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The Kinetics of E.Coli O157:H7 from Feces to Soil and Ground Water, on a Dairy Farm Reared under Field Conditions in Baghdad Province and Its Relation to Public Health

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Abstract
The aims of this study were to investigate the kinetics of *E.coli* O157:H7 from faces and its migration through soil into well- water, and the ability of *E.coli* OI57:H7 to persist on/in soil in Iraqi dairy farms reared under entirely different environmental conditions. The prevalence of *E. coli* O157:H7 in fecal, soil surface (Z1, Z5, and Z10) and depth (D0, D5 and D10) and well-water samples from January through May 2012 were determined. Colonies isolated from fecal, soil, and water samples with morphological characteristic of *E.coli* O157: H7 on CT-SMAC, CHROMagar, were further confirmed as *E.coli* O157: H7 by biochemical reaction, and subjected to agglutination reaction to identifying the O157 somatic and H7 flagellar antigens. Of 32 fecal samples collected over 4 weeks sampling period, 24 (75%) were positive for *E.coli* O157:H7. Of 63 soil samples collected during the period of the study 43 (68%) were positive for *E.coli* O157:H7 in all directions of the farm. The prevalence of *E.coli* O157:H7 contamination transferred to the interior of soil decreased with increased depths, this correlation is highly significant. A total of 45 well-water samples were collected during April and May, 23 (51%) were found to be *E. coli* O157:H7 positive. The highest prevalence was in April (63%) compared to May (33%). A total of 140 samples (fecal, soil, and water) were collected in this study, 90 (64%) were found to be *E.coli* O157:H7 positive. Fecal samples had higher prevalence (75%) than soil and water (68% and 51%) respectively. In conclusion, the results of this study demonstrated that *E. coli* O157:H7 can persist for extended periods of time in the faeces, soil, and well-water in the dairy farm reared under natural conditions. This is important because it suggests that these may contribute to the dissemination of *E. coli* O157:H7 on food products intended for human consumption.
Keywords: *E.coli* O157:H7, dairy farm, kinetics, environmental pollution, public health

1. Introduction

Escherichia coli O157:H7 is a major public health concern. It is associated with human illnesses ranging from uncomplicated watery diarrhea to haemorrhagic colitis (HC) and hemolytic-uraemic syndrome (HUS), which may result in death (Mankin, et.al. 2007; Nicholson, et.al. 2005). Cattle are regarded as the major reservoir of *E.coli* O157:H7 linked to human infection (Berry, and Miller 2005). Cattle can shed the *E.coli* O157:H7 into environment by means of feces (Nicholson, et.al. 2005). *E.coli* O157:H7 can survive in feces, soil and water and animal feces and irrigation water are the main avenues for the spread of human pathogens to field and the crops growing there (Fermium, et.al., 2008). Soil is contaminated due to direct shedding of *E.coli* O157:H7 onto pasture land by animals, especially cattle and sheep (Islam, et.al. 2004; Fremaux, et.al. 2008). *E. coli* O157:H7 is able to move through the soil profile with water after rainfall or irrigation and can even reach the groundwater (Lang and Smith, 2007). The movement and distribution of *E. coli* O157:H7 in soil after application of manure and slurry are still unclear. It is also not clear if and how survival of enteric pathogens is influenced by the depth of the soil where they end up after transport through the soil (Gagliardi and Karns, 2000). However much work has been done already but it is often not appreciated that system involving experimental movement and distribution of E. coli O157:H7 data originate from the laboratory - conditions differ in many respects from the commercial situation. Survival of *E.coli* O157:H7 in different conditions can be influenced by various factors such as temperature, moisture content, pH, and nutrition. This study was designed to investigate the kinetics of *E.coli* O157:H7 from feces and its persistence, distribution, and migration through soil into ground water.

2. Materials and methods

2.1. Description of the farm:-

The farm studied is situated in the college of Agriculture /University of Bagdad. The herd comprised about 83 Holstein-Friesian cows aged from 2 to 5 years and calves at different age of rearing. The animals were housed indoors throughout the year in separate pens. The pens had a concrete floor, and manure and urine were removed manually and used as a fertilizer on nearby crops. There are three wells in this farm, one situated inside, and 2 outside the farm (approximately 25 to 40 meters away from the farm). These wells were used to irrigate the crops surrounding the farm and to wash the concert floors of the pens. The prevalence of *E. coli* O157:H7 in fecal, soil and ground water samples were determined from January through June 2012.

2.2. Study Design

The surface kinetics of *E.coli* O157:H7 onto the soil (surface kinetics), were theoretically achieved by dividing the farm into 3 zones starting from the fence (Z1),and the isolation of *E.coli* O157:H7 were monitored in 5, and 10 m (Z2, and Z3, respectively) from the farm (Fig.1).While the depth kinetics were achieved by taking soil samples from the surface (D0), and at depths of 5, and 10 cm (D5, and D10 respectively) from each zone in all directions. Water samples were collected from the wells immediately after pumping water from the wells at zero time, 5 and 10 minutes (T0, T5, and T10 respectively).

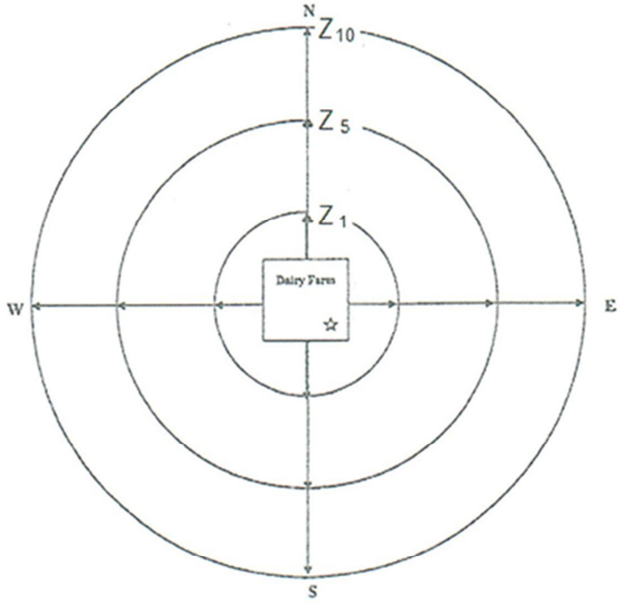

Figure 1 Surface kinetics of *E.coli* O157:H7 onto farm soil

2.3. Collection of samples:-

A maximum of 10 fecal samples of different animals were collected on a weekly basis from 10 pens (cows and 2 bulls). Nine soil samples (100g) were collected in plastic bags for each distance of 1, 5, and 10m from the farm for each depth of zero (surface), 5 and 10 cm at weekly basis. Three water samples (100ml) were collected in a100ml polystyrene containers at weekly basis from each well on zero, 5 and 10 minutes after water pumping.

The samples were kept at 4°C with ice during transportation to the College of Veterinary Medicine /Department of Veterinary Public Health for microbiological analysis.

2.4. Isolation and Identification of *E.coli* O157: H7

Colonies isolated from fecal, soil, and water samples with morphological characteristic of *E.coli* O157: H7 on CT-SMAC, CHROMagar, and nutrient agar were further confirmed as *E.coli* O157: H7 on EMB agar and biochemical reaction (Berry and Miller 2005), and subjected to agglutination reaction to identify the O157 somatic and H7 flagellar antigens (Feng, et.al., 2002; USDANRCS, 2002; and AL-Zubaidi, 2009).

2.5. Data analysis:-

Two sample t-test between percents, one-way analysis of variance (ANOVA) and Pearson's product-moment correlation coefficient (R) and the regression coefficients (R2) with a 5% significance level were used using the computer software by David S. Walonick, Ph.D. (Copyright © 1996-2010, StatPac Inc.).

3. Results & Discussion

3.1. Prevalence of *E.coli* O157:H7in cattle feces.

Of 32 fecal samples collected over 4 weeks sampling period, 24 (75%) were positive for E.coli O157:H7. The highest week prevalence (100%) was recorded in the 4th week, and the second highest peak (80%) was recorded in the 2nd week followed by 70% and 63% in the 3thrd, and 1st week, respectively. Overall, no significant differences in the prevalence of *E.coli* O157:H7 were observed between weeks (Table 1). The results also revealed that there were no significant differences in the prevalence of *E.coli* O157:H7 between pens occupied by bulls (pens 9 and 10) and pens occupied by cows.

Most studies report farm level prevalence of *E.coli* O157: H7 infection to be highly variable, typically comprising sporadic outbreaks, occasional high prevalence, and periods of apparent absence (Nataro and Kasper,

1998; Synge et. al., 2003). Factors that might lead to such fluctuation or heterogenesities include variability in infectiousness, exposure, genetic susceptibility, contact rates, and behavior (Boelle, *et.al.* 2004). On a pen – level basis, supper shedders (100% prevalence) accounted for 50% of cattle shedding the organism, whereas 0% (n=1), 50 %(n=1), 67% (n=2) and 75% (n=1) in pens 4, 6, (5and 8), and 3 respectively (Table 1). Considering the intermittent nature of E.coli O157: H7 shedding (McAllister Et.al. 2006), it is possible that more cattle than were identified may have shed at the level of super shedders. It has been suggested that, period of the highest fecal E.coli O157: H7 prevalence appeared as sporadic events in individual pens (Mechie et. al., 1997; Midgley et. al., 1999). The pens included in this study were relatively close together, which may have facilitated transmission and persistence of *E.coli* O157: H7 within the study group. On the other hand, the pen–fecal E.coli O157: H7 prevalence (100%) detected in pens occupied by bulls (pen 9 and 10) did not differ from the pens occupied by cow (pens 1, 2, and 7). Since all pens were located on the same farm, it is unlikely that climatic changes had a significant impact on the prevalence of *E.coli* O157: H7 between pens. These results are in agreement with Matthews, et.al. (2006) who suggest that transmission rates of *E.coli* O157: H7 may vary by region, climate, season, or management factors.

The overall prevalence of *E. coli* O157:H7 reported in fecal samples was very high (75%; 24/ 32) compared to the prevalence nationwide surveys. (Shere et.al., 1998; Chase- et.al., 2007; Masana et.al., 2010). However, some study yielded very low prevalence data (Albihn et. al., 2003). The reasons for this discrepancy in the prevalence of *E.coli* O157: H7 nationwide may reflect either scarcity of the bacteria in excreta and environment resulting from the sporadic nature of *E.coli* O157: H7 carriage and low number of the bacteria residing in colonized animals or insufficiently sensitive sampling and culturing techniques. One reason for the higher prevalence rate of *E.coli* O157: H7 in this study could be the use of highly selective media (CHROMagar and CT–SMAC) to detect the pathogen in an environment with competitive flora.

Table 1 Prevalence of *E.coli* O157:H7 in cattle feces during the first 4 weeks of the study at the dairy farm.

Pen	Sample collected at Week				Total No.+ve/Total Samples tested	Prevalence (%)
	1	2	3	4		
1	+	+	+	+	4/4	100
2	NA	+	+	NA	2/2	100
3	+	-	+	+	3/4	75
4	-	-	-	NA	0/3	0
5	-	+	+	NA	2/3	67
6	-	+	-	+	2/4	50
7	+	+	+	NA	3/3	100
8	+	+	-	NA	2/3	67
* 9	+	+	+	NA	3/3	100
* 10	NA	+	+	+	3/3	100
Total No.+ve/Total Samples tested	5/8	8/10	7/10	4/4	24/32	
Prevalence (%)	63	80	70	100	75	

NA = Not available (feces were removed from the pens).

* = Bull

3.2. Prevalence of *E.coli* O157:H7 in soil at different directions, distances, and depths

Of 72 soil samples collected during the period of the study 48 (67%) were positive for *E.coli* O157:H7 in all directions (R, L, and Re) of the dairy farm. The highest prevalence was recorded at the L-side (left) of the farm (79%), followed by Re (Rear)-sides (67%) and the R (Right) of the farm (54%). Overall, no significant differences in the prevalence of *E.coli* O157:H7were observed among L×R, or L× Re. The results further demonstrated that the prevalence of *E.coli* O157:H7 in the soil at different distances (Z1, Z2, and Z3) of the farm were 52% (14/27), 63% (17/27) and 94% (17/18) for Z1, Z2, and Z3 respectively. Overall, there was a significant differences ($P<0.05$) between Z1and Z3 or Z2 and Z3 (Table 2).

The overall prevalence of *E.coli* O157:H7 at different depth (0, 5, and 10 cm) irrespective of the directions (L, R, and Re) and distances (Z1, Z2, and Z3) were illustrated in Fig. 2. The highest prevalence was recorded on the surface (0-depth, 85%), followed by 5cm (67%), and 10cm (39%). The prevalence of *E.coli* O157:H7 contamination transferred to the interior of soil decreased with increased depths ($r = –0.97$) of penetration, this correlation is highly significant ($R^2 = 0.94$). Comparing the prevalence of *E.coli* O157:H7 between the surface (0 cm) and 5cm revealed no significant differences, but significant differences were recorded between surface (0cm) and 10cm, or between 5cm and 10cm ($P<0.05$).

Soil and more generally the environment is one of the main pathways of *E.coli* O157:H7 human infections, and a

trend of environmental outbreaks outnumbering burger–outbreaks are actually observed (Strachan, et.al. 2006). Many studies have reported differences in *E.coli* O157:H7 soil survival rates according to diverse experimental conditions, these experiments were mostly conducted in climate–controlled laboratory or greenhouses. Therefore, the data might not reflect the survival rate of *E.coli* O157:H7 in soil under fluctuating weather that would be seen in a commercial Dairy farm (Jiang *et. al.,* 2002; Smith, et.al. 2001).

The highest prevalence of *E.coli* O157:H7 in the soil at different directions (R, L, and Re) and at different distances (Z1, Z2, and especially Z3 (94%), could be explained by the fact that *E.coli* O157:H7 may be introduced into the soil through irrigation water contaminated with cattle feces or through contact with contaminated surface runoff from the dairy farm. These results are in agreement with Buck and Walcott (2003) .Further, Fremaux, et.al. (2008) indicated that animal feces and irrigation water are the main avenues for the spread of human pathogens to field and the crops growing there.

.The movement of *E.coli* O157:H7 into the farm soil decreased with increased depths (0cm, 5cm, and 10cm). These results could be explained by the facts that attachment of the pathogens to manure particles in the upper soil layer probably led to reduced percolation to deeper soil layer (Van Elsas, et.al., 1991) .On the other hand, (Trevors, et.al., 1990; Van Elsas, et.al., 1991) have demonstrated that *E.coli* O157:H7 has the potential to survive and move vertically into the soil with time. Saini, and Lorimor, (2003) suggested that microorganisms found in manure prefer to retain in upper layers of soils and because the preferable pore size between soil particles, pH levels, temperatures, soluble organic materials, and available water favor their growth. Also, movement of pathogens from contaminated manure through the soil profile depends on the type of soil, manure physicochemical, and the climate. Further explanation as suggested by Fremaux, et.al. (2007) was that the presence or absence of oxygen in soil may also lead to differences in survival time of entero pathogens. Other studies have reported that micro flora originating from soils exhibit antagonistic interaction with *E.coli* O157:H7 when introduced into the soil as well as when they were introduced into manure amended soils (Jiang, and Morgan, 2002).

Table 2. Prevalence of *E.coli* O157:H7 in soil surrounding the dairy farm at different distances, depths, and directions.

Distances	Z_1 (1m)			Z_2 (5m)			Z_3 (10m)			Total No.+ve/Total Samples tested	Prevalence (%)
Depths / Directions	0	5	10	0	5	10	0	5	10		
R	3/3	0/3	2/3	2/3	0/3	0/3	3/3	3/3	NT	13/24	54
L	3/3	2/3	0/3	3/3	2/3	3/3	3/3	3/3	NT	19/24	79
Re	2/3	2/3	0/3	2/3	3/3	2/3	2/3	3/3	NT	16/24	67
No+ve/No.tested	8/9	4/9	2/9	7/9	5/9	5/9	8/9	9/9	----		
Total No.+ve/Total Samples tested	14/27			17/27			17/18			48/72	
Prevalence (%)	52 B			63 B			94 A			67	

Z=Zone, O=Surface, NT=Not tested (tilled land), R=Right side, L=Left side, and Re=Rear side of the farm

Different uppercase letters in the same row are significantly different ($P<0.05$)

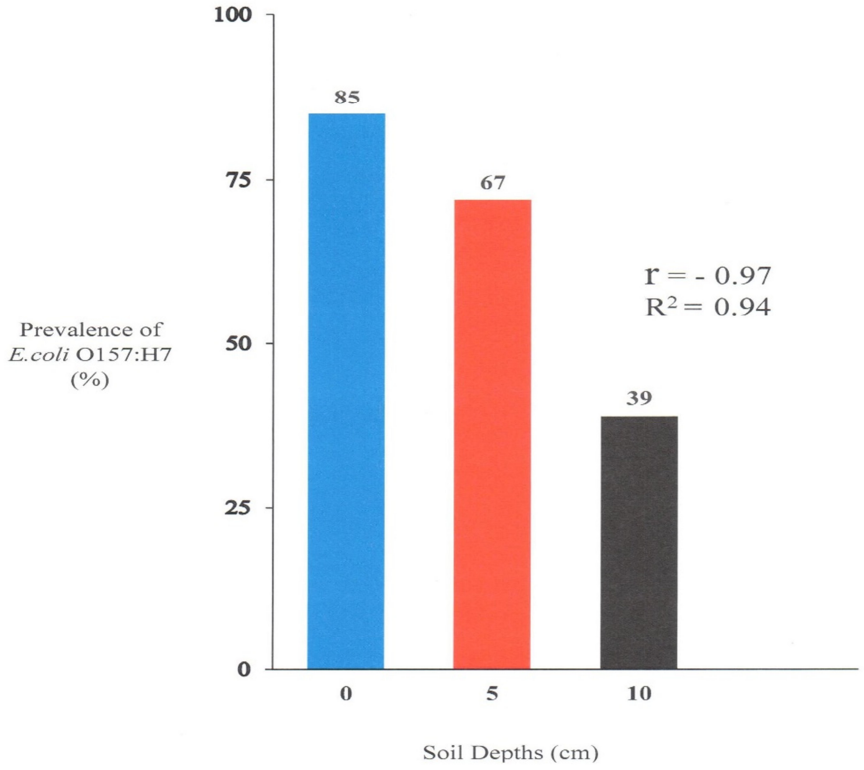

Figure 2 Overall Prevalence of *E.coli* O157:H7 from the soil at different depths (0, 5, and 10 cm) irrespective of the directions and distances.

3.3. Prevalence of *E.coli* O157:H7 in well –water

A total of 45 samples were collected from W1, W2, and W3 during April and May, 23(51%) were found to be *E.coli* O157:H7 positive. The highest prevalence was recorded in W1 (67%) which is located at the R-side of the farm, following by W2 (47%), which is located inside the farm, and W3 (40%), which is located at the L-side of the farm (Table3). Overall, no significant differences in prevalence of *E.coli* O157:H7 were observed between W1× W2, W1× W3, and W2× W3. The prevalence of *E.coli* O157:H7 recorded during April and May is summarized in Table 3. The results showed that the highest prevalence was on April (63%) compared to May, which had a lower prevalence of (33%). This difference was statistically significant ($P<0.05$). Water samples were collected from W1, W2, and W3 on different times (T0, T5, and T10) after water pumping. Of 15water samples collected on T0 (immediately after water pumping), 9 (60%) were positive for *E.coli* O157:H7. While, of 15 samples collected on T5, 6 (40%) were positive for *E.coli* O157:H7, and only 8 out of 15 (53%) were positive on T10. Overall, there were no significant differences between T0×T5, T0×T10, and T5×T10 (Fig.3).

Pathogen release from manure and soil can occur in water as free microbes or in association with manure and/or soil particles (Tyrrel and Quinton, 2003; Dao *et. al*., 2008). Manure contaminated soil and sediment is often implicated as a secondary source of pathogens in flowing water. At the soil surface, pathogen release may occur as a result of raindrop impact and other erosion processes (Boyer, 2008). Many studies have revealed that *E. coli* O157:H7 can survive in water, and numerous human diseases were linked to consumption of contaminated water (Armstrong *et.al.,* 1996; EPA, 2004; EPA, 2009; Scott *et.al.,* 2013). The results of this study (Table 3) showed that the samples of well-water were contaminated with *E. coli* O157:H7 over two months of the study. Although the three wells (W_1,W2, and W3) are situated at different location at the dairy farm ,water samples collected from them yielded high prevalence of *E. coli* O157:H7 (67, 47, and 40 % respectively), this can be explained by the fact that there were high persistence rate of contamination in the fecal and soil which led to the contamination of the well-water either by runoff from agriculture fields after period of rainfall or irrigation, or when animal manure was land applied or when animals have access to crops near the wells (especially wells 1 and 3). Contaminated surface water and groundwater may eventually be diverted or pumped and used as drinking water or irrigation water. (Solomon *et.al,* 2002). These results are in agreements with many studies at different countries where they studied the impact of grazing cattle and land application of manure on the bacteriological quality of runoff and subsurface drainage water (Edwards *et. al*., 1997; Hagedorn *et. al*., 1999)

Of 45 water samples collected over two months sampling period, 23(51%) were positive for *E. coli*

O157:H7. The highest monthly prevalence of 63% was detected on April and 33% on May (Table 3). Statistically this difference was significant (P<0.05). This high prevalence of *E. coli* O157:H7 in well– water samples on April could be explained by the high rate of rainfall which can led to drainage of water carrying significant numbers of *E. coli* O157:H7 to the ground water. Some of the pathogens in manure and/or contaminated soil and sediment may be mobilized into water as a result of diffusion, erosion, raindrop impact, and other release mechanisms (Jamieson *et.al.*, 2002; Tyrrel and Quinton, 2003; Krometis *et. al.,* 2007 Many studies have reviled that, in warm climate, prevalence of *E. coli* O157:H7 in cattle may be less season dependent. (Riley *et.al*. 2003; Dunn *et.al.* 2004). The prevalence of *E. coli* O157:H7 at T_0, T_5, and T_{10} were 60%, 40%, and 53% respectively (Figure 3). The high prevalence of *E. coli* O157:H7 at all collection times may be explained by the fact that there were continuous persistence percolations of *E. coli* O157:H7 from the manure applied to the soil into the ground water during the sampling period. Rice and Johnson (2000) reported that *E. coli* O157:H7 survived for 8 and 16 days in water samples from dairy farms. Other revealed that *E. coli* O157:H7 survived 5-7 weeks in rain/river water (Randall *et.al*., 1999). While Sargenant *et.al*., (2003) reported that water tanks in large cattle feedlot were five times more likely to harbor *E. coli* O157:H7 if a pen was positive for the bacteria.

Table 3 Prevalence of *E.coli* O157:H7 in well water at different collection times and locations surrounding the dairy farm.

Well	April			May			Total No.+ve/Total Samples	Prevalence (%)
	Sampling Time After							
	T0	T5	T10	T0	T5	T10		
W1	3/3	2/3	3/3	1/2	0/2	1/2	10/15	67
W2	1/3	1/3	1/3	1/2	1/2	2/2	7/15	47
W3	3/3	2/3	1/3	0/2	0/2	0/2	6/15	40
Total No.+ve/Total Samples	7/9	5/9	5/9	2/6	1/6	3/6	23/45	
Prevalence (%)	17/27			6/18			51	
	63 A			33 B				

Different uppercase letters in the same row are significantly different (P<0.05)

T = Time (minutes) after water pumping

W = Well, W1 = Right side, W2 = inside the farm, and W3 = Left side.

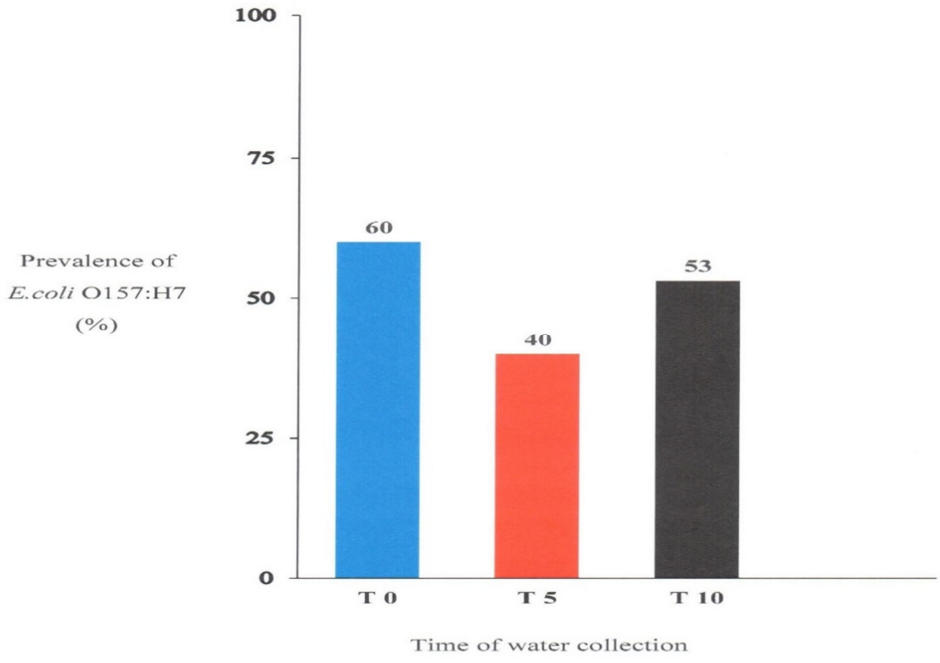

Figure 3 Prevalence of *E.coli* O157:H7 in well water (W1, W2, W3) at different collection time (T 0, T 5, T 10) during the study.

3.4. Overall prevalence of *E.coli* O157:H7 in fecal, soil and water samples

A total of 149 samples (fecal, soil, and water) were collected in this study, 95 (64%) were found to be *E.coli* O157:H7 positive. Fecal samples had the highest prevalence (75%) than soil and water (67% and 51%) respectively (Fig.4). However, no significant differences were observed between fecal × soil, and soil × water, but significant difference was found between fecal × water ($P<0.05$).

The integrated mechanisms and environmental factors that affect bacterial survival time (down to undetectable levels) in naturally deposited livestock waste are unclear (Scott *et.al.* 2013). Lab –based experiments indicated a wide range of potential factors influence bacterial survivability, including soil properties, temperature, sun light, humidity, rainfall, animal feed type, competition among organisms, bacterial density, waste application process, and management practices (Lowe *et. al*.,2010) .In CAFOs, improperly or poorly contained waste is often the primary source of pathogens. The pathogens concentration in animal waste is reported to depend on the species, age, health, stress, and diet (Himathongkham *et. al*., 1999; Nicholson et.al., 2000; Vidovic *et. al*., 2007).

Few survivability studies have been conducted for *E.coli* O157:H7 in naturally deposited cattle manure. Manure and wastewater from CAFOs are routinely applied to agricultural fields as a soil amendment based with little or no treatment to inactivate pathogens (Bradford and Torkzaban 2008; Bradford and Segal, 2009). If these wastes are inadequately treated before land application, then viable pathogens may survive or grow in field soils and contaminate plants (Berg *et.al*. 2005; Avery *et. al*., 2005; Berry *et. al*., 2007). The complex nature of *E.coli* O157:H7 ecology in a farm suggests that a reservoir other than cattle may be important in maintenance of *E.coli* O157:H7. (LeJeune *et. al*., 2004). Survival of pathogens in the environment is dependent upon both the nature of the environment and the organism. (Zhao *et.al.,* 1995; Shere *et.al.,* 1998; Duffy, 2003; Scott *et.al.,* 2013). Substantial data exist to support the ability of *E.coli* O157:H7 to persist in the environment on farms (LeJeune *et.al.* 2001; Lahti *et.al.* 2003). Pathogens traveling from applied manure through the soil and, subsequently, through an underlying aquifer and into water supply wells involves microbial survival in several disparate environments of characteristically different conditions. Consequently, survival throughout the collective transport pathways is exceedingly difficult to predict, resulting in considerable uncertainty concerning survival of pathogens in agricultural farm (Berry and Miller, 2005; Vidovic *et.al.* 2007; Ishii *et.al.* 2010). Thus, the long survival time of *E.coli* O157:H7 in cattle feces and contaminated soils should be considered as an important factor in contamination of surface water, and well–water resources.

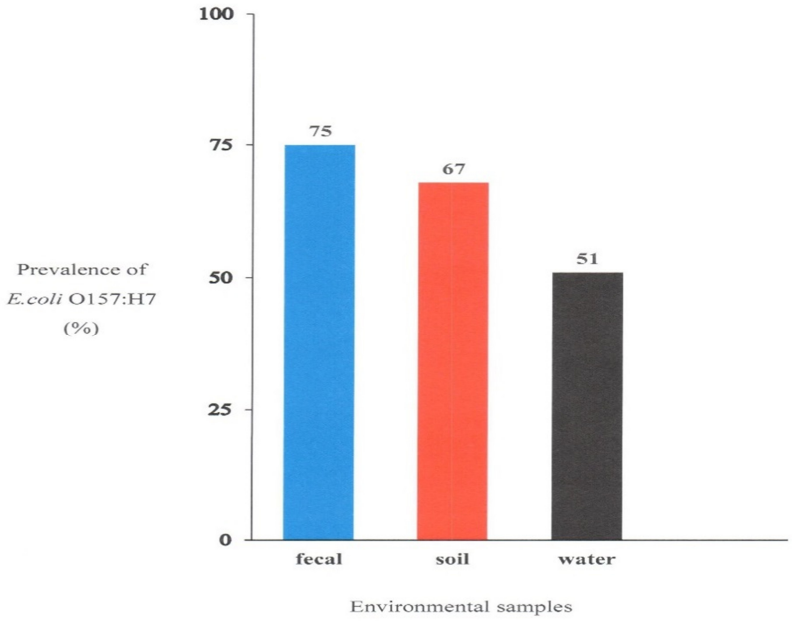

Figure 4 Overall Prevalence of *E.coli* O157:H7 in fecal, soil, and water samples reported in the dairy farm during the study period.

Conclusions

From the results obtained, it can be concluded that *E. coli* O157:H7 can survive for extended periods of time in feces, soil, and well- water in the environments of cattle shedding *E. coli* O157:H7, and there is a high risk of contamination of crops and well-water intended for human consumption and can be a source of exposure to persons attending or participating in cattle husbandry.

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