Yersinia enterocolitica Monographic Study

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Abstract

Germs from *Yersinia* genus have a vast ecologic niche, being met at different domestic and wild animal species, but also in food, water and soil. The majority of yersinis live in the digestive tract of human and numerous animal species, especially rodents, but also in soil, plant debris, waters etc. Numerous species of *Yersinia* genus could produce characteristic infections in human, the main source of infections is represented by rodents and hematophagous insects or, more frequently, by water or contaminated food. In a 1999 study, Mead and coauthors established that the *Yersinia enterocolitica* prevalence in food, in USA, is around 90%. Foods of animal origin more frequently contaminated with *Yersinia enterocolitica* are: pork, poultry, beef and lamb meat, milk, ice-cream, sea fruits etc., among them pork meat and milk represents the sources of the most numerous toxi-infection outbreaks in human, in different world regions. Bacteria determine infections which interest the digestive tract in numerous animal species and human, with diarrhea, lymphadenitis, pneumonia and abortion are the most important symptoms. *Yersinia enterocolitica* enter the human body regularly by oral ingestion, and localize itself with predilection in the distal portion of the ileum and at the ileocaecal appendix and proximal colon level, were determine a terminal ileitis with lymphadenitis, acute enterocolitis, and secondary accompanied with nodosum erythema, poliartritis that could be complicated with septicemia, sometimes leading to death.

Keywords: *Yersinia enterocolitica*, bacteria, epidemiology, incidence.

1. Introduction

During slaughtering and pork meat processing contamination with various organisms can occur, which can depreciate the nutritional value of finished products, and in some cases, can cause consumers illness. Among these microorganisms, genus *Yersinia* species is present, although not all isolates are associated with consumers' disease. This is because not all strains of *Yersinia enterocolitica* are pathogenic for humans.

[1]The incidence of food toxinfections caused by *Yersinia enterocolitica* is far from being known, because of underreporting of all cases. The number of confirmed toxinfections caused by *Yersinia enterocolitica* differs from country to country, depending on the engagement of specialists and the laboratories potential for isolation and identification [39]. Moreover, WHO, through Reference Center for *Yersinia enterocolitica*, presents a situation for *Yersinia enterocolitica* isolates incidence in several European countries, including Romania.

2. History of genus *Yersinia*

Research on the evolution of the genus Yersinia begun over one hundred years ago; the genus name was given by Van Loghem (1944) in honor of A.J. Yersin, a french bacteriologist who, concomitant to Kitasato, isolated the causative agent of human plague in 1894, during great plague pandemy that took as its starting point Canton and Hong Kong. Etiologic agent of this pandemy was originally named *Pasteurella pestis*.

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Pest, plague or "black death" is one of the diseases whose evolution is lost in history, the disease being mentioned BC; the first references are found on the pages of Holy Scripture in the Old Testament [1 Kings 8, 37]. Various epidemics and pandemics that have succeeded decimated the population of some countries and continents [38].
Yersinia enterocolitica was described in 1934 by McIver and Pike as a gram-negative cocobacillus. These authors have described as "*Flavobacterium pseudomallei* Withmore" a gram-negative cocobacillus which they were isolated from two facial abscesses from an old farmer, and from cervical lymph nodes. Since, from the biochemical point of view, the isolate act as glanders agent (*Burkholderya mallei*) as well as *Pseudomonas pseudomallei*, the two researchers concluded that "the possibility to deal with a new species may be less probable than that the organism which we have described to be an atypical form or a variant of a known species". Between 1932 and 1957 about 15 strains were isolated in the USA.
In 1939, Schleifstein and Coleman, working at Department of Health, New York State, drawing attention to the isolate described by McIver and Pike, as well as four other researchers, as being similar to *Actinobacillus lignieri* and, in particular, with *Yersinia pseudotuberculosis*. Because of the five isolates morpho-biological characters were different enough from those of the latter two species, and that the bacteria was isolated from stool of a bacillus carrier, then from a granulomatous lesion on the chest of a 13 years old boy, Schleifstein and Coleman (1947) have proposed the name "*Bacterium enterocoliticum*" for this "unidentified microorganism" [9].
In Europe, Frederiksen has identified one such strain (isolated in 1926-1932) in the Staten Serum Institute Copenhagen. In 1949, Hassig, Karrer and Pusterla were isolated from liver abscesses in two cases of septicemia with fatal outcomes in humans a bacterium similar to the Mallasez and Vignal's bacillus, which was provisionally called Pasteurella X or Germ X. Since 1958, strains were isolated from rabbits in various regions of France (Lucas, 21 strains). In 1960, Dickinson isolated from pigs two other strains, and Frederiksen obtained isolates from sheep in Denmark. During the 1962-1965, epizootics occur in a series of rabbit and chinchilla breeders in North America but also in Western Europe (Switzerland, Netherlands and Germany), by importing animals from North America [10].

3. Description and taxonomy

Germs grouped in the genus *Yersinia* are short bacilli or cocobacilli, with rounded ends, gram-negative, noncapsulated and unsporulated except *Yersinia pestis*, which form a capsule-like shell. They have 0.5 to 0.8 μm wide and 1 to 3 μm long. Grows at 40ºC, do not develop flagella and are immobile at 37°C, but forms peritrich flagella and are motile at temperatures below 30°C, except for some strains of *Yersinia ruckeri* and *Yersinia pestis*, which sometimes are immobile at 30°C. Bipolar stained, and in old cultures pleomorphism was observed. Bipolar staining is observed mainly on smears made from liquid media incubated at 22-28°C. Optimum growth temperature is 28-30ºC [5]. After incubation for 24 hours at 37°C longer forms are observed, both in smears made from liquid media and in those performed from solid media.
Ultrastructure and chemical composition of genus *Yersinia* microorganisms are similar to those of enterobacteria. Thus, 80% water content maintained in a colloidal system proteins, lipids, carbohydrates, minerals and some byproduct of bacterial metabolism. Phospholipids, proteins and carbohydrates have an important role in bacterial metabolic processes [7, 13].
The oldest strains of *Yersinia enterocolitica* appear to have been isolated in 1933 by Gilbert, than in 1934, by McIver and Pike, in the U.S., being reported in "Annual Reports of the Division of Laboratories and Research", Albany. Taxonomic classification of *Yersinia enterocolitica* species dates back to 1963, when Destombes and Mollaret identifies the first strain, isolated in Switzerland by Wyler from a child with diarrhea. Subsequently, strains of *Yersinia enterocolitica* were isolated throughout the world, in various human and animals' infections, and in the environment [13, 32, 34].
In 1964 Frederiksen argued whether the inclusion of *Yersinia enterocolitica* (*Pasteurella* X, *Bacterium enterocoliticum*) in the genus *Yersinia*. In 1967, at the first International symposium Yersinia in Paris, it was recommended changing the name of *Bacterium enterocoliticum* in *Yersinia enterocolitica* and inclusion in the genus *Yersinia*. In 1972, at the Second International symposium Yersinia in Malmo, specialists proposed

classification of genus Yersinia in the family *Enterobacteriaceae*.

Bergey's Manual 2004 [22] group 11 species in genus *Yersinia*: *Yersinia aldovae*, *Yersinia bercovieri*, *Yersinia enterocolitica*, *Yersinia frederiksenii*, *Yersinia intermedia*, *Yersinia kristensenii*, *Yersinia mollaretii, Yersinia pestis, Yersinia pseudotuberculosis, Yersinia rohdei,* and *Yersinia ruckeri.*

4. Epidemiology

Yersinia enterocolitica is frequently encountered in terrestrial and freshwater ecosystems, with higher incidence in cold and temperate climates. The bacterium is widespread in the environment and has been isolated from the intestinal tracts of many species of wild and domestic mammals, but also in rodents, birds, fish, frogs, mollusks, crustaceans and humans [27].

Among animals, the pig is the only species from which Yersinia enterocolitica serotype O3, biotype 4 was isolated with increased frequency, this variety being involved in human illness. Pigs can also be reservoir for serovars O_9 and $O_{5,27}$, particularly in areas in that have been reported human infections with these strains. In countries with a high incidence of human yersiniosis, *Yersinia enterocolitica* has been isolated frequently from pigs in slaughterhouses and butchers [17, 18].

The main way of contamination with *Yersinia enterocolitica* is the oral route, spreading sources being represented in particular faeces and contaminated water and some foods [27].

Both human and animals faeces are an important source of contamination for soil, surface and depth waters, vegetation and food. Some authors noted that contact with animal manure and/or sick people or healthy carriers are the most important source of contamination. Numerous studies have highlighted the species *Yersinia enterocolitica* in surface water (rivers, lakes, fountains) and even in the depth, where it can survive longer because of the low concentration of toxic substances [30, 44].

Regarding the role of food, it can be said that the presence of this species in food is not always associated with illness, so some authors recommend that all food isolates to be subjected to tests in order to highlight the presence of enterotoxins, virulence plasmids, and invasive nature, in order to incriminate the food as a source of contamination [40].

However Kapperud [28] states that *Yersinia enterocolitica* is a food pathogen. Foods that can be contaminated with *Yersinia enterocolitica* are: pork, beef, poultry, and lamb meet, milk and dairy products, especially raw milk, pasteurized milk and milk powder, cream and ice cream, vegetables, seafood etc. [1, 6, 8, 9, 12, 17]. Thus, in Canada and Australia, Schiemann et al. [42] was isolated *Yersinia* both from raw and pasteurized milk and cream. Thomas et al. were isolated 114 strains of *Yersinia enterocolitica* from milk samples in Canada [37]. Instead Fukushima et al. isolated *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from faeces of 618 dairy cows in Japan, but in milk obtained by direct milking from those cows. The authors concluded that the isolates from raw milk do not originate in the mammary gland, main source of contamination being feces and/or contaminated stable [19, 20, 21].

Poultry meat can be the source of *Yersinia enterocolitica* and can contaminate the vegetables, cooked food made in kitchens where products are unhygienic handled or stored in a refrigerator [28, 29]. Robins-Browne [40] presented a cross-contamination model, in which a contaminated raw food (poultry meat), stored in a refrigerator, can contaminate other foods from the same site, that is usually eaten raw.

Slee et al. [43] isolated *Yersinia enterocolitica* biotype 5, serotype $O_{2,3}$ from the gut of 38 sheep and 8 goats in Australia, animals who had diarrhea and a poor state of maintenance. At necropsy were found characteristic micro-abscesses in the intestine of 5 sheep and 3 goats, without being able to establish other causes of death.

Although seafood is not commonly incriminated in the appearance of food toxinfections with *Yersinia enterocolitica* in human, this germ has been isolated from fish and seafood from both the natural environment (seas, oceans) and intensive conditions (aquaculture). *Yersinia enterocolitica* is one of origin enteric pathogens that can contaminate fish and seafood, along with *Salmonella* spp., enteric *E. coli*, *Shigella* spp. and *Campylobacter* spp., says a study conducted in 2000 by Frerk Feldhusen in Germany [15].

In terms of arthropods, so far the researchers don't establish a significant role as vectors for *Yersinia enterocolitica* transmission. However, Fukushima

suggests a possible role of fleas in transmission of bacteria from one pig to another, in farms [19]. Experimentally, *Yersinia enterocolitica* survives four months in artificially infected fleas and ticks [13].
Family outbreaks, with successive cases, involve transmission by direct contact [112, 123, 208]. Remains to accept the indirect transmission, through consumption of food of animal and plan origin or contaminated water, which are main risk factors [5, 11]. Also, in the last years, *Yersinia enterocolitica* become one of the feared agents of transfusion-related sepsis [23].

5. Incidence and pathogenicity

Yersiniosis infections are world wide spread, being both diagnosed in human and animals. Epidemiological factors favoring the spread of infections are not well known yet. The number of bacteria isolations and of confirmed cases varies from country to country, depending on the material possibilities and engagement of specialists and laboratories. In developed countries, the frequency of infection with *Yersinia enterocolitica* is systematized in the laboratories designated for this.
Thus, in Great Britain, Public Health Laboratory Service (PHLS) of Communicable Disease Surveillance Centre (CDSC) receives information from 300 laboratories in England and Wales. A 2007 report from the Health Protection Agency Centre for Infections consists of an analysis of the incidence of *Yersinia enterocolitica* (isolates) from January 1992 to December 2006. Thus, if in early 1992 (January) was recorded a maximum number of isolates (34) by the end of 2007 trend is decreasing, leading to a strain isolated / month [25].
In Europe were reported 11,699 outbreaks of food toxinfections during 1990-1992 and of these, only one caused by *Yersinia* [6].
In 1995 WHO report shows that morbidity by yersiniosis enteritis in Central and Northern European countries in the highest incidence area - Finland, Estonia, Lithuania, Belarus, Denmark - varied between 3.5 and 17.8 during 1988-1992, being three times lower than salmonella enteritis [4]. A study conducted between 1967 and 1996 by two reference laboratories in Belgium led to the isolation of more than 18,700 strains of *Yersinia*, except *Yersinia pestis*, from patients with intestinal infections. Serotype O_3 was dominant (79.4% of strains), followed by O_9 (11.1%). If in the first years of this study the incidence of *Yersinia enterocolitica* increased from 305 cases in 1975, up to 1469 cases in 1986, since 1987 there was a reduction in the number of reported cases, although interest of the laboratories remained constant [47].
Gourdon et al. [24] conducted an epidemiological study about the incidence of *Yersinia enterocolitica* O_9 and *Brucella abortus* in animals and humans in France during 1989-1997. Between 1988 and 1989 there were reported enterocolitis cases caused by *Yersinia enteroco*litica O_9, and cases of brucellosis were almost eliminated. In 1996 it was recorded the maximum incidence of infections by *Yersinia enterocolitica* O_9 in humans (12 cases).
Fukushima et al. [20] performed genotypic differentiation of *Yersinia enterocolitica* O_9 strains isolated in the countries of Eastern and Western Europe by serotyping, ribotyping and restriction endonuclease analysis of virulence plasmid DNA (REAP). It has been establish that is a close correlation between ribotypes, REAP patterns and chronological and geographical distribution of Yersinia enterocolitica O_9 strains. In European countries, clonal types of serotype O9 were found with increased frequency in humans and animals, from the end of 1980.
In North America yersiniosis infections were reported in recent years more frequently as complicated clinical entity, especially serologically confirmed, and often as rare outbreak of food-borne enteritis [6]. Mead et al. [35] shows that the incidence of *Yersinia enterocolitica* in food reaches 90% in the USA, pigs being the main reservoir, but not all serotypes are involved in human disease. The mortality rate is low, estimated at 0.5%, and it is believed that the total number of cases and is about 38 times higher than the reported ones [34].
Following a study conducted in our country between 1990 and 1994 by Vaida et al. [46] on 8363 patients, it was found that *Yersinia enterocolitica* was the etiologic agent of enteritis in only 3.08% of cases (over 20 times less than *Salmonella* and less than nine times than *Shigella*).
In a study conducted in China between 1983 and 1994 on 3601 people, including 956 with enteritis, and 896 animals of different species (pigs, rabbits, rats and guinea pigs), Zheng et al. were isolated 51 strains of *Yersinia enterocolitica*. Of these, 43 were isolated from pigs (all of serotype O_3) and

only 6 virulent strains from humans - two of serovar O_9 the four of O_3 [48].

Sulakvelidze et al. [45], from the Center for Infectious Disease Control, Georgia, were isolated 2493 strains of *Yersinia enterocolitica*, 22 strains of *Yersinia pestis* and 21 strains of *Yersinia pseudotuberculosis* from the 130 574 clinical samples analyzed.

In New Zealand the researchers managed to isolate two new serotypes of *Yersinia enterocolitica*, O_{77} and O_{78} (25 strains) from humans, cattle, sheep and goats [16].

In Japan, Fukushima et al. [21] investigated the prevalence of *Yersinia enterocolitica* in food, resulting values of 3% for pork meat and 0.3% for beef and poultry meat. The authors draw attention to the possibility of introducing new pathogenic serotypes of *Yersinia enterocolitica* by imports [19, 21].

In Norway, *Yersinia enterocolitica* is an important cause of gastroenteritis in humans [29, 41]. Saebo et al. [41] have detected antibodies (IgG) anti-*Yersinia enterocolitica* O_3 by ELISA in 56 (7.4%) of 755 Norwegian soldiers evaluated. An increased incidence of *Yersinia enterocolitica* O_3 was registered in young soldiers in Oslo, with 12 positive patients of 56 (21.4%). Nesbakken et al. [36] studied the presence of antibodies against *Yersinia enterocolitica* O_3 among pig slaughterhouse staff, vets and military in Norway, showing that occasional contact with pigs is an important risk factor for developing infections.

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