli area, supporting the epidemiologic evidence that dairy animals may be the primary reservoir of VTEC 0157. However, it should be been noted that the results of fecal culture in on-farm studies may not reflect the true infection status of the tested animals. Scientific evidence suggests that previously infected but culture-negative cattle may harbor *E. coli* O157 and re-excrete high numbers of the organism following dietary change or food deprivation [25,26] and may therefore be important reservoirs of infection on farms. Within dairy herds, control of factors, such as contact between animals, communal housing, watering of animals and manure management may reduce spread of infection.

Numerous studies have shown that cattle and food produced there from, particularly ground beef, are significant sources and vehicles of transmission of this pathogen. Therefore, one might say that VTEC strains remain a serious threat to public health and an appreciable economic burden to the food industry.

CONCLUSION

A thorough understanding of the population dynamics of VTEC O157 at the farm level is crucial before implementation of pathogen reduction strategies can be expected to be successful since intermittent shedding of food-borne pathogens at the animal and farm level contributes to the challenge of pathogen control during the production stage. For this reason, further study should be designed to investigate the influence of animal factors and management factors on transmission and persistence of O157 VTEC in cattle system.

This study has established the presence of high-shedding animals at farm level, providing an increased risk of contamination to both the food chain and the environment. The need for suitable control measures for such animals cannot be underestimated, and future research is needed to devise mitigation (prevention) strategies that will reduce the risk of farm-level contamination as well as gross or major contamination of the food chain or the environment.

DISCLOSURE STATEMENT

Conflict of interest statement was not declared.

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