

## ABSTRACT

Animal origine aliments are irreplaceable trofines from human food. To corresponde with the aim, these don't have to affect consumer health, meaning these have to be free of any physical, chemical and biological noxe. Plus, these have to present qualitative and nutritional properties and to be fresh.

In order to corresponde to the enounced desideratums it is compulsory, from the beggining, to assure animal origine aliments primary salubrity.

From the bacteria used as biological indicators, *Clostridium perfringens* is the one that can reveal the oldest contamination.

The infections produced by *Clostridium perfringens*, named as Welchiozes as well are sparse in animals, the germ being found almost unfailing in their digestiv tract, as well as in soil. *Clostridium perfringens* is a virulent and toxigen bacteria.

Because of those characteristics it can produce different states of disease, the most important being gasgangrene and food toxiinfection. To produce food toxiinfection, the main pathogenity element is represented by enterotoxin which is elaborated in human small intestine, shortly after ingesting aliments with biggest amount of vegetative cells.

Food toxiinfections produced by *Clostridium perfringens* are toxic type affections, at their origine being the ingestion of contaminated aliments with bacteria and which, from the anatomoclinical point of view, in function of causal agent biotype, there are manifested by ordinary enteritis, or as serious necrozant enteritis.

The presence of *Clostridium perfringens* is possible in all uncooked aliments and in many of the cooked ones. It can be said that this bacteria is present in all plants contaminated by soil, in fresh milk, fresh meat and even in some processed products such as: condensed milk, various cheese sortiments, semipreserved and some preserved. From all of these the meat is most often implicated in food toxiinfections appearence. The incidence of *Clostridium perfringens* is increased (aproximatively 50 %) in the meat of butchery animals and chickens. These animals are almost all intestinal carriers. During slaughter stress or when the time between slaughtering and evisceration prolongues, a part of intestinal bacteria through which *Clostridium perfringens* too escapes from intestin and through lymphatic and blood circulation covers entire organism, inclusively in deep muscle thickness, where after 7-9

hours from slaughter, even strictly anaerobe bacteria will find convenient conditions for multiplication.

The thesis entitled „**Bacteriological, serological and pathogenity researches regarding the presence of *Clostridium perfringens* in animal origine aliments**” contains 153 pages, splited in IX chapters and it is structured conformable with effectual criteria, in two parts.

First part (chapters I, II and III) represents **36,4%** and synthetises main bibliographical datas from speciality literature regarding the elements of taxonomy and morphophysiological peculiarities of *Clostridium* genre bacteria, datas concerning epidemiology of infections produced by *Clostridium perfringens*, the implications of *Clostridium perfringens* in alimentary toxiinfections constuting „The actual stage of knowledge”, and the second part (chapters V, VI, VII, VIII ) is refering to personal researches, representing **63,6%**. Each chapter from the second part is structured in material and method, obtained results with their disscutions and partial conclusions.

In IXth chapter, 10 final conclusions are synthetised, main aspects detached after researches were finished.

The paper is ilustrated with 47 figures, 35 tables and is based on 141 bibliographical titles.

The aim of these researches consisted in *Clostridium perfringens* evidencing in food consumed by humans, in epidemiological conditions previously mentioned.

The researches were based on the following objectives:

- Isolation and identification of *Clostridium perfringens* from various aliments of animal origine: meat and meat products, milk and dairy products, eggs and eggs products;
- Identification of *Clostridium perfringens* biotypes isolated from some animal origine aliments;
- Determination of the pathogenity of strains isolated from some aliments;
- Prevalence of *Clostridium perfringens* strains isolated and identifiacted from some animal origine aliments.

In first 3 chapters are synthetised some bibliographical datas regarding taxonomy morphophysiological elements of *Clostridium* genre bacteria, datas regarding epidemiology of infections produced by *Clostridium perfringens*, implications of *Clostridium perfringens* specie in aliment toxiinfections.

Clostridias are a part of sporogen Gram positive bacillus group, respectively *Bacillaceae* family. These are found as saprophyte in soil and are resistant versus environment factors.

Bacteria from *Clostridium* genre are all anaerobes, mobiles in great majority, and the spore exceeded transversal diameter of the vegetative cells and in function of his position, deforms it engraving sporulated bacillus various shapes, such as lemon or shuttle (central spore), glass of oil lamp (subterminal spore).

*Clostridium perfringens* has multiple somatic antigens, some of them being common and some others being specific for the strains that form a certain serotype. Inside biotypes, various antigenical types can exist. So, biotype A contains 8 antigenical types.

Vegetative forms doesn't present a particular resistance toward various physical and chemical factors, being destroyed in 15 minutes at 63-65°C, spores in change, resisting at more increased temperatures.

*Clostridium perfringens* is the most sparse from all microorganisms that can provoke infections in humans, it has a universal spreading and is found in every environment, soil, water, air, any surface, any aliment that weren't sterilised. In humans and animals is found on the skin, mucous membranes and 100 % in intestinal content. Those facts reveals the importance of this bacteria discovering and determination of probable number in raw and pasteurised milk, such as products of any type.

*Clostridium perfringens* was described by various authors as being present in many aliments with different degrees of freshness and in altered aliments under vegetative form or as a spore.

Concerning the value as a sanitary indicator of *Clostridium perfringens*, comparative with bacteria from coliforms, Enterococci group and with bacteria from *Proteus* genre, *Clostridium perfringens* proved his value as a microbiological sanitary indicator of food contamination because of its existence in human and animals faeces.

In the second part, „Personal researches”, elaborated in V chapters, investigations followed bacteriological researches regarding isolation and identification of *Clostridium perfringens* in alimentary products (V<sup>th</sup> chapter), serological characterisation of *Clostridium perfringens* strains, isolated from aliments (VI<sup>th</sup> chapter), determination of pathogenity in isolated strains of *Clostridium perfringens* (VII<sup>th</sup> chapter), prevalence of *Clostridium perfringens* in investigated aliments (VIII<sup>th</sup> chapter).

Researches were made in Laboratory of Microbiology – Immunology from Faculty of Veterinary Medicine Iași, Laboratory of Food Microbiology and Laboratory of Bacteriology from LSVSA Iași.

During 2005-2009 were harvested samples from various animal origine aliments, respectively bovine, ovine, swine and broiles chickens carcasses, meat products (salami), milk and dairy products, eggs and eggs products, on the carcasses surfaces being realised qualitative and quantitative microbiological exams. The presence of *Clostridium perfringens* was followed.

In order to identify *Clostridium perfringens* European Standard EN 13401:1999 was used.

The study efectuated over bovine, swine, ovine and chickens carcasses revealed the presence of *Clostridium perfringens* in number of 7 positive strains from 60 examined bovine carcasses, 3 strains on the ovine carcasses, 6 strains from swine carcasses and 2 strains on chickens carcasses.

Investigated meat products presented the following values: from the fresh and semismoked salami samples were isolated 10 strains that represented 10%, from the longue term salami samples were isolated 2 strains, representing 2%.

From the category of boiled and smoky specialities, 3 strains were isolated, representing 3% from the total examined samples, and from the category of smoky and boiled specialities 2 strains were isolated, representing 2%.

From the total examined milk and dairy products resulted a number of 38 *Clostridium perfringens* strains.

The samples of curd, yougourt, kefir and sana didn't revealed any positive samples.

Eggs samples revealed a number of 36 isolated strains on the eggs harvested from farm system. Increased number of positive samples reflected eggs contamination during lay.

From the powder egg samples and eggs came from the encasisng centres, not a strain of *Clostridium perfringens* hasn't been isolated.

*Clostridium perfringens* produces 12 toxical fractions that represent the main pathogenity factor. The capacity to elaborate one or more of the major fractions constitutes the criteria to clasify *Clostridium perfringens* strains in biotypes.

There are 5 *Clostridium perfringens* biotypes: A, B (B1, B2), C (C1, C2, C3, C4, C5), D and E. In the speciality literature there is mentioned biotype F as well, which was recommit in C4 and C5 biotypes.

The aim of investigations consisted in making an hierarchy of the most frequent isolated serotypes, because ones of them (A,C,D) are implicated in producing alimentary toxii infections. These biotypes differ one from another by the type of elaborated toxin which is accomplished using seroneutralisation reaction with biotype antitoxic serums, by animal specie which is infected and morbid entity that determines.

As a result of immunofluorescence on smears with different cultures of anaerobe microorganisms, a number of 88 *Clostridium perfringens* strains were revealed.

Using seroneutralisation reaction, strains of *Clostridium perfringens* isolated and bacteriological identified were included in the 5 serotypes: A, B, C, D, E.

From the overall analysis of obtained datas revealed the fact that from the total isolated strains, predominates biotype A - 8 (44,5%) strains and biotype C – 5 (27,8%) strains, biotype D -3 (16,6%) strains and biotype E - 2 (11,2%) strains, being isolated more rarely.

After bacteriological investigations 34 strains of *Clostridium perfringens* were isolated and included in various biotypes.

Serotypisation of *Clostridium perfringens* strains isolated from the eggs shell obtained from farm system, revealed the presence of 21 strains from biotype A and 15 strains from biotype C.

In all examined products, from the 88 *Clostridium perfringens* strains, 46 were confirmed by seroneutralisation and 55 positives alphatoxigene strains were revealed using immunoenzymatic method.

Toxicity testing using intravenous inoculation of *Clostridium perfringens* strains, is more sensitive, evidenced the presence of 68 (81%) toxigen strains, comparative with testing strains using intraperitoneal way where only 65 (77,4%) strains were considered positives, being less efficient.

The results obtained using ELISA revealed the fact that specific anti – enterotoxin antibodies were present at 71 (84,5%) *Clostridium perfringens* strains, from the entire 84 examined strains.

Identification of *Clostridium perfringens* specie, varied from a aliments group to another in function of aliment type from this category.

The prevalence of positive samples was 6,76% from the entire 1300 examined food samples.

The prevalence of *Clostridium perfringens* biotypes was of 47 (43,40%) strains classified as biotype A, 25 (28,40%) strains as biotype C and 10 (11,36%) strains as biotype D. In biotype B were included only 2 (2,27%) strains and in biotype E 4 (54%) strains.