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Review Article

Escherichia coli O157:H7, Genetics Assessments, Prevalence and Importance for Health and Food Chain Supply

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Abstract

Escherichia coli O157 is a food borne Shiga toxin-producing human entero pathogenic pathogen known to cause severe diarrhea often accompanied by cramps, nausea, vomiting and slight fever in humans. This bacterium is one of the most aggressive entero hemorrhagic *E. coli* strains and probably evolved through horizontal transfer of genes encoding for Shiga toxins and other virulence factors. This strain is commonly found in the faces of healthy cattle, its major natural reservoir, and is transmitted to humans through contaminated food, water, and direct contact with infected people or animals. Human infection is associated with asymptomatic shedding, non-bloody diarrhea, hemorrhagic colitis, hemolyticuremic syndrome, and death. The antimicrobial treatment is not well established and opinions about its efficacy diverge. Some studies suggest that antimicrobials may accelerate the development of complication known as Hemolytic Uremic Syndrome (HUS), while others suggest no adverse effect and health recovery. Alternative treatments are in progress, including vaccine development, the bacteriophage- based bio control and the use of probiotics and prebiotics. The understanding of the bacterium's biology and social internalization about the hazards of consuming undercooked ground meat or unpasteurized milk products and juices and the awareness to personal and collective cleaning are essential to successfully face this public health problem. Prevention measures as well as information to public-health authorities in case of bloody diarrhea is very important to successfully handle this public health problem.

ABBREVIATIONS

VTEC: Verocytotoxin-Producing *E. coli*; STEC: Shiga-Toxin Producing *E. coli*; EHEC: Entero Hemorrhagic *E. coli*

INTRODUCTION

The increases of intensive animal production implies increment intensive manner to handle and maintain livestock. This situation is well correlated with the risks of zoonotic infections in animal population and animal-origin food. At present, the world is growing smaller as a result of globalization with the expansion if human activities and transport facilities, the unprecedented growing of economy, trade goods including living organism of byproducts raw or processed and greater movement of persons making more extensive and complex the Food Chain Supply. As a consequence, hosts are facing growing level of exposure to different pathogen agents. Great number of different global human health problems has animal infections as origin [1]. Usually research about zoonotic diseases and their impact on human health, environment and other form of life has been conducted isolated matter. The pathogen transition , intra and inter animal species and then to human, can cause a zoonotic outbreaks with a great possibilities to be transmitted to human by human-animal contacts or exposure [2]. It's clear that exposure of humans from animal can be resulted in pathogen transmission by variation in original pathogen or by changing in human susceptibility to specific infectious agents [3].

Escherichia coli is an intestinal bacterium commonly found in human and animal intestine. This bacterium were discovered in the human colon in 1885 by German bacteriologist Theodor Escherich, that also showed that some of strains were the causing pathogen for child diarrhea and gastroenteritis, of the most important public health problems up to the moment. During some time *E. coli* bacteria were denominated *Bacterium coli*, but making justice to the founder and in his honor the name was

finally changed to *Escherichia coli* [4]. It's not possible to conceive the life science without this bacterium. It is the most-studied free-living organism a model to the most important life science, including microbiology, genetic, molecular biology, and genetic engineering. Routine test for *E coli* O157 in stool specimens is based on isolation by platting and growth on specific media, complemented by serological analysis and/or PCR. Up to now more than 700 serotypes of *E. coli* have been identified. The "O" and "H" antigens of the bacteria and their flagella were used to distinguish sero typically the different strains [5].

Under normal conditions, the intestinal micro flora, including many *E. coli* strains, is harmless and some of bacteria are beneficial, while some other can cause illness like slight diarrhea, gastrointestinal and urinary tract infections. Such *E. coli* strains are denominated enterohemorrhagic *E. coli* (EHEC) and are particularly aggressive when the bacteria proliferate over the normal level. There are six EHEC patho types that cause diarrheal diseases in human and animals. The strain *Escherichia coli* O157:H7and some non-O157 serotypes of *E.coli* also produce verocyto toxins, also known as Shiga-like toxins because of their similarity to toxins produced by *Shigella dysenteriae*. These strains are called verocyto toxin-producing *E. coli* (VTEC) or Shiga-toxin producing *E. coli* (STEC). EHECO157:H7causessevere diarrhea often accompanied by several abdominal pain, cramps, nausea, vomiting and slight fever. This serotype was classified according to his O and H flagellar as O157:H7 and represent the most common entero hemorrhagic serotype of this bacterium. Inside the organism, this strain has the ability to produce attaching and effacing lesions [6,7]. Adverse evolutions of pathologic process may be occurring and about 5% of the infected persons develops hemolytic uremic syndrome (HUS), with a typical hemolytic anemia, thrombocytopenia and also renal malfunction. Other important complications are thrombotic thrombocytopenic purple, pancreatitis and diabetes mellitus. The infection with this strain is occasionally fatal [8].

Serotypes of VTEC bacteria include strains with different level of virulence because in addition to the toxin are other factors influencing the virulence of strains. The high frequency of infection with enteropathogenic *E. coli* in persons and animals, is a source of big economic loses for two main reasons: the treatment costs and the labor hours or day lost during the diseases, there also negative economic impact in animal production and in many susceptible steps of Food Chain Supply. It has been estimated that EHE Care the causal agent for the numerous case of contaminated foods and beverages. The key strain in this health problem is the strain EHEC O157:H7.From approximately 100,000 illnesses, 3,000 hospitalizations, and 90 deaths by the EHEC reported annually in the United States [9] the *E. coli* O157:H7 covered the major part of this statistic. EHEC O157:H7 is responsible of approximately 73,000 cases and 60 deaths every year while non-O157 VTEC serotypes cause about 37,000 cases annually [10]. A report published in 2005 estimated the annual cost of EHEC O157:H7 illnesses to be $405 million (in 2003 dollars), which included $370 million for premature deaths, $30 million for medical care, and $5 million for lost productivity [11].

This work is focused in *Escherichia coli* O157:H7, its biology, genetics, prevalence and impact in public health and social life as well as the measures for prevention and treatment. The need of the interdisciplinary work to control the gastrointestinal diseases is also reviewed, understanding the problem of co infections and multi parasitism. Some aspects related with safety in Food Chain Supply are considered important, particularly when facing the climatic change and globalization. We also made an assessment to different methods to handle *Escherichia coli* O157:H7 infections, including antibiotic treatment, vaccination, bacteriophage based bio control, administration of probiotics and prebiotics.

ESCHERICHIA COLI O157:H7

Escherichia coli O157:H7is the serotype that has most often been associated with severe forms of diarrhea, but other non O157 sero groups causing similar illnesses, have been reported [12](Figure 1). The main target of research has been focused on O157:H7 serotype, but the rest of Shiga Toxin Producing *E. coli* (STEC) serotypes are also associated to this human illness. Cattle and other ruminants as well as healthy swine can be natural reservoir for this bacterium. The infection by such bacterium is asymptomatic in these and other animals and they can be carriers of the bacterial strain for long period [13].

Escherichia coli O157:H7 (Figure 1) was first associated with human disease after a multi-state outbreak in 1982 involving contaminated hamburgers. The strain EDL933 was isolated from Michigan ground beef linked to this incident, and has been studied as a reference strain for O157:H7 [13,14]. In human, *Escherichia coli* strains O157:H7 causes severe diarrheal disease frequently accompanied by bleeding, intestinal obstruction, nauseas, vomiting and fever symptoms. In most chronic situations, the patients can develop Hemolytic Uremic Syndrome (HUS), hemolytic anemia (HA), thrombotic thrombocytopenia purple, renal impairment, pancreatitis and even diabetes mellitus. These severe chronic affections are caused by Shiga toxins produced by this bacterium. Shiga toxins affect systemically sensitive cells in different organs including kidneys, brain, and other organs and tissues [15,16]. There are other members of enteropathogenic *E. coli* strains that produce these toxins, but the strain *E. coli*157:H7 is especially virulent and aggressive and it´s the causal agents of for the majority of bacterial infections reported in the world

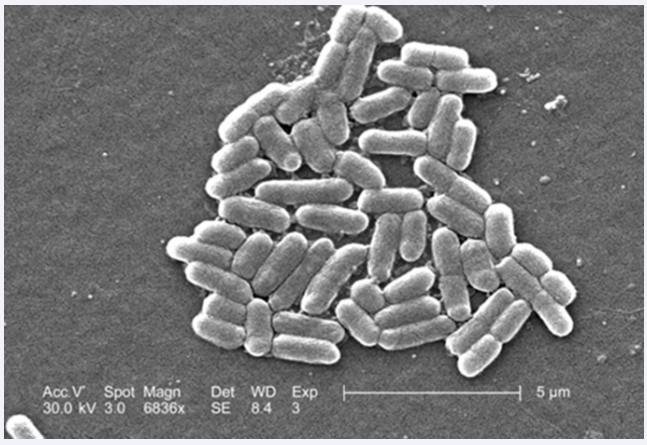

Figure 1 Microscopy photography of *E. coli* 0157:H7. Courtesy of CDC (Free image collection at USCDCP, author: Janice Haney Carr).

[15,17]. Such virulent characteristics can be explained by its extraordinary ability to colonize human and animal intestines together with the great capacity to survive in the different environmental conditions [18-21]. Other important aspects are the ability to use animals, particularly, farm animals as reservoirs and carriers, making the farm and animal economic activities in important contact liker for the transmission of this infectious agent to humans.

Animal reservoir and human infection sources. The adaptive bacterial responses of Escherichia coliO157:H7

Cattles have been considered the major reservoir, natural carriers and transmitters of Stx-producing microbes, directly in farms and also as a source of contamination bovine-derived food chain supply. In consequence, prevalence studies of *Escherichia coli* O157:H7 are important for monitoring safety in food processing and preservations activities. The low number of colonizing bacteria in intestine of animal carrier resulted in difficulties for quantification by sampling and culturing approaches [22,23]. Because the ability to detect and quantify the number of *E. coli* O157:H7 depends of the methodology used. This dependence explains the difficulty to provide complete view of the bacterial prevalence and circulation in animals whose role in transmission of the pathogen remains undetermined. The bacteria can be isolated and grow by plating on sorbitol MacConkey agar supplemented with cefixime and potassium tellurite (CT-SMAC) [22,23]. Them the resulted colonies are serologically and biochemically analyzed as colonies positive for O157 and H7 antigens. In some case the CT-SMAC is supplemented with 4-methylumbelliferyl-beta-d-glucuronide that is transformed in 4- methyl umbelliferone [25] or 8-hydroxyquinoline-beta-d-glucuronide [26] by the majority of *E. coli*, including most non-O157:H7. The first conversion product is UV-fluorescing while the second one product can be observed with light-visible. Selective plating can occasionally generate false-positives and false-negatives because some *E. coli* O157:H7 isolates may be sorbitol-fermenting and exhibit glucuronidase activity [27].

In our laboratory, we were able to standardize many of these experimental procedures, each methodology was normalized but when the same samples were analyzed by different methods we obtained different quantities of UFC. The prevalence studies carried using different detection methods resulted in different estimated quantifies of this strain. We have develop a multi-primer real time PCR method discriminating the different *E. coli* in intestinal, stool and other samples and we obtained encouraging but preliminary results and the method needs to be validated. In general, genetic analysis of *E. coli* O157:H7 is hampered by a high degree of variation and need to study if the proposed method has practical significance. Low prevalence results usually reflect a reduced probability of detecting *E. coli* O157 due to one or more aspects of methodology including single sampling visits, small numbers of tested animals per farm, absence of comprehensive farm surveys, and selective or no enrichment applied to stored samples.

The relative population of animals per farm and its correlation with positive identified cases was not well established; probably the general livestock health and living conditions vary from farm to farm enough to affect correlation studies. Other aspect of prevalence studies are related with the fact that the bacteria can be found out of the intestine and that the period of intestinal carriage is usually short [28,29]. We have carried a study in bovines compared the intra-intestinal and extra-intestinal prevalence of *E. coli* O157:H7 in 30 animals during winter and summer and winter seasons, using traditional selective plating combined with multi primer real time PCR (data not shown, method in process of validation). We found that intra-intestinal prevalence is higher during summer season (10%) than during winter season (3%) while extra-intestinal prevalence decreases during summer (1 %) compared with the results obtained during the winter (5.25 %). Probably external climatic factors such as insolation, temperature and climatic seasons resulted in the reduction of bacterial colonies found outside intestine but stimulate rapid spread and transmission of bacteria. These results are preliminary but showed us the same tendency obtained earlier by Dunn [30].

Healthy domesticated ruminants are the major animal reservoirs and carriers, particularly cattle [15], followed by sheep and possibly goats [31]. Human isolates associated with severe diarrheic diseases represented a minority of *E. coli* O157:H7 found intra-intestinally in cattle [19]. Genetic analysis of *E. coli* O157:H7 isolates resulted in the identification of two different linages according to the fact that 11 distinct genetic regions were found in 80% of human harboring isolates (Linage I) and in 92% of cattle harboring isolates (Linage II). In consequence the Linage I is more associated with human disease than the strains of lineage II, usually found in cattle and even in other ruminants [32]. Both linages share many genetic characteristic and virulence factors but the majority of bovine isolates doesn't infect or have low prevalence in humans [33]. But nerveless the strains belonging to linage II are potential human pathogens [34,35]. The general concern is that cattle are the main source of human *E. coli* infections [36,37] and the probability of pathogen transmission and infection to human from cattle is high due the role of beef and dairy cattle consumption among domesticated animals, and size and economic importance of bovine in food industry. Isolation of enteropathogenic *E.coli*, including the serotype O157:H7, have been reported in other farm and domestic animal but in lower frequency. The studies that measured intestinal prevalence of *E. coli*O157:H7in cattle and sheep at slaughter consistently show higher prevalence in cattle. For example in the United Kingdom, the bacteria were found in 4.7% of cattle and1.7% of sheep [41], in 15.7% of cattle versus 2.2%of sheep [42], and in 4.7% of cattle and 0.7% of sheep [43]. The same was reports for other countries although numerically the results show differences from one country to other [34]. Small ruminants can be a significant source of human infection too [31], and of strains of *E. coli* O157:H7were identified in sheeps [42], lambs [43] and goats [44]. In the case of pigs, usually they carry *E. coli* strains and intra-intestinal colonization by *E. coli* O157:H7 have been observed but at low frequency [45]. This bacteria can also colonized chickens but shed for up to 11 months [46]. This fact is considered as poor transmission event rather than host incompatibility, builds a wall to infection. Prevalence in domestic animals and animals living in anthropogenic environments, like urban birds, rodents

and petsis very infrequent [46,47]. Because of bacterial high mutability reflected in the potential adaptive capacity it can be possible making these species as reservoir and transmission organisms. In the nature wild live is not considered an important animal reservoir of this bacterium and only sporadically isolation of *E. coli* O157:H7 strains were found other than deer. This can be carried by amphibians and fish, as well as invertebrates, such as insects and mollusks. It has reported in fish caught in place near to cattle's slaughter in Africa [48]. In another report this strain was found in American bullfrogs (*Rana catesbeiana*) [49]. Insects may be especially important for its high proliferation rates, mobility and even for its adaptive responses to climatic change. That found the bacteria in 11.4% of cattle (n¼1407), 1.2% of swine (n¼1102), 3.6% of sheep and goats (n¼364), and in 5.2% of 154 fly pools; at some fairs, isolates from cattle, swine, and flies shared indistinguishable subtypes [50]. The transmission of this strain mediated by animal vectors is not clear, but all evidences indicate that this is an important step accounting for transfer without direct contact between native and colonized carriers. An extensive compilation of *E. coli* O157:H7 findings in farm, domestic and wildlife animals have been reported showing remarkable differences in infestation values [16].

According to our experience the manipulation of samples is a crucial fact explained the presence of "false negative". In our case the samples were taken from 100 healthy cattles samples obtained from diarrheic calves. We introduced different variations in sample preparation, including bacterially enriched cow fecal samples and the results were similar with those obtained by other authors when optimized sample preparation method was established [51-54].

Characterization of shiga toxin subtypes and virulence genes in Escherichia coli O157:H7

The Shiga toxin, also called verotoxin, is produced by *Shigella dysenteriae* and enterohemorrhagic *Escherichia coli* (EHEC), but the strain O157:H7 has become the best known for its implication in public health and food security. They are classified in two major antigenic forms, defined as Stx1 and Stx2, but variants were defined in both classes: three subtypes for Stx1: Stx1a, Stx1c, Stx1d, and seven subtypes for Stx2: Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f and Stx2g respectively [55,56]. The Stx1a has been directly related human illness but others like subtypes Stx2a, Stx2c, and Stx2d are frequently found associated with the development of most extreme form of illness in human [55,56]. "*In vitro*" experiments carried in cell lines showed that the subtypes Stx2a and Stx2d were more toxic that Stx2b and Stx2c. Experiments in mice confirmed also these results [57]. There are reports indicated that toxicity of different subtypes varies in different animals [58-60]. For example, Swine STEC strains commonly produce Stx2e [61-63], which may cause edema disease in weaned pigs, often lead to ataxia and death [64]. Another subtype, the Stx2e, not represent a particular threat for humans [13,55].

Shiga Toxin is considered an essential virulence factor in human disease mediated by *Escherichia coli* O157:H7 [64]. Since its identification, in 1982, the incidence of *E. coli* O157:H7 in human diseases annually grow in the world. Two antigenically distinct Stx variants, the Stx1 and Stx2, produced by *E. coli* are related to the Shiga toxin produced by *Shigella dysenteriae* [65], which was first isolated and identified over 100 years ago [66]. At amino acid level the similarity between the sequences of *E. coli*-derived and the Stx of *S. dysenteriae* is very high and Shigella and *E. coli* Shiga toxin genes have been expressed by other bacteria: Enterobacter, Citrobacter, Acineobacter, Campylobacter, and Hamiltonella [67].

The broadly distribution of these Shigella-derived genes encoding was achievement probably by horizontal transfer. In the original host, *S. dysenteriae*, Stx genes are placed on the bacterial chromosome. In *E. coli*, the Stx genes are associated with active or cryptic lambdoid prophages. The lambdoid or lambda-like phages are a group that includes properly lambda phage, phi80, and also several so-called Hong Kong phages, like HK97 and HK022, among others. This group includes also some *Salmonella*ssp-phage [13,64]. The lambdoid phages are grouped together because they share a common genetic map [67]. Recombination between different species of this group, including partial and complete prophages, resulted in viable hybrids. Genes encoding both Stx1 and Stx2 toxins in *Escherichia coli* O157:H7 are located on different lambdoid bacteriophages that lysogenize this strain. Bacteriophages that separately encode Stx1 and Stx2 have been isolated from various EHEC strains. The overall construction of Stx1 phages is similar to that of Stx2-encoding phages, and their genes display some similarity [68], whereas other Stx1 phages contain different sets of homologous genes. The component genes of individual lambdoid phages are arranged modularly and are generally a mosaic of genes from a particular gene family despite that most component genes are homologous. The sequence of the DNA binding domain at the N-terminus of the repressor of phage 933W is nearly identical to that of the non-toxic lambdoid phage HK022, but the sequence of the oligomerizing C-terminal domain of this protein is identical to that found in the repressor of the Stx1-encoding phage H19-B [69]. By recombination can be possible the opportunity to create new species by analysis of the genomic sequence of the *E. coli* O157:H7 [70]. This strain harbors two active bacteriophage, each expressing stx1 or stx2 genes, showing that a Shiga toxin-producing *E. coli* (STEC) strain can harbor more than one Shiga toxin encoding bacteriophage. In addition, this strain is lysogenic for many cryptic phage genomes, providing ample material for recombination and a strategy for rampant spread of Stx in the environment [72].

E. coli O157:H7 isolates from the bovine reservoir was considerably broader than it was expected on the basis of analysis of human isolates [72]. Many of the genotypes distinguished among bacterial isolates from cattle and human composed the major representation in both cattle and human derived samples. Analyzing different cattle samples, it was found that more represented genotypes isolated in cattle were well correlated with data over the genotypes identified in clinical cases. Bresser et al. [72], considered that some events like phage lysogenization and excision in the short terms could result in changes in the Stx-encoding bacteriophage insertion site in *E. coli O157:H7*. The reported studies indicated that in *E. coli* O157:H7 the acquisition of Stxen coding phages plays a crucial role in the evolution of this pathogenic clade from precursor strains and thus predated the divergence detected on the basis of these genotypes within the clade [73,74].

Among the others virulence factors, the Shiga toxins (Stx) and the ability to colonize the epithelial colonic surface through the functions are the most important. Both factors are believed to play a crucial role in the patho physiological process caused by *E. coli* O157:H7. The attachment to epithelial colonic cells, are the results of the expression of genes placed in a pathogenicity island named the Locus of Enterocyte Effacement (LEE) [75]. The heterogeneity of *E. coli* O157:H7 genotypes in bovine seem to have implications for pathophysiology, evolutionary genetics, and food safety. The majority of isolates, including those with geno types underrepresented in clinical isolates, displayed both of these factors but significantly differ in the expression level of Stx or LEE encoded protein [72]. More than 250 serotypes of Stx-producing *E. coli* have been detected in the bovine reservoir, but less than 100 have been associated with human illnesses, and only a few sero types cause most human infections [75,76]. It is possible that expression of virulence factors determines character and distribution of nonclinical genotypes of *E. coli* O157:H7 and in addition the distribution of nonclinical Stx-producing *E. coli* serotypes. This fact supports the idea that the vast majority of *E. coli* O157:H7 isolates reside in the animal reservoirs and not in humans [73].

Differential distribution of *E.coli* O157:H7 lineages among isolates from cattle and humans were consistent with differences in human infectivity orpathogenicity in certain isolates has been reported [73]. Using octamer-based scanning was identified two major class of *E. coli* O157:H7 lineages, one more frequently found in human clinical isolates and the second one, that are more frequently in bovine isolates. The variation among genotypes was linked with bacteriophage-related sequences. The *E. coli* O157:H7 sequentially acquired Stx-encoding bacteriophages in specific chromosomal locations [78,79]. The nonrandom distribution of *E. coli* genotypes lineages in cattle and human hosts occur but at the degree that is considerably lower than expected [73]. Strains of *E. coli* O157:H7 exhibit high genetic variability but typically a small number of genetic types predominate in groups of cattle and a farm environment. Transmission to people occurs primarily via ingestion of inadequately processed contaminated food or water and less frequently through contact with manure, animals, or infected people. The interest and knowledge about non-*E. coli* O157:H7STEC and its importance inhuman Illness have increased. There is a need to develop a model for molecular risk assessment associated with STEC [80]. Knowledge of the virulence gene combinations that distinguish highly pathogenic *E. coli* from less virulent strains remain sun clear, particularly for LEE-negative STEC Additionally, new virulence-associated and put a tivevirulence-associated factors are being identified [81-84].

Expression of phage-harboring stx genes in host organism

In bacterial chromosomes there are genomic sequences that show high frequency of both cryptic and active temperate phage. Despite that Stx toxins affect mammals, including human, these phage-harboring stx genes are also found in free phages and lysogenic bacteria isolated from environments, where the presumed corresponding targets (mammals) are not present [85]. The most important priority of bacteriophages is to survive and reproduce by following lytic or lysogenic ways (Figure 2). Once the bacteriophage progeny is obtained these phages kill their host and the resulted progeny goes abroad to find another host cell. Despite phage DNA encodes exotoxins they are tolerated in the host bacterial chromosome probably because their presence helps bacterial population to prevent predation providing advantages to bacteria. This hypothesis tray to explain why the exotoxin genes are always located in active bacteriophages and according to this, humans and susceptible animals are neither the original nor primary targets of these toxins [86].

In the stx-encoding phages, the stx genes are located in the late region of the phage, downstream from PR. The PR' is inducible promoter, only active during lytic growth but not during lysogenic fate, the lysis-lysogenicway of growth is a kind of control fact over the Stx production [87-90]. Once the hos organism, like *E. coli* O157:H7 is transfected and the phage begins lytic development way, the transcription initiates at the early promoters PL and PR. The PL promoter allows the transcription and translation of the gene N. The N activity allows the RNA polymerase transcribing from PR to read-through transcription terminators and extends transcription into the Q gene. Q binds DNA at a site partly overlapping PR' and acts as an anti-terminator thereby allow RNA polymerase to transcribe an oper on that includes the stx genes [88-91]. Synthesis of Q depends on the activities of the early lytic promoters PR and PL. These promoters are repressed in lysogenic fate by repressor DNA binding. During induction to lytic fate, repressor became inactivated, making possible the expression of Q and them the expression of stx genes. The synthesized Stx lacks any secretory signal for bacterial secretory systems, it's accumulated in the cytoplasm until the lytic development is completed and the host bacterium is lysed according to the phage-encoded genetic program, and the Stx is released.

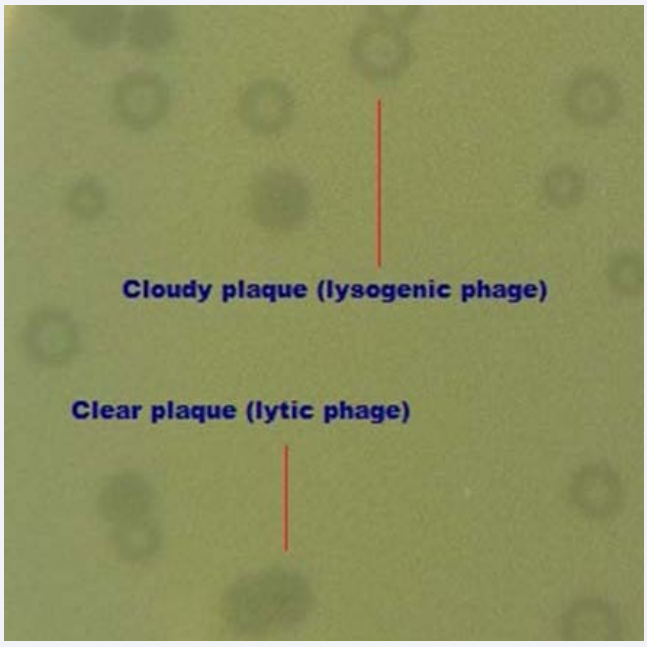

Figure 2 The lytic and lysogenic phase of phageson *E. coli* O157:H7.

The phage's choice to follow lytic and lysogenic development is induced by external conditions. The influence of local factors over the lifecycle of lambdoid prophages in the environment is linked and well correlated with the Stx production [88-90]. All lambdoid bacteriophages have a common developmental program. Upon infection of a host bacterial cell, the lambdoid phages choose between two developmental fates: lytic and the lysogenic fate. The lytic fate ends with the lysis of host cells releasing new generation of prophages. In lysogenic growth, the phage chromosome is inserted into the host chromosome and is replicated along with it, until a signal that induces lytic growth is perceived by the lysogenized cell. The genes for Stx are encoded in the late region of the phage. Late genes, including stx genes, are only expressed while the bacteriophage is growing lytically [71].

In the lysogenic phase, the repressor cI protein is produced and the synthesis of Stx is blocked. The protein cI acts as repressor for others gene, including some involving in lytic way of development. To inactivate cI repress or during lysogenic fate, the phage takes advantage of part of the host's SOS response; the pathway involved in general response to DNA damage, particular important is the interaction with host Rec A, the crucial regulator of the host's SOS response system. RecA stimulates the intrinsic auto proteolytic activity of the phage's repressor protein cI, making possible the transcription of early repressed phage genes including stx genes [92]. The RecA stimulated intrinsic auto proteolytic activity of the repressor cleavages the repressor's C-terminal oligomerization domain separating it from the N-terminal DNA binding domain. In that condition cI loss the capacity to bind DNA depressing the expression of all the genes involved in lytic growth, and the stx genes. In infected mammals by *E. coli* or other Stx producing bacteria, hosted the prophages with stx genes, the leukocytes and neutrophils activates the SOS response in Stx encoding *E. coli* (STEC) by release superoxide. This process leads to the release of the Stx toxin and to the subsequent cell death [89,93]. Without external stimulation, prophages have the ability to produce free phages by spontaneous induction. But the number of spontaneously produces phage in very low compared with the inducing external factors are present. But even in spontaneous phage induction the active RecA is crucial because the process is also mediated by changes in repressor levels [94]. The spontaneous induction in also designed as DNA damage-independent (spontaneous) induction occurs in other related bacteriophages but in stx-encoding bacteriophages the frequency of this process is higher than in the rest of bacteriophages. The explanation of that behavior has been attributed to possible lower requirements in concentration of active RecA necessary for induction [94].

Repressor levels in a lambdoid phage lysogen are regulated by autogenously positive and negative control where differential site binding by repressor and hence repressor gene activity is the key for understanding such regulation. Repressor cI binding to one subset of binding sites leads to activation of repressor synthesis, whereas repressor occupancy of additional sites leads to repression of repressor expression. In the well-studied non-STEC lambdoid phages, differential site binding by repressor and hence repressor gene activity depends on the cooperative binding of the repressor to multiple sites, some of which are separated by up to 2.5 kb but in other bacteriophage, like 933W, DNA binding does not cooperatively [92]. May be bacteriophage 933W has evolved by other alternative way of repressor regulation.

There are other than Stx virulence factors in illness, including genes encoding for proteins involving in cell adhesion, proteases, and toxins, particular combination of all these factors and the proportion in the composition among these factors may be important in severity of human illness [64,95]. Some of these factors aren't well studied, that is the case on eibG, lpfA, saa, and sab genes, encoding for proteins acting as virulence factor, like the adhesion of the bacteria to the intestine wall [96].

Distribution and role of Shiga toxin in environment

In the environment, microbial biodiversity emerges as a set of large and complex living networks closely linked to specific ecological niches, which are defined as microbiomes. Shiga toxin (Stx) producing microbes are part of such microbiomes and can exist in multiples environmental conditions and ecological niches. But there are difficulties to isolate and grow microorganism from microbiomes. The core of the problem besides in the fact that only small proportion of microbiome could be grow at laboratory conditions. Shiga toxin producing microbes are present in terrestrial and aquatic environments, in free living condition or in organism-carries like cattle, pigs, farm and domestic animals and even in wildlife animals and plants [97-99]. The actual biodiversity among Stx-producing microbes in animals, including Cattle and other farm, domestic and wildlife animals, is difficult to define due of differences in methodologies used for Stx determination that offer different quantifiable results. Usually the number of samples resulted positive in verocell cytotoxicity assay from different fecal samples from healthy animals, only 5% were tested positive to Stx production, whereas with PCR and real time PCR, using phage-specific primers, the proportion of samples harboring Stx producing bacteria the proportion of positive samples increased to 9% and 11% respectively. Other farm animals can be considered in this regard [16,100]. The presence of Stx producing microorganisms in farms have proved in different farms animals including sheep [101,102] goat [31,103], pigs [104, 105]. Chickens, turkeys, and rabbits, have also been classified as reservoirs for Stx-producing microorganisms too, but at lower level, according to our experience [34,106-108]. There are evidences addressing the existence of Stx-producing microorganisms in other domestic and wild-free animals [27,109,110]. Birds populations have been shown to harbor organisms which possess the stx gene and probably the *E. coli* O157:H7, we tried to study the fecal samples of 10 bird species, but up to now we found stx gene in only one bird, thrush (*Turdus migratorius*) (preliminary results). We must to increase the number of processed fecal samples because actually is too low to formulate any conclusion. Hughes et al. [111], make a test in wildlife birds, 12 different species tested positive for stx1 gene and 30 bird species tested positive for the stx2 gene. Airborne particulates in a contaminated building have been shown to contain bacteria with stx genes [111].

It seems that Stx-producing organisms are very abundant in terrestrial ecosystems but also were found in aquatic environments including in potable water source and supply lines [112]. Stx producing microorganisms are a possible pathogenic causal agent in gastrointestinal illness outbreaks [112-118].

In general aquatic environment including fresh water sources, potable and drinking water systems as well as lakes and rivers using for recreational activities has also been found to contain organisms which produce Stx and may cause human illness [120-121]. From all these studies we can conclude that stx gene distribution in aquatic ecosystems can be highly variable, likely owing to local conditions which impact organism survival, variation in organism population, in the lifecycle of lambdoid prophages harboring stx genes, its expression in suitable hosts and the production of Stx [122-124].

What is the role of Shiga-toxin in the ecological niches occupied by such producing microbes? One of must accepted hypothesis resides in the necessity of microorganism colony to self-defense against other microorganisms but bacterial predator. Phage-encoded exotoxin as a defense anti-predator activity include the sacrifice of part of bacterial population succumbing to produce a toxin to kill a population's major predators but preserving the greater part of bacterial population. In the logic of this hypothesis we can consider induction factor as a signal to predator´s presence. This hypothesis was tested in *Tetrahymena thermophile*, a unicellular eukaryotic predator model. When co-cultured with *T. thermophile* Stx-encoding bacteria are able to confront and kill this predator. It is necessary to extend this kind of study to other exotoxin-encoding bacteriophage resident within bacteria influences the growth and survival of the bacterial population [93]. As we already observed, the ant predator defense by using phage-encoded Stx as an ant predator defense, the lysogenic bacterial host dies releasing new phage progeny and exotoxins, protecting the overall bacterial population-a population that would include cells that are not lysogenic for the toxin-encoding bacteriophage. But this act means the sacrifice of a few for the welfare of the majority because Stx-encoding phages lysogenize hosts at low frequency in relation with whole bacterial population [94]. And some data suggest that there is some mechanism over the proportion of suicidal cells. These lysogenic populations sacrifice a few cells to produce a toxin that kills its major predator and produce infectious phage that have the potential to eliminate bacterial competitors [89]. Apparently growing prevalence of Stx-encoding bacteria and phages within the biosphere may be considered as an example that such hypothesis is highly effective collective defense mechanism although we don't know the number and identities of microbial predators, as some protozoa and even nematodes, which are sensitive to Stx or other cytolitic exotoxins [125,127,128]. This is a promising way to better understand the environmental factors that activate the genetic machinery to produce Stx, and the consequence this has on other microbes within an ecosystem, are needed to provide greater clarity regarding the functionally of Stx in the environment, its role and haw the complex phage-host population works in the nature.

Molecular characterization of Escherichia coli O157:H7

Genomicis undoubtedly the best source of information and the basis to develop better methods to advance in understanding the evolution of *E. coli*, through comparison with the genome of the non-pathogenic laboratory strain *E. coli* K-12 laboratory strain MG1655. The genome of *Escherichia coli* O157:H7 strain EDL933 was sequenced was and taken as a reference strain for O157:H7. This strain is very pathogenic and it was isolated from ground beef during outbreak incident allowing the study genes involved in pathogenicity [129].

The sequence of the genome of *E. coli* O157:H7 allows identify genes putatively responsible for pathogenesis (Gene Bank Accession NumberAE005174) [14]. According to the reported data, the chromosomal size of *E. coli* O157:H7 is 5.5 Mb. This genome includes a 4. 1 Mb backbone sequence highly conserved in all *E. coli* strains while the remaining 1.4 Mb, is specific to *E. coli* O157:H7. Compared *E. coli* O157:H7 with nonpathogenic E. coli K12 shows that 0.53 Mb of DNA is missing for *E. coli* O157:H7 probably as a result of genomic evolution [96]. Horizontal transfer is very extensive, in fact, 1,387 new genes encoded in strain-specific clusters of diverse sizes were found in O157:H7. These include candidate virulence factors, alternative metabolic capacities, several prophages and other new functions-all of which could be targets for surveillance. These strains last shared a common ancestor about 4.5 million years ago6. The two *E. coli* genomes revealed an unexpectedly complex segmented relationship, even in a preliminary examination. They share a common 'backbone' sequence which is co-linear except for one 422-kilobase (kb) inversion spanning the replication terminus [129-131].

It is considered that both strains share a common ancestor and a complex segmented relationship was evident. They share a common `backbone' sequence (4.1 Mb), which is co-linear except for one 422-kilobase (kb) inversion spanning the replication terminus. Homology is punctuated by hundreds of islands of apparently introgressed DNA numbered and designated K-islands' (KI, 0.53Mb) or `O-islands' (OI, 34Mb), where K-islands are DNA segments present in the reference *E. coli* K12 strain MG1655 but not in *E. coli* O157:H7 strain EDL933, and O islands, composed by segments exclusively found in are unique segments present in EDL933 [132]. An important part of genes located in O-islands have unknown function, but exclusivity of this segment as well as the fact that many classifiable proteins are related to known virulence-associated proteins, from other *E. coli* strains or related enterobacteria. For those reasons this segment is considered important bacterial virulence. Each island might be ancestral and lost from the reciprocal genome; but analyzing base composition an atypical base composition were found suggests horizontal transfers of relatively recent origin from a donor species with a different intrinsic base composition. Undoubtedly this segment contains the some of the key to establish the molecular basis of adaptation and virulence of *E. coli* O157:H7, but we cannot limit the study to this large genome segments since probably associated with virulence are not limited to the largest islands and some of them interacts in trans forms.

The majority of *E. coli* O157:H7-specific DNA sequences (1.4 Mb) are horizontally transferred foreign DNAs such as prophage and prophage-like elements. *E. coli* O157:H7 contains 463 phageassociated genes compared with only 29 in K-12 strain [13]. A change in G+C contents is one of the indications that a genomic region has been acquired by horizontal transfer, and estimated that at least 53 different species have contributed to these unique sequences in *E. coli* O157:H7. Virulence-associated

genes between two sequenced *E. coli* O157:H7 strains are nearly identical (99%) [133]. Both the acquisition and loss of DNA have played an important role in the evolution of pathogenesis of *E. coli* O157:H7.These evidences helps to broad array the whole genomic information for obtaining a complete set of genes potentially related to the pathogenicity of O157:H7 and is crucial for better understanding the evolution of *E. coli* strains.

The plasmid O157 in *Escherichia coli* O157:H7

In addition to chromosomally encoded Stx and LEE proteins, all clinical isolates of *E. coli* O157:H7 possess a putative virulence plasmid called pO157. This plasmid contains genes encoded for pathogenesis-related proteins that are required in many enteropathogenic bacteria including Shigella, Yersinia, Salmonella, and *E. coli* species [131]. This function allows us to understand why this plasmid is highly conserved in those types of bacteria. It is a non-conjugative F-like plasmid and his size varies from 92 to 104 kb. The complete pO157 sequences in strains isolated from two different outbreaks have been published [134]. This plasmid has different mobile genetic elements including transposons, prophages, insertion sequences (IS), and parts of other plasmids, evidencing recombination and horizontal transfer events. In the plasmid it is possible delimit the co-responses to functional regions of pO157. The IS or remnants of IS are frequently associated with the virulence-related segments, which are similar to compositions of the large virulence plasmid in *Shigella* spp. [131-133]. According to sequence analysis and comparison with homologue elements in other enteropathogenic bacteria we can say that actual pO157 is formed by integration of fragments from multi plesevolutional and species origins into an F-like plasmid, and thus virulence factors or putative virulence factors on the different segments of pO157 may be from different origins. In this plasmid were described 100 open reading frames (ORFs) [132], of them 43 ORFs presented sufficient similarities to known proteins, suggesting putative functions for each. But 22 ORFs had no convincing similarity with any known proteins. Thirty-five proteins are presumably involved in the pathogenesis of *E. coli* O157:H7 infections, but of which only 19 genes have been previously characterized. However, the biological significance of pO157 in pathogenesis is not fully understood [131].

MANAGEMENT OF ESCHERICHIA COLI O157:H7 INFECTIONS

Antibiotics and antibiotic resistance in *Escherichia coli* O157:H7

Antibiotics have been an essential component of the infectious disease treatment resistance. In 1928, Alexander Fleming identified penicillin, the first chemical compound with antibiotic properties while working on a culture of disease-causing bacteria and realized that the spores of a little green mold (*Penicillium chrysogenum*) killed the bacteria in one of his culture plates. Antibiotics are not antimicrobial but antibacterial compounds. Frequent confusion of both terms and easy access to antibiotics together within correct follow of medical guidelines; have led to misuse and overuse of these antibacterial substances. Antibiotics are not effective against viruses such as the common cold or influenza, and even common diarrheic illness. This inappropriate management allows the emergence of resistant organisms. Today as a consequence of a development and spread of antibiotic and the lacks of control in their use, become also a global problem in human and veterinary medicine through the development of multi resistance [135]. World Health Organization to classify antimicrobial resistance as a serious threat that is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country. When bacteria become resistant to three or more antibiotic classes is defined as a multidrug-resistant bacteria strain. Multi resistance is a term also defined as multidrug-resistant. An antibiotic is a secondary metabolite used in bacterial control and antibiotic can eliminate certain a group of bacteria when it is used, be it for human, animal or plant, but it also can promote the emergence of resistance by selection of resistant population and further promote the dissemination of resistant bacteria genes and their horizontal transfer [136,137]. An extensive and detailed review of this important for public health and safety of food chain supply has been published by Colello et al. (2015) [138], and periodic report appears in WHO and FAO official bulleting [137].

Bacteria are able to acquire antibiotic resistance genes that provide protection against most antibiotics. The dissemination and acquisition of such genes by horizontal gene transfer has led to the rapid emergence of antibiotic resistance among bacteria [138]. This process is promoted when the wrongly antibiotic administration takes place: administration of sublethal doses, incomplete treatment, and inadequate disposal of antibiotic residues. In the antibiotic resistance transfer play a crucial role the genetic structure named Integron, a mobile DNA element that can capture and carry genes, particularly those responsible for antibiotic resistance.

Integrons are a part of bacterial DNA harboring the genetic determinants for a site-specific recombination system that recognizes and captures the mobile genes cassette. The antibiotic resistance genes that integrons capture are located on gene cassettes, which can exist as free circular DNA. By site-specific recombination the cassette can be integrated into the integron and additional gene cassettes can be integrated, resulting in the integration of several genes. Structurally an integron is minimally composed by a gene encoding for a site-specific recombinase: intI, belonging to the integrase family, a proximal recombination site: attI, which is recognized by the integrase and at which gene cassettes may be inserted and a promoter: Pc, which directs transcription of cassette-encoded genes. There are several classes of integrons based upon which integrase gene they contain but so called Classes I and II are the most frequent in clinical *E. coli* isolates and they are directly involved in the dissemination of antibiotic resistance [139-141].

The Integron has a 5 conserved segments called functional (5 CS), a functional platform contained elements necessary for site-recombination. The int1 gene encoding a site-specific recombinase, an adjacent site, that is recognized by the integrase and is the receptor site for the cassettes encoded gene, and a promoter suitably oriented for the expression of the cassette [141]. The integrons have the ability to recognize and associate with insertion sequences present in transposons or in conjugative (helper) plasmids that act as transfer tool for their

intra- or inter-species transferences [142-144]. There are five classes of integrons are known to play a role in the dissemination of antibiotic resistance genes [145-148]. The most common structure is the Class I follows by Class II. Class I integrons are characterized by the presence of two conserved fragments, separated by a segment of variable length (sequence) which includes inserted antibiotic resistance gene cassettes. The 5'-CS contains the int I gene, the att I site and the promoter, while the 3'-conserved segment (3'-CS) where usually is placed the sul1 gene, conferring resistance tosulphonamides and the qacE1 gene, conferring resistance to quaternary ammonium compounds used as disinfectants[147,148]. The Class II 2 integrons are embedded within transposable elements Tn7 and its derivatives. The gene coding for the integrase class 2 (intl2) is located at the 5-CS [148]. Its 3 conserved segment usually contains five genes involved in the movements of the transposon (tnsA, tnsB, tnsC, tnsD, tnsE) [140]. Some reports indicate that STEC multi resistant strains isolated from humans and animals have acquired antibiotic resistance genes almost twenty years ago [149,150]. It is clear that the Class 1 and 2 integrons are linked multi resistance to antibiotic in STEC isolated [138,151,152].

In the USA 93 (34%) of 274 STEC from poultry, cattle, swine, and humans were resistant to streptomycin, 89(32%) to sulfamethoxazole, 83 (30%) to tetracycline, 48(18%) to ampicillin, 29 (11%) to cefalothin, 22 (8%) to trimethoprim/ sulfamethoxazole, 18 (7%) to gentamicin, 13(5%) to chloramphenicol, and 10 (4%) to cefoxitin. Forty three (16%) of the STEC isolates harbored class 1 integronsand 41 (95%) of STEC positive to class 1 integrons were resistantto one or more antibiotics [153]. In USA during 2005 on24 *E. coli* isolates from dairy farms. From that, 14 *E. coli* were isolated from dairy cows with mastitis (ECDM), 9STEC O157:H7 from dairy cow and only one from bulktank milk. These strains were evaluated for sensitivity to 19antibiotics used in human and/or veterinary medicine. Class 1 integrons were found only in eight of 10 isolates (one STEC O157:H7 and seven ECDM). Eight of 10 STECO157:H7 and six of 14 ECDM were susceptible to all tested antibiotics [154]. Since 1983 to 2003, a total of 105 epidemiologically unrelated STEC isolates from humans and cattle from Germany were tested for susceptibility to 17 different antibiotics agents by microbiological and molecular methods, the last one to observe if the class 1 and 2 integrons were present. Resistance was found in 76% of the isolates, with a prevalence of 72% for multi resistance. The most prevalent resistance patterns were to streptomycin, sulfamethoxazole and tetracycline (72-68%), followed by spectinomycin, ampicillinand kanamycin/neomycin (39-25%). The molecular screening showed the presence of Class 1 integronsin 41% of the isolates while class 2integrons were detected in only one isolated [138,151].

Derived from this situation and for public health interests, it´s clear the needs to increased surveillance and the development of adequate prevention and treatments strategies based in the multi parasitism character of diarrheic diseases and using different alternative procedures to antibiotics, such as vaccine development, bacteriophage based biocontrol, the administration of prebiotics and probiotics, an preventive epidemiological and sanitary measures.

Vaccines

Vaccination has proven to be the most cost-effective strategy for controlling a wide variety of infectious diseases in humans and animals. The new vaccines are greatly demanded to effectively control newly- and reemerging pathogens in livestock. Development of veterinary vaccines is a challenging task, due to a variety of pathogens, hosts, and the uniqueness of host-susceptibility to each pathogen. Some surface proteins, including intimin, are important antigens which give bacteria the ability to adhere tightly to host cells [155-157]. It can be assumed that vaccine-induced antibodies against these surface antigens would effectively hamper the adherence of the challenge bacteria to the target cells [158,159] Bacterial ghost (BG) is produced by the expression of PhiX174 lysis gene E, and results in cellular lysis and cytoplasmic loss. BG maintains the cellular morphology and native surface antigenic structure, and displays the adjuvant property [160,161]. The effects of BGs vaccines have been demonstrated in various pathogens, such as *Vibrio cholera* [162], *Pasteurella haemolytica* [163] and *Helicobacter pylori* [164]. The Bacterial ghost has been developed to be the candidate vaccine against the infection of *E. coli* O157:H7 [165,166]. The use of such procedure could induce Stxs-specific antibodies in sera induced by the surface expression of Stxs antigens in BALB/c mice but failed to induce Stxs-specific IgG antibodies in irrigating solution [167]. May be the reason for this results is the interruption of intestinal mucosa against IgG antibody and the low titer of IgG antibody. The Stxs-specific antibodies could neutralize the Stxs, and the antisera could provide effective cross protection against other subtype of Stxs but the low titer of the Stxs-specific antibodies is a problem to reach by increasing of toxoid on the surface of *E. coli* O157:H7 [166].

Immunization in catles with vaccine candidate composed by recombinant *E. coli* O157:H7EspA, intimin and Tir resulted in the generation of antibodies capable of cross-reacting with antigens from non-O157 EHEC serotypes, suggesting that immunization with these antigens may provide a degree of cross protection against other EHEC serotypes [168]. Further studies are now required to test the efficacy of these vaccines in the field, and to formally test the cross-protective potential of the vaccines against other non-O157 EHEC. Interesting could be the use of different carries and adjuvants to test the effectiveness this vaccine candidates including the Heat-labile toxin B subunit (LTB) of enterotoxigenic *E. coli* (ETEC). The LTB can be used as an adjuvant, carrier of fused proteins, and antigen itself. Immunization of B Subunit-Whole Cell Cholera Vaccine LTB protein induced humoral and secretory antibody immune responses and resulted in cross-protection against Diarrhea Associated with Heat-Labile Toxin-Producing *E. coli* in humans [169]. This indicated that LTB protein associated with other antigens can be a promising vaccine candidate against ETEC. Another promising adjuvant and carrier is Stx1B. The adjuvant property of Stx1B is well known, this protein can mediates adhesion between O157:H7 and target cells by Stx2 [170]. The two Stx toxins are complex holotoxins within the basic 1A:5B structure and two subunits [171,172]. At amino acid level both Stx toxins show high similarity: the A and B subunits of Stx1 and Stx2 are 68% and 73% similar. But immune logically they are different [173,174]. A hybrid StxA2/StxB1 holotoxoid and a genetic Stx2B-Stx1B could elicit neutralizing

antibody response, and a safer and higher production of genetic toxoid SAmB which could induce cross-neutralizing antibodies has been found recently [167,175,176]. Intimin, like other surface proteins, are important antigens which give bacteria the ability toad here tightly to host cell and in consequence it is considered antibodies induced against these surface antigens would effectively prevent adherence of the challenge bacteria to the target cells [158,159]. The use of this model of vaccine candidate, higher intimin-specific IgG antibody was induced and higher titers of antibodies were obtained generating stronger protection against *E. coli* O157:H7 [166].

The development of vaccines for cattles, a natural reservoir of *E. coli* O157:H7 may be the most important target of vaccine development programs. Epidemiological studies indicate there is an association between higher rates of human infection with *E. coli* O157:H7 and the areas associated with higher cattle densities. This is the result of the logical linking of high cattle densities and the greater chance of infestation by human-animal and human-human contacts, as well as the extension contamination throughout the different steps of food chain in animal production: contamination of meat, vegetables, fruits and water, etc. Livestock, including cattle, are routinely immunized against common pathogens to prevent infection and disease. Incorporation of an additional vaccine against to *E. coli* O157:H7 into existing animal health management protocols will reduce the possibilities of foodborne outbreak cause by this bacterium.

Bacteriophage-based biocontrol

Among the various assessment available to control enteropathogenic *E. coli* (i.e. probiotics, prebiotics, oligosaccharides, antimicrobial peptides and essential oils) the use of bacteriophages are starting to receive increased attention due to their special biological characteristics. Bacteriophages are viruses causing lysis of the host bacteria and were discovered by Twort in 1915 and d'Herellein 1917. Since that bacteriophages have been extensively studied and in recent years have exploited as a tool to control bacteria [177,178]. Bacteriophages have widespread distribution, self-replication and a lack of effects on the normal microflora of treated animals [179]. They can horizontally transfer genetic material and affect bacteria. They are very common in all natural environments and play an important role in bacterial evolution [180], including in the evolution of enteropathogenic bacteria *E. coli* O157:H7. The increase in development of multidrug, particularly multi antibiotic, resistant bacteria has become a global public health problem which has prompted research into the development of alternative disease control strategies for the animal production industry and has received more attention by the food industry and medical science [181-183].

The U.S. Food and Drug Administration (FDA) approved Listex P-100 (Micros Food Safety, Netherlands, www.foodsafetynews.com/files/2013/05/Listex) and EcoShield (Intralytix Inc., USA, www.businesswire.com/news/home/20110614007247/en/Intralytix) for the commercial use of one or more specific bacteriophages to clean hard surface in food processing plant and to reduce the risk of meat contamination by *Listeria monocytogenes* (*L. monocytogenes*) or *Escherichia coli* O157:H7, respectively. The test of such technology showed the inhibitory effect of bacteriophage cocktails on E. coli O157:H7 (on hard surfaces and in tomato, spinach, ground beef, and meat [184,185] Bacteriophage BPECO19 strain was selected for the bio control of *E. coli* O157:H7 under in vitro condition and on beef, pork, and chicken meat the results [186,187].

Bacteriophages can be an alternative approach against bacterial pathogens with the flexibility of being applied therapeutically or for biological control purposes by affecting host bacteria without induction selection pressure for resistance, without exerting selective pressure resistance, as do antibiotics. The establishment of Good Practices in the different operations of animal production as well as in controlling zoonotic human diseases by reducing the bacterial load spread prevents the *E. coli* O157:H7 spread from food chain supply to humans through the milk, meat and residues. The use of phage to control pathogenic *E. coli* in pigs, calves and lambs are very encouraging [180]. *E. coli* O157:H7 and other Shiga toxin producing bacteria are relative abundant in their natural reservoirs and carries and for obvious reason, specific phage was isolated from swine feces [188]. In a previous work a 4.2 × 107 PFU/g of the *E. coli* O157:H7 specific phage PP01, indicating that phage PP01 might suppress its host *E. coli* O157:H7 in the gastrointestinal ecosystem isolated from swine stool sample have been isolated [189].

The antibacterial ability of phages in *E. coli* has been investigated [190]. The efficacy of a two-phage mixture against infection induced by the ETEC strain P433 in neonatal pigs has been evaluated. In an in vitro experiment, both phages showed a high capacity to lyse bacteria with nine particles of P433/1 and four particles of P433/2 required to completely lyse broth cultures of their respective hosts [32,190]. In addition, the results of this work indicated that phages that targeted colonizing pili were more effective in controlling a larger proportion of the porcine ETEC than phages that target other pili. These observations show us the importance to isolate specific phages from specific hosts to obtain better results in phage-mediated control of enteropathogenic *E.coli* [191]. conducted an experiment in swine using anti-ETEC phage therapy. Six phages lysing the ETEC strain O149:H10:F4 and three phages lysing the ETEC strain O149:H43:F4 were isolated with 10strains of ETEC used in total. For 85 strains of O149:H10 ETEC, Phage GJ1-GJ6 lysed 99–100 % of them, while for 42 strains of O149:H43 ETEC, only 0–12 % strains were lysed by phage GJ1-GJ6. Three other phages (GJ7-GJ9) selected against an O149:H43 hoststrain lysed 86–98 % of 42 strains of O149: H43 and 2–53 % of strains of O149:H10 [192]. Subsequently, the phages (GJ1 to GJ7) were individually assayed for their capacity to treat an experimental infection with an O149:H10:F4 enteropatho genic *E. coli* in weaned pigs an important reduction in severity of symptoms (diarrhea and the composite diarrhea) was observed. Better results were obtained in prophylactic treatment supplemented with a combination of three phages, a clear indication that specific selected phage cocktail was effective in controlling the experimental infection induced by ETEC strainO149:H10:F4 [192]. Waddell et al. [193], successfully control *E. coli*O157:H7 in experiment with similar characteristic but carried out in calves. They orally inoculated a mixture of six phages on days: −7, −6, −1, 0 and 1 after oral inoculation of animals with pathogenic *E. coli* O157:H7. (10^9 CFU). The results obtained with pigs and calves reinforce the idea that treatments with multiple doses and different

administration times are important in effective phage therapy, which will make significant differences to the effectiveness of phages: This experiment has other important results. The results proved the effectiveness of phage treatment in controlling *E. coli* in polygastric (ruminant) animals, the suitability to use this approach as an important preventive treatment.

One important source of food contamination by *E. coli* O157:H7 is the transmission of the bacterium from fecesonto meat during slaughter [32,194]. Could be a phage cocktail be used to remove or decrease bacteria on meat car casses? O'Flynn et al. [185], demonstrated that a phage cocktail which consisted of phages e11/2, e4/1c, and pp01 pipetted medially onto nine slices of meat contaminated with a rifamp in-resistant derivative of *E. coli* O157:H7strain P1432 can decrease the of this bacterial strain in the meat. It was significant tat among those phage-treated phage samples; seven of the nine samples were completely free of bacteria but control non-treated pieces of meat were positive, exhibiting counts of *E. coli* O157:H7 of 10^5 CFU/mL [185]. The results are very promising and probably this approach can be viable to use in other types of animal production. A phage cocktail (PC1), able to lyse a variety of *Salmonella enterica* (formerly *Salmonella choleraesuis*) was modified to use the broad host-range phage Felix O1 and three phages isolated from sewage sludge and water. Phage cocktail of PC1, which was applied to pig skinartificially-contaminated with multi-drug resistant *S. typhimurium* U288, produced a significant ($P < 0.05$) decrease in *S. typhimurium* U288 [195]. The use of a MOI (Optimal Multiplicity of Infection) in excess of the bacterial concentration seems to be closely related to the effectiveness of the treatment [32]. It is significant that the low temperature (4 °C) required for meat storage did not decrease the passive activity of the phage, indicating that the contaminating *Salmonella* ssp. could be eliminated by phage before potential exposure of consumers to meat products.

The effectiveness of bacteriophage-base anti enteropathogenic bacterial control depend not only to the host-range of phage but also in bacteriophage concentration and conditions of preservation before use or administration [187,197, 198].

Prebiotics

Prebiotics are non-digestible food ingredients that stimulate the growth of bifidogenic and lactic acid bacteria in the gastro-intestinal tract and typically, the prebiotics consist of dietary fibers and oligosaccharides [199]. Prebiotics have a positive influence on the gastrointestinal tract's immune system, and is often referred to as gut-associated lymphoid tissue or GALT and works to protect the body from invasion [200-202]. The GALT is an example of mucosa-associated lymphoid tissue. Gut-associated prebiotic have distinct origins and manners of action. Some of the most well-known prebiotics are: Inulin, mannooligosaccharides, fructooligo saccharides, arabino galactans, β-glucans [203,204]. Prebiotic preparations have been used for the optimal gut function, for favoring the proliferation of normal bacterial flora, and for impeding the growth of pathogenic organisms preventing different diseases [205]. The consumption of prebiotics can modulate immune parameters in GALT, secondary lymphoid tissues and peripheral circulation. Probiotic and prebiotic administration manipulate the intestinal bacterial community, accelerating the growth of commensal bacteria. *In vivo* experimental result from animal studies and human trials suggest that probiotics decrease the incidence of *E. coli* [204,206]. Prebiotic supplemented formula increase stool colony counts of bifido bacteria and lactobacilli in preterm neonates without adversely affecting weight gain [207]. Probiotics do reduce *E. coli* O157:H7 shedding in experimental-challenged immature calves and adult cattle. To be effective as pathogen mitigation agents, probiotics or prebiotics must escape fermentation in the rumen and digestion in the abomasum prior to reaching any pathogen colonization sites in the intestinal tract [208]. There is evidence to suggest that prebiotics do survive the rumen environment [150] and as such, these agents may be useful for addressing pathogen issues and disease. Fructooligo saccharides (FOS) are being increasingly included in food products and infant formulae due to their laxative effect. Their consumption increases fecal bolus and the frequency of depositions, reducing instances of constipation, considered one of the growing problems associated with inadequate fiber diet consumption in the modern society and neonates [209,210]. Eco-friendly alternatives to the therapeutic use of antimicrobials are always being investigated. *Salmonella typhimurium* and *Escherichiacoli* O157:H7 are one of the major etiological agents of food-borne illness in humans. The prebiotic, CelmanaxTM, formulated with a non-living yeast cell walls or MOS, acts as an anti-adhesive for Shiga toxin producing *E. coli* O157:H7 colonization and a mycotoxin in vitro. The CelmanaxTM also improves milk production and feed conversion efficiencies in dairy cattle [211,212].

Probiotics

Probiotics, a living "microorganisms which when administered in adequate amounts, confer a health benefit on the host' [213]. The interest for those organisms and their use is increasing in the world during the last two decades. They play a role in the stabilization of the intestinal micro flora by competition against pathogens [214], reduction of lactose intolerance [215] prevention of antibiotic-induced diarrhea [216] and stimulation of the immune system [217], are just some of commonly recognized benefits. Microorganisms categorized as a probiotic must exhibit resistance to technological processes used in preparing the vehicle of probiotic delivery and produce antimicrobial substances [218]. It is clear that the inhibition of *Escherichia coli* by ingestion of a selected consortium of specially adapted probiotic microorganisms. A combination of *L. plantarum*, *L. fermentum, B. bifidum* LMG 11041, *B. longum* LMG 13197 and *B. longum* Bb 46 reduced *E. coli* resulted better to control *E. coli*. For the control of *S. aureus*, *Lactobacillus ssp.* strains were displayed better than others inhibitory effect. Lactobacilli strains are very flexible for growing and show a great adaptation to different conditions of application [219-221].

Theantagonistic effects of the probiotic cells towards the pathogensare mostly related to the ability of the strain to excrete the broad spectrum antimicrobial substances [222]. Therefore, the results suggest that exposure of the probiotics did not have negative effects on the ability of the probioticsto excrete the antimicrobial substances, a phenol type that is directly linked to pathogen inhibitory abilities of probiotics. The pre-adaptation of probioticsto multiple stresses enhanced their anti pathogenic

effects. The main advantage of using combination of probiotic is that they display beneficial effects against a wide range of nutritional, pathogenic, disorders and water regulation disorders [223]. We were able to detect better rates of weight grow rates when a mixture of probiotic was administrated to chickens during 12 weeks, but in our experiment the best results were obtained when the probiotic combination was supplemented with two prebiotic compounds enhanced hepatic function and immunological activity at the intestinal membrane level [de la Riva and Lopez, 2016, personal communication]. Probiotic is already important in clinical protocols and in nutritional practice in both humans and animal. It was reported that he administration on single probiotic as well as probiotic consortium can inhibit pathogen adhering to the human intestinal mucus [224]. As well as other authors I think that a consortium of probiotic, with different type of action, can better inhibit pathogen.

Probiotics inhibit the virulence-related gene expression in *Escherichia coli* O157:H7 [225] affecting the attachment of this enterohemorrhagic bacterium to host intestinal epithelial cells, an essential step for the development of hemorrhagic colitis and hemolytic-uremic syndrome in humans. Genes involved in attachment are carried within a LEE (Locus of Enterocyte Effacement) known to be directly activated by QS (Quorum Sensing). In presence of a *Lactobacillus acidophilus*-secreted production and the expression of several virulence-related genes in EHEC O157 are affected at transcriptional level. *L. acidophilus* strain La-5 showed significant inhibition in the expression of the LEE genes and AI-2 production by EHEC O157 affecting the ability of E. coli to adhere to and cause lesions on mammalian cells [225].

The enhancement of the pathogen inhibitions will therefore be useful in the probiotic concept, but also is important if their action is focused not only to pathogen control but also to promote beneficial microbiota, promote digestion and nutrient absorption and to protect hepatic and kidney functionality.

The problem of coi nfections and multiparasitism, the need of interdisciplinary approaches

Confection is a recent definition based on the facts that pathogens or parasites co-exist with other sharing the same environment in both the free life and inside of the same host. But probably the first the first attempt resulted when in the medical practice appeared the term of “syndrome”. This term allowing defining syndrome is a set of medical signs and symptoms that are correlated with each other and, often, with a specific disease according to their symptomatology, when host are independently infecting by more than one parasite.

More than 80% of all known species are considered parasites, including viruses, bacteria, flatworms, nematodes, protozoa and fungi. They are pathogen agents that depend to a host species to live and survive, provoking pathogenic process of different degree of health impact to that host [226]. Most parasites co-occur with other parasites and they regulate the populations of a wide number of hosts in all ecosystems by establishing certain life conditions for survival [227-229]. For this reason, parasitism becomes a tool of natural selection and drives evolution playing a significant contribution to biodiversity [186,230]. Organisms are a living community sharing specific environmental conditions forming co-evolutionary units. In those units include environmental and climatic conditions, host density levels, host behaviors, or host physiological conditions can promote co-infections because the same factors promote their presence without any synergistically interactions. It is interactions, rather than associations, among parasites that play a major role in structuring both parasite populations, both within and among hosts, and host populations [231,232]. Such situation implies several methodological challenges. When the multilateral assessment is evident, the selection of research model became an obstacle and represents a serious challenge to be reached at the beginning of the research process.

Gastrointestinal diseases, such as causing by *Escherichia coli* O157: H7 and other ETEC are one of the fields that clearly show the necessity of that interdisciplinary approach. Those diseases implicate a group of pathogens sharing not only the human being as a host by also farm animals, natural sources, reservoirs and routes of infection mainly through the domestic animals, poisoned food, contaminated water source, etc. There are also numerous similarities in symptoms, possible complications and affectations of these vital organs and body systems. The combination of several of these pathogens possibly leads to widespread organ failure with a fatal end and it should be analyzed and manage as a multi parasitism event. There are two non-etiological factors complementing this situation, one is the climatic change and the other is the globalization. The climatic change effects will differ from one pathogen to other. But there are coincidences that warner temperature in the world affects disease transmission enhancing the reproduction of pathogens, host or vectors. That is the case West-Nile virus, a mosquito-borne vital diseases and its transmitting agent [233]. It’s also clear the affectations over the land and water source, and over the animal, plant and human habitats and their pathogens [234]. The biology of each organism will be determinate their behavior to climate change. But it’s clear that it’s clear that predict how climate change influences on pathogens and their life cycles are uncertain [235].

Fragmentation of research and knowledge on different pathogenic agents allow the modeling of the studies given the possibility to understand individual process but them we must be to integrate the obtained information and put it into a more complex model, a network resulting from complex intra and inter relationship between different organisms and environmental conditions. Another important factor is environmental situation will change economic, political and social interrelationship between diseases and individuals and communities. Another very important factor is globalization. Globalization is a reality where the improvement in economy, transport, communication, political y social life cut the distances and cultural differences. But the increasing in relationships, movement of goods and persons is accompanied by the movement of pathogens and their transmitting vectors, asymptomatic host and reservoir, raw and processed food, products, animal, plants and seeds. All these together contribute to quickly spread of diseases and to transform outbreak into epidemic diseases. The climatic change also stimulates the pathogens to jump from habitual hosts and reservoirs to other species, becoming into new hosts or reservoir [235].

ANIMAL PRODUCTION AND HUMAN HEALTH

Animal production and Escherichia coli O157:H7

The root of understanding the epidemiology and behavior of *Escherichia coli* O157:H7 is to explore the original reservoir, or ecological niche, where this bacterium lives according to their natural life cycle. The second point is to know the circumstances of contact of humans with this pathogen. Although is most frequent the isolation of enteropathogenic EHEC from animal to human, and from human to human, the incidence in food and water safety have been reported [7,236].

This review surveys the literature on carriage and transmission of enterohemorrhagic *Escherichia coli* EHEC serotype O157:H7. EHEC O157:H7 strains are hosted by healthy cattle and other ruminants. But in general, the majority of bovine strains are not transmitted to people, and for this reason they don't present virulence factors responsible directly with human disease. This fact led to underestimation of prevalence in livestock of *Escherichia coli*O157:H7 and other EHEC. But taking the farm as a particular type of environmental system shared by different host species, the prevalence may extend for months or years. There are also some carriers displaying the ability to harbor high quantities of this bacterial pathogen for significant larger periods. Polygastric or ruminants, and particularly cattle are considered the major reservoir of EHEC O157:H7. Other ruminants like sheep, goats and deer, are also considered reservoir. With a low frequency EHEC O157:H7 have been isolated from other animals probably because of ingestion of meat, food, and water contaminated by ruminants' stools [97] but in those reservoir the infection is asymptomatic with a few exceptions such as diarrhea in calves [237].

The prevalence of EHEC in animal seems to be seasonal. In cattle the peak of EHEC O157:H7 is observed in the summer and is higher in post weaned calves and heifers than in younger or older animals. The virulent strains of EHEC O157:H7 is not frequently host of pigs or chickens, but isolation in turkeys has been reported. In the wildlife EHEC O157:H7 hasn't been isolated, with exception of deer and sporadically occur in amphibian, fish, and invertebrate carriers More exceptionally are the reports in domestic animals and can colonize plant surfaces and tissues via attachment mechanisms different from those mediating intestinal attachment. Undoubtedly, the prevalence in farm and food producing animal remain us that any step in animal-based food chain supply is susceptible and can be invaded and contaminated by EHEC. This is an important aspect to be considered when sanitary, safety and quality controls must be adopted and updated in any of productive step. These controls must be integrative and suitable form to warrantee the safety of production chain throughout Good Production, Manufacturing, Processing, Transporting, Storage and Commercialization Practices, and the establishment of suitable safety and quality control system like Hazard Analysis and Critical Control Points (International HACCP All) [238]. The system was conceived during the 1960s when the US National Aeronautics and Space Administration (NASA) asked Pillsbury to design and manufacture the first foods for space flights. In 1994, the organization of International HACCP Alliance was established initially for the US meat and poultry industries to assist them with implementing HACCP. Probably the concern appeared since the 1982 outbreak causing by *Escherichia coli* O157:H7 over the importance of EHEC bacteria as pathogenic agents was one of the reasons that led to that decision.

Hazard Analysis and Critical Control Points or HACCP system is a complex preventive approach to food safety from biological, chemical, and physical hazards in production processes that can cause the finished product to be unsafe. This system can be adapted to any specific kind of food production. The system, which is continually being improved and updated, designs measurements to reduce these risks to a safe level. Today in USA, the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) [239] require mandatory HACCP programs many of chain production including, juice and meat, seafood, etc. All other food companies in the United States those are required to register with the FDA under the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. Such regulation also acts for companies that export food to the US [238, 239].

Public health perspective

The One Health approach investigates the complexities surrounding the interplay between the animal, human and environmental domains (Figure 3). Many new and emerging diseases are zoonotic, meaning they can be transmitted from animals to humans. The approach addresses better control of these pathogens by recognizing connections between the animal, human and environmental domains. *Escherichia coli* are a bacteria those are commonly found in the gut of humans and many warm-blooded animals, because of 36-37°C of optimal temperature for growing and mostly they are harmless but other are enterohemorrhagic or EHEC, and are able to cause severe foodborne diseases. The abundance of such strains in terrestrial, aquatic and in air, made the EHEC an important food supply water source and distribution systems, contact with plants, animal and other potential source of EHEC and de diversity in food supply chain operations and trade activities are a potential opportunities for *E. coli* O157:H7 and other EHEC. Foodborne diseases causes approximately 76 million illness just in the United States, including 325´000 hospitalization and 5'000 deaths, with annual medical cost estimated in 5.5-9.4billion. The U.S. Department of Agriculture's, Animal and Plant Health Inspection Service and the National Animal Health Monitoring System (NAHMS) have a program to determine the prevalence of such bacterial strain along the different steps of this production chain, analyzing fecal stool from states with the highest production of swine in the food chain supply. Such kind of surveillance programs exist in many countries including those that were animal production is one of the most important sectors in their economy [238-241].

E. coli O157:H7 is one of the microorganisms classified as Category B. The microorganisms of this group are relatively easy to spread and disseminate, causing moderate morbidity rates, and with some specific requirements in diagnostic capacity and enhanced care and surveillance. There are many pathogens and substance, causing foodborne illness as virus, bacteria, worms and parasites, fungi, animal toxins, plant substances and metals inorganic and organic compounds. It is transmitted to humans primarily through consumption of contaminated foods, such

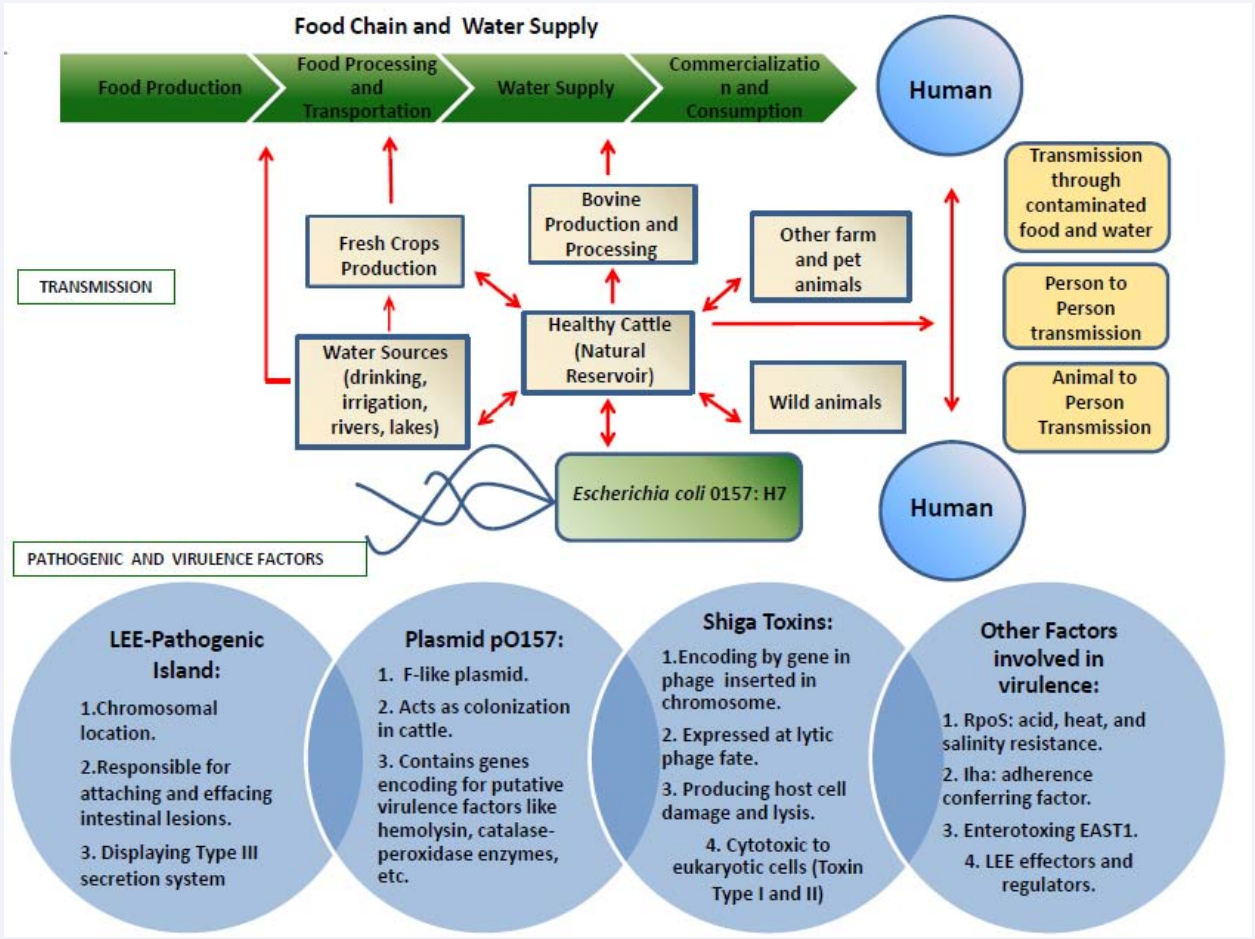

Figure 3 Schematic representation of *Escherichia coli* 0157:H7 transmission to human and the four groups of pathogenic and other virulence factors displayed by the bacteria. The most important routes for its transmission are shown, particularly through cattle (natural reservoir) and other plants and animals serving as reservoir too, and the contamination of food chain and water supply.

as raw or undercooked ground meat products, raw milk and contaminated raw vegetables and sprouts. it was recognized in 1982, following an outbreak in the United States of America. EHEC produces toxins, known as vero toxins or Shiga-like toxins because of their similarity to the toxins produced by *Shigella dysenteriae*. EHEC can grow in temperatures ranging from 7°C to 50°C, with an optimum temperature of 37°C. Some EHEC can grow in acidic foods, down to a pH of 4.4, and in foods with a minimum water activity (Aw) of 0.95. *Escherichia coli* O157:H7 is the most important EHEC sero type in relation to public health; however, other serotypes have frequently been involved in sporadic cases and outbreaks. This bacterium is a worldwide threat to public health. As already was mentioned this strain has been implicated in many outbreaks of food diseases and their derived complications such as hemorrhagic colitis, vomiting, hemolytic uremic syndrome, etc. Some of these diseases provoke fatalities caused by generalized organ failures. The severity of disease, the absence of effective treatment and the latent and potential outbreaks from contaminated food supplies have encouraged an intensive research on whole aspects concerning de behavior of E. *coli* O157:H7, its biology, transmission, and pathogenesis, prevalence in living organisms and open environment. Outbreaks of this strains is caused by ingestion of contaminated meat, contaminated fruits and vegetables; unpasteurized milk and fruit juice; potable and recreational water such as lakes, rivers and streams; and animals at fairs, direct contact with its natural reservoir and carriers. The growing knowledge about this pathogen enhanced the development of prevention, detection and treatments at several moments of food because of the complexity of food supply chain from primary producers to final consumption, with numerous entry points and supply routes, susceptible for the introduction of contaminants and pathogens. In addition in any national food supply system are present items that are brought from other countries, crossing borders in different times according to the characteristic and caducity of imported food, some time in less than one day. The food supply chain system is a delicate economic and social network susceptible to human negligence, technological mistakes and even sabotage and terrorism [244-245].

In 1996, the outbreak occurred in primary schools of Sakai City, Osaka prefecture in Japan. During that incident more than 6000 schoolchildren were affected. EHEC causes not only hemorrhagic colitis but also serious complications such as hemolytic uremic syndrome (HUS). In the Sakai outbreak, approximately 1000 patients were hospitalized with severe

gastro-intestinal symptoms and about 100 victims had complications of HUS, resulting in 3 deaths. Different outbreak involved this pathogen has been developed since the discovery of this strain, and since 1982 it is considered significance as a public health problem [242,245].

This problem is accentuated when additional when outbreak is caused by antibiotic resistant bacteria. In 2011 in Germany, a large outbreak of HUS associated with the rare hybrid strain of Shiga toxin producing *Escherichia coli* (STEC) and bacteria (EAEC) *Escherichia coli* serotype O104:H4 (248). Compared with other outbreaks there are some differences in this case and previous outbreaks of STEC infection, particularly with the incident in Sakai, Japan (1996). The majority of the cases of the HUS (90%) occurred in adults, particularly in women, rather than in children. A total of 3816 cases (including 54 deaths) were reported, 845 of which (22%) involved HUS [244,245]. All isolates classified as the outbreak strain were resistant to all penicillins and cephalosporins and susceptible to carbapenems. The implication of other *Escherichia coli* strain, serotype O104:H4, instead of O157:H7, the antibiotic resistance horizontal transfer and particularly the emerging problem of multi antibiotic resistance in bacteria represent a serous fact increasing the difficulties to manage outbreaks.

An increasing number of outbreaks are associated with the consumption of fruits and vegetables (sprouts, spinach, lettuce, coleslaw, salad) whereby contamination may be due to contact with feces from domestic or wild animals at some stage during cultivation or handling. EHEC has also been isolated from bodies of water (ponds, streams), wells and water troughs, and has been found to survive for months in manure and water-trough sediments. Waterborne transmission has been reported, both from contaminated drinking-water and from recreational waters [239,240].

Person-to-person contact is an important mode of transmission through the oral-fecal route. An asymptomatic carrier state has been reported, where individuals show no clinical signs of disease but are capable of infecting others. The duration of excretion of EHEC is about one week or less in adults, but can be longer in children. Visiting farms and other venues where the general public might come into direct contact with farm animals has also been identified as an important risk factor for EHEC infection. The number of cases of disease might be reduced by various mitigation strategies for ground beef (for example, screening the animals pre-slaughter to reduce the introduction of large numbers of pathogens in the slaughtering environment). Good hygienic slaughtering practices reduce contamination of carcasses by faeces, but do not guarantee the absence of EHEC from products. Education in hygienic handling of foods for workers at farms [240], abattoirs and those involved in the food production is essential to keep microbiological contamination to a minimum according to Codex Recommended International [241-243]. The only effective method of eliminating EHEC from foods is to introduce a bactericidal treatment, such as heating (e.g. cooking, pasteurization) or irradiation [243-245].

Outbreak investigations, especially for emerging pathogens such as *E. coli* O157:H7, pursuits a better understanding these pathogens' epidemiology, because they can affect policy and behavior changes in public health systems particularly in epidemiological assessments and security in food supply chain [244-245]. The study of transmission routes, food vehicles, outbreak size, and clinical outcomes over time empowers public health officials, regulatory agencies, and health educators to target appropriate interventions and reevaluate current prevention strategies. To reduce this public health risk, immunization of cattle would be an effective intervention. Several models have shown that on-farm pathogen reduction programs would significantly reduce the risk of human illness.

CONCLUDING REMARKS

The *Escherichia coli* O157 is one of the major public health problem because of its prevalence within the multiple steps in the food supply chain, social feeding and the risk for animals and people. The problem is complex; the possible outbreaks are latent due bacterial prevalence in animal production sector and derived food processing system, in animal reservoir, animal carries and diverse ecological niches. Although many aspects of their pathogenicity, genetic and its prevalence in the environment have been studied and clarified; many important aspects remain under investigation. In 1996, I was witness of a prominent example of *E. coli* O157:H7 caused outbreak occurred in primary schools of Sakai City (Osaka Prefecture, Japan), telling us that even in countries with the best health care and sanitary standard and well educated society the problem still latent. The control of *Escherichia coli* O157 requires an integral plant of measures of different kinds and scope: from health education and public informative policy to the complex programs of zoonosis and public health epidemiological surveillance and the quality control and biohazard control in food chain supply. The control of this strain by using biotechnological approach such as vaccine development, bacteriophages, prebiotic, probiotic represent a promising approach to keep this problem under appropriate limits of control.

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