Key points for the successful control of campylobacter

Campylobacter is a bacterial genus composed of Gram-negative, microaerophilic bacilli. Campylobacter species are typically spiral-shaped, non-sporulating and able to move.

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This bacterial genus is widely distributed in the environment, its main reservoir being the gastrointestinal tract of domestic and wild animals. Birds are more prevalent due to their higher corporal temperature. Campylobacter can also infect humans leading to an infectious disease called campylobacteriosis.

The genus Campylobacter includes, at least, 25 species, with C. jejuni (responsible for more than 95% of diagnosed cases of campylobacteriosis) and C. coli, being the most frequently detected in humans.

Other species such as C. lari and C. upsaliensis have also been isolated from patients suffering from diarrhoeal diseases, but their notification is less usual.

Campylobacteriosis

At present, campylobacteriosis is the most commonly reported zoonosis (disease transmitted to humans by animals or products from animal origin) in the European Union, with 220,209 cases in 2011 (EFSA, 2013).

No statistically increasing or decreasing trend in the number of cases of campylobacteriosis was observed when analysed by month during a five year period (Fig. 1).

Nevertheless, EFSA affirms that less than 10% of campylobacteriosis cases are notified. Thus, it is calculated that every year in the EU around nine million infections caused by campylobacter occur, which represents an approximated cost of €2,400 million.

Most cases of campylobacteriosis in humans are associated with consumption of products of animal origin, such as undercooked meat (mainly poultry meat due to birds' role as asymptomatic carriers), raw milk, and cross contamination (for example, food processed with contaminated water). Direct contact with animals and/or people carrying campylobacter is another possible route of infection.

The infective dose of campylobacter is relatively small. Ingestion of less than 500 micro-organisms (an amount that can be easily found in one drop of sauce) may develop the disease. In spite of such a low infective dose and the ubiquity of campylobacter in the environment, most cases occur as isolated events. However, some zoonotic outbreaks have also been documented all over the world (Washington State Penitentiary, Kinkin Dairy Raw Milk, etc).

Anyone is vulnerable to campylobacter, but particularly children, old people and immune-compromised people are more likely to contract the illness.

Most common symptoms of campylobacter infections are diarrhoea, abdominal pain, high temperature, headache, nausea and/or vomit. These signs usually appear between 2-5 days after infection, and they persist for 3-6 days. Other than rehydration, no specific treatment is necessary. Nevertheless, antibiotic therapy is needed in cases with complications caused by the infection (reactive arthritis, Guillain Barre Syndrome, etc).

Biofilm as a barrier

Chicken meat and derivatives are the main source of transmission of campylobacter to humans. This fact is due to the great amount of poultry flocks that are positive to this micro-organism at slaughter date, which causes raw chicken meat contamination during the slaughter process. It is necessary to take into account that once the infection establishes in poultry, campylobacter multiplies very fast, reaching infective doses very soon.

Taking into account that most poultry carriers are asymptomatic, the diffusion of the infection through contaminated water and contact with faeces is very frequent. This fact, together with the high intensification level of poultry production at this moment, makes campylobacter an infectious agent very difficult to control.

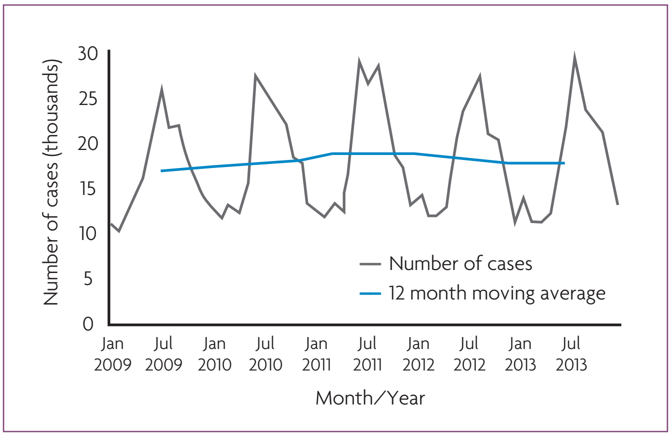

Fig. 1. Trend of campylobacteriosis incidence in EU 2009-2013 (EFSA, 2015).

Although C. jejuni is the main cause of infection in humans transmitted by animal origin products, outside a host, in the environment, this micro-organism is not very resistant. This fact makes it difficult to explain its ability to survive and spread.

In order to survive under adverse conditions, some pathogens (Listeria monocytogenes, salmonella, shigella, Staphylococcus aureus, etc) develop the capability of forming organised structures called biofilms.

A biofilm is a complex community of micro-organisms that grows inside an organic polymeric extracellular matrix which allows them to adhere to alive or inert humid surfaces. A biofilm may contain approximately around 15% of micro-organisms and 85% of extracellular matrix. This matrix is usually constituted of exopolysaccharides able to form canals to facilitate the circulation of water, nutrients and residues.

It has been demonstrated that biofilms act as protective barriers against desiccation, disinfectants and antibiotics. Moreover, they facilitate nutrition and genetic exchanges. As a consequence, common disinfectant methods and antibiotic products are very often not effective against micro-organisms structured in biofilms.

Campylobacter is able to form biofilms in aquatic environments and also on other surfaces. The environment created inside the biofilm offers the optimal micro-aerophilic conditions for C. jejuni development. It has been shown that this micro-organism is capable of surviving one week at 10°C inside a biofilm with limited nutrient level.

Despite its sensitivity to atmospheric conditions (presence of oxygen), campylobacter is able to develop biofilms more quickly under aerobic conditions (20% O_2) than under microaerophilic conditions (5% O_2 and 10% CO_2). Thus, biofilm may act as a reservoir of campylobacter viable cells.

This fact makes relevant the role played by biofilms in order to maintain the active presence of campylobacter in any type of surface in farms and food processing facilities (wood, metal, stainless, plastic, crystal, etc), which supposes an important health risk to consumers.

In addition to this hygiene risk, the formation of biofilms may affect different processes and reduce shelf life of equipment and materials.

Biofilm formation in water storage and distribution systems supposes a

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continuous spreading of viable micro-organisms to water, but also may cause obstructions (affecting energy consumption and water transport). Fortunately, biofilm formation along the food chain ('from farm to fork'), may be controlled by the implementation of intelligent biosafety management systems. These work programs are essential in order to prevent campylobacter contamination.

Fig. 2. Transversal section of pipes and conduits with biofilm.

Biofilm control

Nowadays, it is accepted that biofilms, far from being a rarity, are a usual way of microbial growth, and their presence might have a massive impact, with several hygiene and technological implications. Therefore, the knowledge of methods in order to eliminate and control biofilms, is at present a key issue.

Biofilm tridimensional structure is involved in their resistance to disinfectants and other chemical products. The thicker and older the biofilm, the higher its resistance.

As a consequence, the effectiveness of a chemical product in order to eliminate biofilm will be directly linked to its capability to disorganise extracellular matrix.

Biocidal products most usually used to control biofilm are:

- **Sodium hypochlorite:** this substance is very often used for water treatment due to its low cost. Nevertheless, the necessary dosage of sodium hypochlorite in order to eliminate biofilm is remarkably higher than those recommended for water treatment. When high dosages of sodium hypochlorite are used, it provokes serious corrosion problems.

- **Chlorine dioxide:** its long term stabilisation is chemically impossible. Thus, in order to use it properly, it must be generated in situ. This fact increases its cost and is inconvenient from a prevention of occupational hazards point of view.

- **Ozone:** it is effective as biofilm removal, but its use leads to many problems derived from its high instability (ozone is explosive). In addition, its low solubility in water is also inconvenient.

- **Hydrogen peroxide:** this chemical oxidant, due to its mechanism of action, guarantees the complete destruction of extracellular matrix that provides resistance to biofilm. At common recommended dosages for water treatment, it eliminates biofilm and it does not lead to toxicity or corrosion problems. It is 100% biodegradable.

Other biocidal products, which are not allowed for water treatment, are described below. These active principles can still be used as biocides in order to treat surfaces, materials and water distribution systems during non-productive periods:

- **Quaternary ammonium compounds:** they are effective surfactants that may help to remove biofilm. Nevertheless, their high toxicity demands very exhaustive rinse protocols after their use.

- **Aldehydes:** they are very effective against planktonic micro-organisms, but not against micro-organisms organised in biofilms.

- **Peracetic acid:** it is a very powerful chemical oxidant that may be stabilised with hydrogen peroxide resulting in peroxyacetic products. These types of mixture are 100% biodegradable and have demonstrated a great effectiveness eliminating biofilms even under hard conditions. In fact, stabilised peroxyacetic products are recommended for biofilm control in farms and the food industry.

Having in mind this information, the optimal work protocol in order to control biofilm requires continuous water treatment using products formulated with stabilised hydrogen peroxide (Grupo OX recommends the use of OX-AGUA 2G) and periodic cleaning and disinfection treatments of surfaces and water distribution systems using peroxyacetic products (Grupo OX recommends the use of OX-VIRIN).

To avoid toxicity risks and guarantee long shelf life of materials and equipment, it is necessary to work with stabilised products integrated in a complete biosecurity work program.

Grupo OX specialise in offering integral solutions adapted to specific necessities to achieve a very interesting return on investment.

Finally, it should be noted the importance of biofilm detection in order to pay special attention to critical points where it is more easily developed. Nowadays, there are products on the market in order to demonstrate the presence of biofilm, making the implementation of an intelligent biofilm control strategy easier .

Conclusions

The presence of biofilm throughout the food chain is a very important technological problem related to food safety. It has been proven that campylobacter is able to grow creating biofilms. The characteristics of this particular way of microbial growth lead to the necessity of implementing specific cleaning and disinfection processes in order to overcome biofilm protective barriers.

Biofilms can be eliminated by the establishment of specific biosafety work protocols applied regularly. Nevertheless, the difficulty in eradicating well instituted biofilms makes prevention the most intelligent control strategy.

The application of peroxyacetic products in order to disinfect surfaces and water distribution systems during non-productive periods combined with a continuous water treatment with stabilised hydrogen peroxide based products, are key factors in order to develop a complete strategy to control campylobacter. ■

References are available from the author on request