

ABSTRACT

The role of *Escherichia coli* bacterium, for a long time known under the main denomination of coli bacillus or colibacillus, in producing alimentary toxi-infections at human beings, had been a controversial role during the time. Some researchers have been reserved regarding the incrimination of this bacterium, which is naturally so ubiquitous and present in a large number, in the digestive tube of human beings and warm blooded animals, in starting up the alimentary toxi-infections. For this reason the cases of diseases at people where only *Escherichia coli* was isolated from aliments, have been reported as alimentary toxi-infections for a long time, but with an unknown etiology.

However, *Escherichia coli* was a very important germ and it was taken into account by the alimentary microbiology since its beginnings, this bacterium being considered the most frequently sanitary indicator used to estimate the dejecture contamination of the aliments. The hygienic rendering of the bacterium presence in aliments and in water, suffered modifications as new data have been obtained. At the beginning there was the tendency to take into account only *Escherichia coli* species as indicator for the dejecture contamination of aliments and of water. Gradually the researchers data have proven that also the species within *Enterobacter* and *Klebsiella* are frequently present in human beings and animals excrements and therefore these bacteria which are present in aliments and water, to be considered as indicator of de dejecture contamination.

Being considered at the beginning only as an opportunist pathogenetic germ, at present *Escherichia coli* is considered as one of the most frequent enteric agent at people and animals.

The significance of *Escherichia coli* species for the public health and for the alimentary hygiene enormously increased within the last years, due to the identifying of O₁₅₇ : H₇ serotype, as an agent leading to severe diseases at people. The pathogenetic action of this serotype at people is bigger than the other's, because it can multiply rapidly in human intestine, it elaborates more toxins which seriously injure the intestinal mucous membrane and other organs, causing not only colitis or enterocolitis, but also haemolytic and uraemic syndrome and thrombotic and thrombocytopenic purpura, having very severe results. The human being can also

get ill when he ingurgitates together with the aliments also small number of bacteriological cells, the minimum poisoning dose being smaller than in case of the most salmonella serotypes. *Escherichia coli* O₁₅₇ : H₇ serotype cannot be found in the digestive tube of healthy people. The bacterium is sometimes carried by the healthy bovines, considered as a main natural reservoir, they eliminate it through dejecture and it can contaminate the bovines' flesh during the slaughtering or other products.

Starting with the description for the first time in 1982 of two episodes of alimentary infection with unusual severe diseases, epidemiologists and microbiologists made the connection between *Escherichia coli* O₁₅₇ : H₇ and haemorrhagic colitis of the patients from whom they isolated it. Due to the association with haemorrhagic enterocolitis, this bacterium has been named Enterohaemorrhagic *Escherichia coli* (EHEC). This pathotype presents a special importance for the human and veterinary medicine. Within the human pathology *Escherichia coli* O₁₅₇ : H₇ became shortly one of the most dreadful agent of human alimentary toxo-infections. For veterinary medicine, the studies referring to *Escherichia coli* O₁₅₇ : H₇ have proven the the main reservoir is represented by the apparently healthy animals, more frequently at the baby calves and suckler cows.

The bacterium has been isolated also from other animal species, such as swine, sheep and poultry (Francis D.H. and col.,1989; Karcher HL., 1996; Kudva I.T. and col.,1997).

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At present, it is settled that the diarrheic disease produced by *Escherichia coli* non O₁₅₇:H₇ is frequent with the developing countries, as a following a overall and alimentary hygiene deficiencies and very rare within the developed countries, where there can be more often noticed the illnesses produced by O₁₅₇ : H₇ serotype.

On the one hand, taking into account the gravity of the alimentary toxo-infections at people and on the other hand the role of the animals as germs reservoir, we'll search for the presence of O₁₅₇:H₇ serotype in some products of animal origin (dejecture, meat and products from meat, milk and products from processed milk) come from different animal species.

The dissertation consists of two distinct parts. In the first part, based on the bibliographical data, it is done a synthesis of the speciality literature. Among these, there are included references regarding taxonomy, morphology, biochemical particularities, multiplication and factors affecting the development, but also the epidemiology of the infections with *Escherichia coli* O₁₅₇ : H₇.

The second part “Personal Research” is elaborated during seven chapters, including 106 pages and it approaches a large variety of facets.

In chapter 4 entitled “ The methodology of isolating and identifying the strains of *Escherichia coli* O₁₅₇:H₇ from different products of animal and non animal origin “, it is presented the used method (recommended by FSIS-USDA), based on the slow fermentation of the solbitol , the negative beta glucuronized feature of the bacterium and the agglutination with a specific antiserum regard the antigens O₁₅₇ : H₇. The method includes 3 stages : for the probable detection through a screening test, using ELISA technique for the isolation and confirmation of the bacterium.

In chapter 5 it was investigated the presence of *Escherichia coli* O₁₅₇:H₇ serotype in the dejecture at some categories of bovines. In this respect, a number of 652 samples of dejecture has been collected from three farms for breeding bovines for milk production, from baby calves (224 samples), weaned calves (241 samples) and from suckler cows (187 samples).

The analysis of the obtained results reveals that from the 652 samples of dejecture which were examined, 306 strains of *Escherichia coli* were isolated and identified, this number representing 45.39%. From these 17 (5.55%) were biochemically and serologically identified as belonging to *Escherichia coli* O₁₅₇ : H₇ serotype, and 289 (94.45%) *Escherichia coli* non O₁₅₇ : H₇ , 6 (35.29%) were isolated from dejecture collected from weaned calves, 4 (32.35%) from baby calves and 4 (32.35%) from suckler cows.

The resulted differences between age categories (baby calves, weaned calves and suckler adult cows) can be explained by the different development grade of rumina and immunitary system , by the diet and the lack of ingurgitated first days milk or of other factors. The results can also be explained through the difference existing in the rumina functioning at a young bovine and at an adult one. At the adult bovines rumina is a compartment in which the association of a strong concentration of volatile fatty acids and a weak pH, inhibits the growing of *Escherichia coli* O₁₅₇ : H₇.

The obtained results reveal the presence and the identification of *Escherichia coli* O₁₅₇:H₇ serotype within the samples of dejecture collected from different categories of healthy bovines, which were not presenting gastro intestinal manifestations, but it was proven they were bearers and eliminators of this bacterium, so we can suppose this bacterium is a component of the conviva intestinal flora especially of the calves and the cows grown for milk.

The obtained results reveal the presence of *Escherichia coli* O₁₅₇ : H₇ within all the three examined farms and at all the age categories, in different percents, between 4.50% and 6.12%. Thus, within the farm “T”, from 209 examined samples of dejecture, there were isolated 128 (61.24%) *Escherichia coli* strains, from which 6 (4.28%) O₁₅₇ : H₇ serotype and 122 (95.32%)

non O₁₅₇ : H₇. From the obtained results we can notice the large number of isolated and identified *Escherichia coli* strains, which do not belong to O₁₅₇:H₇ serotype, but which can be potentially pathogenetic.

The presence and the isolation of *Escherichia coli* - O₁₅₇ : H₇ serotype from dejecture from different age categories, reveal the risk of spreading of bacteria from the contaminated animals through the environment, or through the equipment from the shelters.

In chapter 6, it was followed the presence and the incidence of *Escherichia coli* O₁₅₇ : H₇ serotype in excrements of swine. The investigation was made on a number of 362 samples collected from two farms for cows breeding. The dejecture were collected from baby piglets, weaned piglets and suckler sows.

From these 362 sample of excrements there have been morphologically, culturally and biochemically isolated and identified 272 *Escherichia coli* strains. From these 9 (3.30%) strains have been identified as *Escherichia coli* O₁₅₇ : H₇ serotype and 273 (96.70%) strains belonged to other serotypes met at swine.

Escherichia coli O₁₅₇ : H₇ serotype was isolated and identified at both farms, but with a different incidence. Therefore, from the analyse of the recorded data, it can be noticed that in farm “P” , from those 194 samples 137 (70.10%) strains of as *Escherichia coli* have were isolated and identified, from which 4 (2.85%) belonged to O₁₅₇:H₇ serotype and 133 (97.15%) to other non O₁₅₇:H₇ serotypes. In exchange, in farm “B”, from 168 samples of excrements there were isolated 135 (80.35%) strains of *Escherichia coli*, from which 5(3.70%) belonged to O₁₅₇:H₇ serotype and 130(96.30%) non O₁₅₇ : H₇ serotype.

The incidence of *Escherichia coli* O₁₅₇ : H₇ serotype differs also depending on the examined swine category. Most of the strains of *Escherichia coli* O₁₅₇ : H₇ were collected from dejecture of the weaned piglets 3(5.17%) strains in farm “B” and 2(4.08%) in farm “P”, followed in a decreasing order by baby piglets and suckler sows, where there were isolated from each category one by one strain of *Escherichia coli* O₁₅₇ : H₇.

The investigations regarding the presence and isolation of *Escherichia coli* O₁₅₇ : H₇ serotype in meat and in products from meat (chapter 7), have been done on a number of 558 samples of aliments in the frame of the “Programme of surveillance and prevention of diseases transmission from animals to human beings”. The samples of aliments have been represented by minced beef and fresh wursts, pork, lamb and different salami.

From the analyse of the obtained results it is noticed that from 558 analysed samples, there have been isolated 286(51.25%)strains of *Escherichia coli*, from which only 7 strains were biochemically and serologically identified as O₁₅₇ : H₇ serotype , which represented 2.45%.

The strains of *Escherichia coli* O₁₅₇ : H₇ serotype were isolated from minced beef 2 (3.38%), fresh wursts 2 (4.65%), different salami 2 (2.75%) and fresh lamb meat 1 (1.25%). To be mentioned that in the fresh pork there wasn't revealed the presence of *Escherichia coli* O₁₅₇ : H₇.

The obtained results prove a reduced incidence of bacterium within meat and meat products, between 1.25% (lamb) and 4.65% (sausages), compared to the presence of the serotype in aliments in other geographical areas of the globe, but similar to other investigations in Romania.

The research regarding the presence and isolation of the species *Escherichia coli* O₁₅₇ : H₇ serotype from milk and some dairy products (chapter 8), have been done on a number of 572 samples represented by 167 samples of crude milk, 128 samples of fresh cheese (cow), 47 samples of sour cream, 105 samples of ewe cheese and 125 samples of sheep milk.

From the obtained results it results that from 572 analysed samples, there have isolated 364 (63.46%) strains of *Escherichia coli*, from which only 6 strains biochemically and serologically identified as O₁₅₇ : H₇ serotype, which represented 2.45%, and 358 (98.36 %) strains – other serotypes non O₁₅₇ : H₇.

The strains of *Escherichia coli* O₁₅₇ : H₇ serotype were isolated from raw/crude milk 2 (2.04%), fresh cheese 1(1.03%), ewe cheese 1(1.39%) and sheep milk 2(2.44%). To be mentioned the presence and isolation of *Escherichia coli* O₁₅₇ : H₇ serotype in all the examined assortments.

The presence and isolation of *Escherichia coli* O₁₅₇ : H₇ serotype in some alimentary products of animal origin, even if in a small number, must warn and increase the interest for the respecting strictly the hygiene measures in order to prevent the alimentary toxi infections at people.

The investigations regarding the identifying the strains of verocitotoxigenic *Escherichia coli* O₁₅₇ : H₇ (VTEC) using the method PCR multiplex (chapter 9), made on a number of 33 isolated strains coming from products of animal origin, revealed the presence of gene *eae* codifies verotoxins VT₁ and VT₂ and so the pathogenity at 24 strains, which represents 71.50 %.

At a number of 7 (20.55%) strains it was accentuated only the VT₂ verotoxin, and at 2 (5.95%) strains the presence of verocitotoxicity being negative and non pathogenetic.

Most of the strains owner of *eae* genes, were identified at those isolated from excrements of bovines and swine-17, followed in a decreasing order by the genetic segments from fresh meat and meat products - 4 and the least at those from milk and dairy products 3 strains.

In order to reduce the risk of contamination and to prevent the admission of alimentary toxi infections determined by *Escherichia coli* O₁₅₇ : H₇ serotype, they tried to elaborate and

apply strategical measure of surveillance (chapter 10), where there are stipulated both general and specific measures, but also measures for the case of enterohaemorrhagic serotypes appearance.

In chapter 11 “ Final conclusions”, there are synthetized the effected research in all 20 formulations.