



Report of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) on microbiological risks associated to consumption of different foods by pregnant women

Section of Food Safety and Nutrition

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Abstract

Pregnant women are considered a special risk population group for food-borne illnesses, due to their specific susceptibility to certain infectious diseases and the severity of the complications that may appear. In Spain, official incidence of food-borne disease outbreaks in this population group is low. However, due to their special vulnerability and the strict sanitary control associated to pregnancy, they constitute a group in which prevention measures based on risk communication can be very effective.

Listeriosis is one of the more relevant food-borne diseases during pregnancy since, although rare, it affects seriously the foetus, causing abortion or premature birth, stillbirth, meningitis or sepsis.

Toxoplasma gondii can also cause foetal serious infection, resulting in miscarriages, perinatal mortality or congenital lesions in the brain, eyes and other organs. Other pathogens transmitted by food which, although infrequently, may seriously affect pregnant women or foetus are *Brucella* spp., Hepatitis E virus, verotoxigenic *E. coli*, *Salmonella* spp. and *Campylobacter* spp.

Health education, together with prevention measures taken during production, processing and distribution, is the main factor to reduce microbiological hazards derived from food consumption. Therefore, in order to establish the basis for performing management and risk communication activities, the Scientific Committee has studied the main food-borne pathogens of special risk during pregnancy, analysing the factors affecting their survival and growth, as well as the foods most frequently involved in their transmission.

This report includes a list of foods to avoid during pregnancy in order to minimize the risk of infection mainly by *Listeria monocytogenes* and *Toxoplasma*, but also by the rest of studied pathogens.

The need to include instructions about the hygienic handling of food at home in any risk communication campaign is highlighted.

Key words

Pregnancy, microbiological risks, *Listeria*, *Toxoplasma*, food borne pathogens, cross contamination, hygienic food handling practices.

1. Introduction

Toxinfections are a serious and growing problem for public health (Van de Venter, 2009). Nearly all the countries with food outbreak information systems have detected a significant increase in illnesses caused by microorganisms transmitted through food in recent years. The World Health Organization (WHO) has estimated that there are around 200 diseases that can be transmitted through food. Main pathogens include *Salmonella*, *Campylobacter jejuni*, verotoxigenic or enterohemorrhagic *E. coli* (VTEC), parasites such as *Cryptosporidium*, *Cryptospora*, some platyhelminthes and nematodes and viruses such as hepatitis A and E and gastroenteritis virus such as Norovirus. In the EU (European Union) alone, in 2012, 5 363 cases of toxinfections outbreaks were reported, affecting more than 55 000 people (EFSA/ECDC, 2014).

The importance of toxinfections for public health does not only relate to their occurrence in humans. Various factors, such as the seriousness of the illness, death rate, subsequent complications and possible prevention measures are also key issues when establishing the importance of different illnesses (EFSA/ECDC, 2013). Particular population groups may be affected to a greater degree as a result of these factors, making them what is known as “vulnerable population groups”. The University of California defines vulnerable populations as population subgroups with a relatively higher risk (a greater exposure to risk factors) or a greater susceptibility to specific health problems (UCLA, 2013). That is, a vulnerable group shows signs of being more susceptible to a specific illness or within that group, the consequences of this illness are more serious.

Pregnant women are considered to be a vulnerable population group given the seriousness of the complications they can suffer and their susceptibility to specific infectious diseases. This susceptibility would appear to be related to a unique immunological condition caused by the pregnancy. For a long time it was believed that pregnant women had a suppressed immune system, however, it has been demonstrated that this is not the case. Therefore, some authors prefer to state that pregnant women have a modulated immune system. Hormonal changes during pregnancy can alter a number of cellular and innate immunity mechanisms. This unique behaviour would explain why they respond differently to the presence of microorganisms or their metabolites, being more susceptible, for example, to intracellular parasites (Pejčić-Karapetrović et al., 2007) (Mendz et al., 2013) (Sappenfield et al., 2013). Placental immune response and its tropism for specific viruses and pathogens increase this susceptibility to certain infectious diseases, which tend to involve more serious complications (Mor and Cárdenas, 2010).

Having seen the particular relevance of pregnant women as a high-risk population group for certain microbiological risks and with the aim of providing comprehensive and thorough information for both health professionals and pregnant women and also carrying out risk management and communication activities, a request has been submitted to the Scientific Committee by the Executive Director of AECOSAN (Spanish Agency for Consumer Affairs, Food Safety and Nutrition) in order to address the following issues:

- For the specific cases of *Listeria monocytogenes* and *Toxoplasma gondii*:
 - Main foods that can transmit these microorganisms.
 - Other sources of infection.

- Preventive measures that can be taken, particularly with regard to foods to be avoided and conditions for handling and preparing food.
- Effects on pregnant women and/or fetuses.
- Other relevant pathogens that need to be included in risk communication campaigns.

2. Main foodborne biological risks for pregnant women

2.1 *Listeria monocytogenes*

In the European Union (EU), listeriosis is a rare but potentially life-threatening foodborne disease, compared with other foodborne processes such as salmonellosis. In 2012, 1 642 cases of human listeriosis were reported in the EU, a slightly higher number than in previous years. In the EU between 2008 and 2012 there was a significant upward, seasonal trend when compared with previous years (EFSA/ECDC, 2014). There are also high levels of morbidity, hospitalisation and mortality among vulnerable population groups (EFSA, 2013a). Mortality rates can reach 20-30 %. In the EU, in 2012, the mortality rate was 17.8 %; this is the highest since 2006 (EFSA/ECDC, 2014). The disease mainly affects specific and more vulnerable segments of the population. Basically, *L. monocytogenes* is an opportunistic pathogen that almost always affects people with underlying serious pathologies (for example, immune suppression, HIV/AIDS, chronic illnesses that lead to immune deficiencies, such as cirrhosis); pregnant women; fetuses and newborns; and the elderly. Specifically, in pregnant women, listeriosis can cause nonspecific symptoms in the mother, similar to flu symptoms (fever, shivering, myalgias, arthralgia, back pain and headaches). The bacteria spreads until it reaches the placenta which is relatively protected from the defence system and causes serious alterations in the foetus that can result in miscarriage, preterm birth or stillbirth (FAO/WHO, 2004). In newborn babies the most serious and earliest form is granulomatosis infantiseptica, characterised by widespread abscesses and granulomas which are particularly prevalent in the liver, spleen, lungs and the brain. The mortality rate is usually very high (mortality rates of between 10 and 50 % have been documented (Farber and Peterkin, 1991). *L. monocytogenes* has a tropism for nerve tissue, particularly the meninges and the brainstem, which is why it is a cause of meningitis in neonates. There is a neonatal form that often appears later, between the 1st and the 8th week of life of the child, in which the disease was probably acquired in the birth canal. The most frequent manifestation is meningitis, with lethargy, unwillingness to eat and irritability.

Listeria is a genus of bacteria that contains 10 species, but human cases of listeriosis are almost exclusively caused by the species *Listeria monocytogenes* (EFSA, 2013a). *L. monocytogenes* has very unique and specific characteristics (FSAI, 2005) (Luber et al., 2011). It is a Gram positive bacillus, a facultative anaerobe that does not form endospores. It can grow in a wide range of temperatures, including cold temperatures (minimum -0.4 °C and maximum 50 °C) (Farber and Peterkin, 1991) (FAO/WHO, 2004) and shows a certain temperature dependent acidophilia, unable to grow at pH lower than 4 and higher than 9.6 (Farber and Peterkin, 1991). The bacterium is destroyed at cooking temperatures above 65 °C (EFSA/ECDC, 2013).

Its ability to resist various dysgenic conditions (such as acidity and high levels of salt), explains its omnipresence in the environment and thus, in water and fresh and processed foods, as well as in food processing facilities that are not correctly sanitised. It is therefore widespread, mainly found on

the floor, fodder and surface water. *Listeria monocytogenes* can come into contact with food during various stages of the food chain, from primary production (Nightingale et al., 2004), slaughterhouses (Jemmi and Stephan, 2006) and the processing plant (Reij and De Antrekker, 2004). Microbiological cross contamination is one of the main problems concerning *L. monocytogenes*. It can occur as a result of direct contact with the raw material, staff, aerosols, utensils or contaminated equipment (knives), etc. Cross contamination can occur in any phase in which the product has been exposed to the environment, including manufacturing, transportation and retail sales, food services to groups and at home.

The main route of transmission to humans is believed to be the consumption of contaminated food (FAO/WHO, 2004) (EFSA, 2013a). Nosocomial infections and person-to-person transmission are recognised but they are rare. Direct contact with animals apparently poses a relatively small risk to members of non-vulnerable groups, which is why the World Health Organisation (WHO) once stated that animals are not important direct sources of infection for humans (Low and Donachie, 1997). In sporadic cases when there is direct transmission of *L. monocytogenes* from animals to humans, symptoms are cutaneous and do not lead to systemic infection (Blenden et al., 1987).

Its multiplication and survival capacity at refrigeration temperatures enables it to survive inside or on the surface of ready-to-eat (RTE) foods with a relatively long shelf life, such as fresh or smoked fish products, heat-treated meat products or raw cured meat and cheese (Farber and Peterkin, 1991) (FAO/WHO, 2004) (EFSA, 2013a).

EC Regulation No 2073/2005 (EU, 2005) indicates that the maximum limit for ready-to-eat foods intended for infants and those intended for special medical purposes shall be an absence in 25 g for market products during their shelf life. For RTE foods that cannot increase the development of *L. monocytogenes*, that are not those intended for infants or for special medical purposes, the maximum limit is 100 cfu/g. For RTE foods that can increase the development of *L. monocytogenes*, that are not those intended for infants or for special medical purposes, the absence in 25 g is required for products before they have left the immediate control of the food processor when the latter cannot prove, in a satisfactory way for the relevant authority, that the product will not exceed the limit of 100 cfu/g during its shelf life. This criterion is applicable before the foodstuff has left the immediate control of the food business operator that has produced it.

Another possibility allowed by the Regulation is to demand a limit of up to 100 cfu/g during the shelf life for RTE foods that can favour the development of *L. monocytogenes*, which are not intended for infants or for special medical purposes, if the manufacturer can prove, in a satisfactory way for the relevant authority, that the product will not exceed the limit of 100 cfu/g during its shelf life. In this case, the producer can set intermediate limits during the process that should be sufficiently low in order to guarantee that the limit of 100 cfu/g is not exceeded at the end of its shelf life.

In a recent reference study prepared by EFSA (European Food Safety Authority) on the prevalence of *L. monocytogenes* in the EU in different ready-to-eat foods (EFSA, 2013a), it was found that the proportion of samples that exceeded the microbiological criteria of 100 cfu/g established by this legislation at the end of the shelf life of the product was 1.7 %, 0.43 % and 0.06 % in fish, meat and cured soft and semi-soft cheeses respectively. As shown, the proportion is relatively low and also, the

risk for humans is mainly from exposure to specific foods that contain bacteria above this level of 100 cfu/g. However, given the survival and multiplication capacity of this microorganism in these products, even a low and uncommon proportion of samples that exceed this level can be a cause for worry for public health (EFSA, 2013a) (EFSA/ECDC, 2013). It is important to point out that this reference study did not include samples of unripened cheeses.

With regard to fish, in 2009, the Scientific Committee of AESAN issued some recommendations concerning raw or rare fish consumption, which include: Risk populations should avoid eating these types of products (for example, pregnant women) and the handling of fresh or frozen fish in households with risk populations should be performed strictly respecting basic prevention and cross contamination measures (AESAN, 2009). The French National Health and Nutrition Programme recommends that pregnant women specifically should avoid consuming raw molluscs, raw fish (sushi, surimi, tarama) and smoked fish (salmon, trout) (PNNS, 2007).

In the case of soft or semi-soft cheeses, the French National Health and Nutrition Programme recommends that pregnant women should avoid the consumption of cured soft cheeses with mouldy rinds (Camembert and Brie for example) and soft cured cheeses with washed rinds (Munster for example), if they are made with unpasteurised milk. They also recommend avoiding the consumption of industrial grated cheeses and removing the rinds from all cheeses (PNNS, 2007). Other public health institutions, such as the United Kingdom's Food Standards Agency, recommend avoiding blue cheeses and unripened cheeses (such as Burgos, Villalón, Mexican style unripened cheeses) (FSA, 2002) (CDC, 2013a). In general terms, it recommends avoiding the consumption of cheeses made with unpasteurised milk, such as traditional cheese varieties like Feta or cottage cheese. In one of the main outbreaks in the EU in 2011, (11 cases and 4 deaths), the foodstuff that was identified as the cause was a homemade cheese, although information is not available concerning the thermal treatment to which the milk was subjected, or concerning the cheese curing procedure (EFSA/ECDC, 2013). Although EFSA did not include unripened cheeses in its reference study of 2010-2011 (EFSA, 2013a), there have been a number of recent outbreaks of listeriosis in other countries as a result of the consumption of this type of cheese (Danielsson-Tham et al., 2004) (Fretz et al., 2010) (Jackson et al., 2011) (Castro et al., 2012).

Although cheeses are the dairy products that have traditionally been associated with outbreaks of listeriosis, there have also been cases related to other dairy products, such as butter; for example, in Finland (Maijala et al., 2001) and in the United Kingdom (Lewis et al., 2006).

With regard to meat products, reports indicate that there is a greater risk from the consumption of refrigerated pâtés, cured raw meat products, raw or rare meat, some meat products prepared "in their jelly" (cow tongue, pork tongue) and other local specialities such as *rillettes* (de Valk, 2000) (InVS, 2000) (FSA, 2002) (PNNS, 2007). In Finland in 2012, a serious outbreak was detected through the consumption of red meat and derived products (gelatine) (EFSA/ECDC, 2014). There have also been cases detected as a result of the consumption of cooked ham (Hächler et al., 2013).

To a lesser degree, there have been cases reported as a result of the consumption of other foods, such as pastries (EFSA/ECDC, 2013) or melons (CDC, 2012).

Given the types of foods involved and the unique characteristics of *L. monocytogenes*, in many

cases the outbreaks are the result of the consumption of RTE foods made with the aforementioned products (fresh or smoked fish, cured foods or cheeses); in other words, foods that are acquired already prepared and do not need to be cooked, such as sandwiches (Dawson et al., 2006) (Coetzee et al., 2011) or mixed salads, where its ability to grow has been demonstrated (Skalina and Nikolajeva, 2010). Also, an analytical epidemiological study carried out in England and Wales between 2007 and 2011 identified the consumption of packaged mixed salads (Little et al., 2010) and non-packaged mixed salads (Gillespie et al., 2010) as a significant risk of the appearance of sporadic cases of listeriosis.

With regard to the cases of listeriosis in which ready-to-eat foods are involved, these may be classified as follows in order of relevance: (i) ready-to-eat foods made from vegetable, dairy, meat and smoked fish products, (ii) foods that are kept refrigerated for long periods of time, (iii) foods that are not submitted to *Listeria* testing during conservation and processing, (v) foods with contamination risks after processing (slicing), (vi) foods with ingredients that support the growth of *L. monocytogenes* and (vii) foods consumed by individuals with suppressed immune systems and pregnant women. In the case of minced meat products and emulsified foods with complex preparation and handling methods, the possible contamination with *L. monocytogenes* is very high. In fermented meat products, the presence of *L. monocytogenes* is probably lower although its survival ability is higher (FSAI, 2005).

Retailers and consumers are responsible for treating ready-to-eat foods as they are intended to be used (following the preservation, preparation and storage indications on the food packaging). Maintaining good standards of hygiene in the kitchen can help to prevent listeriosis. For example, avoiding cross contamination and cleaning refrigerators on a regular basis.

In a review concerning food safety studies carried out in the United States, the United Kingdom and Australia (Yang et al., 2006), it was shown that a considerable number of consumers do not carry out correct food handling procedures in the household: Insufficient cooking time, storing for long periods of time at high temperatures, cross contamination and bad personal hygiene practices. Clearly consumers are the last part of the "from the farm to the table" chain and food handling and consumption behaviours are critical in order to minimise the risk of contracting foodborne diseases, including listeriosis (Yang et al., 2006). Also, consumers need to know whether they belong to a risk category. This includes not only pregnant women and the elderly but also people with underlying pathologies. In a study carried out over 10 years in England, cancer patients represented more than one third of the cases of listeriosis and a higher risk was observed for the majority of the cancer subgroups (Mook et al., 2011).

Food producers should consider a certain safety margin in order to prevent consumers from cooking at incorrect temperatures, given that in the initial stages of the chain (i.e. preparation and distribution) temperatures are controlled correctly, while in some display cabinets aimed at the general public and in particular in domestic fridges, there is less control (Afchain et al., 2005). RTE foods sometimes spend a considerable amount of their shelf lives in a domestic fridge, instead of in a commercial or industrial fridge. Therefore, domestic fridge temperatures can have a significant effect on the safety of refrigerated foods. In a study carried out on refrigerator temperatures in Greece, it showed that in 66.7 % of the refrigerators tested, the temperature was above 4 °C, while in 3.6 %, the temperature was above 10 °C (Koutsoumanis and Angelidis, 2007).

There are a number of factors that affect the survival and growth of this microorganism. A particularly determining factor is food composition. There are now scientific studies that indicate that *L. monocytogenes* will not multiply at a water activity (*aw*) of <0.92 or a pH of, <4.39 (Steven et al., 2004). Other factors to bear in mind are the storage temperatures of food either at room temperature or under refrigeration and the length of time these foods are stored at room temperature or under refrigeration (Codex Alimentarius, 2007). The production of many ready-to-eat foods associated with foodborne listeriosis includes a listericidal phase. Therefore, the frequency and degree of contamination of these products with *L. monocytogenes* are typically associated with the re-contamination of the product before the end packaging or with the subsequent handling when on sale (slicing) or use in the home. It is likely that controlling the frequency or degree of contamination are strongly affected by factors such as attention given to the design and maintenance of the equipment and the integrity of the cold chain, with the latter being clearly identified as a risk factor. Some ready-to-eat foods do not include a listericidal treatment. The safety of the product in these cases depends on the measures taken during primary production, manufacturing and distribution and subsequent use in order to reduce contamination or recontamination to a minimum or to a certain degree and to limit the proliferation by maintaining the cold chain and limiting the refrigerated storage time.

Table 1. Growth and survival limits for *L. monocytogenes*

Parameters	Minimum	Maximum	Survival
Temperature	-1.5 a 3 °C	45 °C	-18 °C
pH	4.2	9.4-9.5	3.3-4.2
Water activity (<i>aw</i>)	0.90 a 0.93	>0.99	<0.89
Salt (% NaCl)	<0.5	12-16	>20

Source: (FSAI 2005).

Temperature is another important factor affecting the growth of *L. monocytogenes* in foods and the detailed information concerning temperature conditions in the food chain is a prior requisite for efficiently assessing the risks of *L. monocytogenes* in ready-to-eat foods.

Listeria spp. is sensitive to heat and can be eliminated by pasteurisation (Wiedmann et al., 2003). In cooked ham, the pre-packaging pasteurisation process, (68-72 °C, 15-30 min) alone or combined with the post-packaging pasteurisation (60 or 90 seconds), enables a reduction of 3.2 to 3.9 logarithmic cycles of *L. monocytogenes*. This method seems to be effective for controlling contamination by this bacterium on the surface of meat (Gande and Muriana, 2003). In the post-packaging process of ready-to-eat meats by immersion, a reduction of 2 to 4 logarithms of *L. monocytogenes* is achieved after exposure at 90.6 °C, 93.3 °C and 96.1 °C between 2 and 10 minutes (Muriana et al., 2002).

The high hydrostatic pressures have become more relevant in recent years as an alternative to the thermal treatment in some types of products (meat products, fruit juices and sauces), given that promising results have been obtained on the inactivation of microorganisms and enzymes. The

population of *L. monocytogenes* in muscle pork is completely eliminated at pressures higher than 414 MPa. The treatment of contaminated ground pork with *L. monocytogenes* through this process, together with temperatures of 50 °C during 6 minutes, can achieve a six-fold extension of the shelf life of this product (Murano et al., 1999).

Antimicrobials have also been used to control *Listeria* spp. Lactic acid and its salts have been widely used in the meat industry for increasing the taste and shelf life of the product. It has been seen to work against *C. botulinum*, *L. monocytogenes*, *S. aureus*, *Salmonella* and *E. coli* O157:H7 (Aymerich et al., 2005). Nisin, combined with 2 % sodium chloride has shown to be efficient as an antilisterial agent in minced raw buffalo meat (Pawar et al., 2000).

The efficiency of the irradiation in meat products and ready-to-eat (RTE) meals has been proven (Ahn et al., 2006).

L. monocytogenes is a facultative anaerobic microorganism (it can grow at low oxygen concentrations) and the use of modified atmospheres (75:25, CO₂:N₂ and 72.5:22.5:5, CO₂:N₂:O₂) does not prevent its growth (Scif et al., 2009).

2.2 *Toxoplasma gondii*

Toxoplasmosis is the most common parasitic zoonotic disease in humans. Despite this, in the EU it is still an underestimated disease both in terms of detection and communication (EFSA, 2007) and in other countries, such as the United States, it is classified within the group of neglected parasitic infections (CDC, 2013b). Pursuant to EC Directive 2003/99/EC (EU 2003), monitoring toxoplasmosis and the ethological agents thereof shall be performed if the epidemiological situation of the Member state justifies it, which is why there are no known data of this parasite in the EU in humans, animals or in foods (EFSA, 2007). In a recent evaluation study carried out in the United States, toxoplasmosis was identified as the second most important cause of death related to the consumption of foods and the fourth in relation to hospitalisations resulting from toxoinfections (Scallan et al., 2011). In another recent study in Greece, it was established that toxoplasmosis is among the five foodborne diseases that have the highest morbidity burden (Gkogka et al., 2011).

In the EU in 2009, 1 259 confirmed cases of toxoplasmosis were reported; the majority of these were among women of child bearing age (24-44 years). Of these, only 23 cases were children under the age of 12 months and only two of these cases were the result of a congenital illness (EFSA/ECDC, 2011).

Since 2010, EFSA and ECDC only include the cases of congenital toxoplasmosis in their annual report on zoonoses, zoonotic agents and food outbreaks, pursuant to the case definition established in Decision 2008/426/EC (EU, 2008), thus only reporting the cases of children under the age of 1 year (EFSA/ECDC, 2012). During 2011, 29 cases of congenital toxoplasmosis were confirmed in 19 Member States, although this figure is thought to be lower than in reality, because some countries had not provided their figures at the close of the 2013 Annual Epidemiological Report (ECDC, 2013). In 2010, there were no deaths reported for children under the age of 1 year, although in the majority of the reported cases, there was lack of information concerning the consequences of the illness (EFSA/ECDC, 2012). The epidemiological monitoring systems for this illness are diverse, which makes it difficult to estimate its real burden and comparisons between countries (ECDC, 2013).

Infection by *Toxoplasma* is common in animals and humans (EFSA/ECDC, 2012) (Jones and Dubey, 2012). It is thought that between 50 and 80 % of Europeans are infected. The majority of the infections are asymptomatic or have symptoms similar to mild flu symptoms. The resulting protective immunity is long-term. The most common manifestation is lymphadenitis, accompanied by fever and headaches. Sometimes *Toxoplasma gondii* can lead to a serious illness in people with compromised immune systems or serious foetal infection, leading to miscarriage or congenital damage to the brain, eyes or other organs of the foetus. The seriousness of the damage is greater if the mother becomes infected in the first trimester of pregnancy (EFSA/ECDC, 2012). Congenital toxoplasmosis tends to be obvious during the initial days of life, characterised by four main types of symptoms: Chorioretinitis, hydrocephalus, intracranial calcifications and prenatal meningoencephalitis damage. There may also be symptoms of a generalised infection with liver enlargement, jaundice, fever, heart, breathing and haematological disorders, when the infection is caught within the last months of pregnancy. A quarter of newborns affected by toxoplasmosis die within the first few days and 80 % of children affected by a congenital infection subsequently have loss of hearing and chorioretinitis.

The agent that causes it is an obligate intracellular protozoan parasite: *Toxoplasma gondii*. Its biological cycle includes an asexual multiplication in the intermediary host and a sexual reproduction in the definitive host. Many homeothermic species can act as intermediate hosts for the parasite and may be asymptomatic carriers of the parasite's intra-tissue cysts. Cats, both wild and domestic, are the only definitive hosts excreting oocysts in the cat's faeces (CDC, 2013c), which need to sporulate in the environment before becoming infective. In the intermediary hosts, *Toxoplasma* undergoes two phases of asexual development; in the first phase an active multiplication of the tachyzoites in different types of cells takes place and in the second phase tissue cysts are formed which contain bradyzoites. All the hosts, including humans, can be infected by the three different types of stages: Tachyzoites, bradyzoites (found in the tissue cysts) and sporozoites (found in the sporulated oocysts).

The organotropism of tissue cysts varies according to the different intermediate hosts. In many of them the cysts are more prevalent in muscle and nervous tissues, situated mainly in the central nervous system and in the eyes, as well as in the skeletal and heart muscles; however they have also been detected, to a lesser degree, in viscera such as lungs, liver and kidneys. Although the cysts are the terminal stage in the host in which they are located, they can remain infective during the life thereof, therefore the consumption of raw or rare meat, from infected animals, poses a serious risk of transmission of toxoplasmosis to humans. Although *Toxoplasma* has been historically associated with pork, there is also evidence to suggest possible contamination as the result of consuming other meats (minced beef, rare ovine meat, homemade sausages, dried meat or smoked meat). The consumption of food or water contaminated with sporulated oocysts and the congenital infection are other forms of transmission of *T. gondii*, including direct contamination through handling earth or sand contaminated by feline faeces (Bayarri et al., 2012) (CDC, 2013c).

Although there is not much epidemiological data, some authors (Slifko et al., 2000) suggest that around 50 % of all the cases of human toxoplasmosis are related to food infections. Pereira et al. (2010) establish animal and poultry meat (sheep, goat, beef, poultry and pork) as a source of infection and directly relate the presentation with the consumption of meat indicating values of related cases

ranging between 30 and 63 % depending on consumption habits, with a variation of the type of meat involved according to countries. Bayarri et al. (2012) indicate that the figures provided by the bibliography suggest a higher rate of risk with regard to the consumption of sheep and goat meat in countries such as France and Norway while in Poland the consumption of rare pork is the main risk factor.

Although to a lesser degree, the role of vegetable contamination with oocysts from cat faeces should not be ruled out, or the theoretical contamination through contaminated waters; Pereira et al. (2010) cite a number of outbreaks that occurred in Brazil, the United States and Canada through drinking water and these same authors do not rule out the possibility of *Toxoplasma* transmission from bivalve molluscs through contaminated water.

Routine *post mortem* examination methods make it difficult to identify this parasite in meat products; however, *Toxoplasma gondii* has been considered a high-priority pathogen in the risk assessment reports carried out by the EFSA with regard to the inspection of sheep and goat meat (together with Verotoxigenic *Escherichia coli* (VTEC); (EFSA, 2013c)), porcine meat (together with *Salmonella*, spp., *Yersinia enterocolitica* and *Trichinella* spp.; (EFSA, 2011)) and game meat (deer and wild boar) (together with *Salmonella* spp.; (EFSA, 2013b)); the final conclusions from these reports characterise the risk of *Toxoplasma gondii* in the medium risk categories for porcine meat, low for poultry, high for small ruminants and high severity and low incidence rates for beef, although it clearly states the impossibility of detecting it in routine inspections.

2.2.1 Effect of the control systems on *Toxoplasma gondii*

The majority of the recommendations related to control systems for toxoplasmosis are based on the use of preservation methodologies or culinary treatments that ensure the destruction of viable cysts and in the use of measures to prevent cross contamination through contaminated materials.

In general terms, the efficiency of the destruction methods is considered to be variable and it is often related to the duration and intensity with which these methods are implemented.

The main systems tested could be summarised as follows:

Use of low temperatures

1. *Effect of refrigeration on the viability of Toxoplasma gondii.* The resistance of *T. gondii* to low temperatures depends on the vegetative state thereof (sporulation or not). Lindsay et al. (2002) carried out a study to evaluate the survival of nonsporulated *Toxoplasma gondii* oocysts under refrigerator conditions. The studies showed that *T. gondii* remained viable after the refrigeration period under study (11 weeks at 4 °C) and was still able to sporulate when returned to room temperature conditions.
2. *Effect of freezing on the viability of Toxoplasma gondii.* Freezing at temperatures below -12 °C has a negative effect on the viability of *Toxoplasma*, as illustrated by Dubey (1988) who studied the effect of freezing on oocytes in pork verifying the loss of vitality after 3 days of storage at -12 °C. Similar conclusions have been obtained in subsequent studies (Kotula et al., 1991) (Lundén and Uggla, 1992) (El-Nawawi et al., 2008). However, recently Gencay et al. (2013) detected viable cysts

in frozen buffalo meat, although they do not offer information regarding the temperatures and commercial freezing times.

Use of high temperatures

Initial studies concerning the action of heat on *Toxoplasma* cysts were carried out by Jacobs et al. (1960), which showed the inactivation of cysts after being exposed to 50 °C for 1 hour. Later, Dubery et al. (1990) and Dubey (2000) studied the effect of different combinations of time and temperature (49 to 67 °C for periods of 0.01 to 96 minutes) in samples of minced meat and packaged in plastic bags. Their results proved that *T. gondii* cysts are less heat resistant than those of *T. spiralis*, and are not viable when the internal temperature of the samples reaches at least 67 °C, while lower temperatures needed times of 9.5 minutes at 58 °C and 3.6 minutes at 61 °C.

Cooking at over 67 °C is considered sufficient to destroy intra-tissue cysts (Dubey et al., 1990), although for minced meat cooking temperatures should be increased to 71 °C (CDC, 2013c). The survival of these cysts at lower temperatures will depend on the cooking time and, in domestic conditions, heating times may have to be extended in order to ensure that suitable temperatures are reached inside the food (Dubey et al., 1990). These measures will also help to prevent other toxoinfections. It is important to point out that some studies indicate that cysts can continue to be infectious when limited homogeneous heating methods are used, such as microwaves, probably because the product is not uniformly heated (Lundén and Ugglá, 1992).

Effect of salting and/or curing meat products. Technological factors that affect the viability of *Toxoplasma*

Very few studies have examined the efficiency of curing processes in the inactivation of *Toxoplasma gondii*, but the majority of these establish a correlation between the duration of the salting/curing and the viability of *Toxoplasma*.

Initial contradictory information offered by scientific studies was that obtained by Sommer et al. (1965), which indicated that encysted *T. gondii* could survive for 4 days in 8 % NaCl although not even Sommer or Work (1968) could demonstrate the presence of viable parasites in pork meat infected with *T. gondii* and submitted to different curing processes. Similarly, Lundén and Ugglá (1992) reported the absence of viable *Toxoplasma* in lamb after curing and smoking processes, although the parasite survived in fresh products cooked in the microwave. Later, Warnekulasuriya et al. (1998) in a study on the detection of *Toxoplasma gondii* in cured meats, detected viable cysts in one of the 67 samples of ready-to-eat cured meats that were analysed. This sample showed a pH of 6.98 and water activity of 0.945.

Dubey (1997) studied the survival of *T. gondii* cysts in salt solutions with concentrations ranging between 0.85 and 6 % at temperatures ranging between 4 and 20 °C. At temperatures of 4 °C, survival ranged between 56 and 21 days for concentrations ranging between 0.85 and 3.3 % and between 14 and 3 days at 20 °C for the same concentration, with no viability detected in any of the tested samples with a concentration of 6 % salt. Similarly, Hill et al. (2004, 2006) studied the effects of time/temperature combinations on the viability of *Toxoplasma* cysts injecting pork fillets with different types of saline solutions and they noticed that concentrations of 2 % NaCl or 1.4 % sodium lactate were

efficient for destroying cysts in 8 hours at 4 °C, while concentrations of 0.85 % NaCl determined that after 40 hours the parasite was still viable; this same study at 0 °C and lower, enabled *Toxoplasma* viability for a maximum of 7 days.

The viability of *Toxoplasma gondii* in sausages was studied by different authors, concluding that the parasite stops being viable once they have been made, depending on the concentration of salt and storage times. Thus, Jamra et al. (1991) concluded that *Toxoplasma* ceases to be viable in sausages with concentrations of 3.0 % salt for periods ranging between 4 and 7 days; Navarro et al. (1992) confirmed the non-viability of *Toxoplasma* in sausages after 48 hours of storage, attributing this lethal effect to the salt content and indicating that other condiments (black pepper and garlic) had no effect whatsoever. De-Oliveira et al. (2004) demonstrated that *T. gondii* ceased to be viable in pork sausages sold in Brazil, despite the fact that 47 % of the samples of the raw materials they were made with were positive.

With regard to the use of curing salts, Neumayerová et al. (2014) assessed the survival of *Toxoplasma gondii* cysts in vacuum-packed goat's meat and fermented and dried cold meats made from goat's meat. The meat was vacuum-packed with or without 2.5 % curing salts (6 % sodium nitrate and 94 % NaCl) and stored at 4 °C and -20 °C. In the vacuum-packed meats stored at 4 °C without salt, the analysis of *Toxoplasma* was positive after 6 weeks. The same meats stored at -20 °C lost *Toxoplasma* viability after 4 hours. These same authors verified the successful elimination of *Toxoplasma* cysts after a controlled fermentation of 12 days, testing fermented cold meats seasoned with spices, sugars, initiators and with 2.5 % curing salts. In the samples cured with a mixture of 2.5 % curing salts, *Toxoplasma* was viable at 7 days, stored at 4 °C and unviable after 14 days, taking into account that the lethal capacity was reached in samples with an aw of 0.960, a salt content of 1.86 % and a pH of 5.82.

Bayarri et al. (2010) discovered that at the end of the ham curing process (14 months of curing), there were no viable parasites, despite the fact that the pigs used as raw material were infected with *T. gondii*, therefore these authors concluded that the consumption of cured ham depending on the conditions of the process used, poses an insignificant risk of contracting toxoplasmosis, although they do suggest the need for additional studies to assess the safety of cured ham products obtained under different curing periods, salt and concentrations of nitrites and nitrates.

Forbes et al. (2009), studied the survival of *Toxoplasma gondii* in various seal meat products: Fermented (*igunaq*), dehydrated (*nikku*) and savoury and spiced sausages. Viability tests carried out after storing at 4 °C for fermented or dehydrated meats and at -20 °C for sausages for periods ranging between 41 and 121 days, showed that no contamination capacity existed in cats in any of the tested samples.

Other preservation treatments

The use of high pressures enables *T. gondii* to be destroyed at relatively low pressures. Lindsay et al. (2006) studied the survival *T. gondii* cysts in tissues as a result of applying high pressures to ground pork. The ground meat was exposed to 0, 100, 200, 300 and 400 MPa treatments for 30, 60 or 90 seconds. The results indicated that pressures of 300 MPa or above, render the *Toxoplasma* nonviable for any of the time periods studied.

Dumètre et al. (2008) studied the effect of the use of ozone and UV light radiation on the infectivity of *T. gondii* oocysts present in water. These authors verified the inefficiency of the ozone (9.4 mg/min/l in water at 20 °C) and the effect of 4 decimal reductions for the use of UV radiations in water (dose of more than 20 mJ/cm²); however, other authors, such as Wainwright et al. (2007a) demonstrated the inefficiency of treatments of 500 mJ/cm² in the inactivation of oocytes. This same author (Wainwright, 2007b) verified the inefficiency of sodium hypochlorite (100 mg/l) for periods ranging between 30 minutes and 24 hours and of the ozone itself (6 mg/l) for periods ranging between 1 and 12 minutes.

Dubey and Thayer (1994) studied the efficiency of using irradiation under different conditions for inactivating *T. gondii* cysts, testing doses ranging between 0.1 and 0.9 kGy at 5 °C, with a gamma radiation source of Cs¹³⁷. Doses of 0,4 kGy, rendered the cysts nonviable, while a dose of 0.25 kGy was inefficient at temperatures ranging between -4 and 16 °C.

2.2.2 Prevention

The French National Health and Nutrition Programme specifically recommends that pregnant women should avoid eating raw or undercooked meats as well as smoked or marinated meat that is not going to be cooked (PNNS, 2007).

In relation to the risks involved in consuming non-animal based products, a number of analytical epidemiological studies show a significant relation between the presence of outbreaks of toxoplasmosis and the consumption of fruit and vegetables that have not been washed (Kapperud et al., 1996) (Lopes et al., 2012). In France, an analytical epidemiological analysis of certain cases of sporadic toxoplasmosis identified the consumption of vegetables outside the home as a significant risk factor (Baril et al., 1999), to the point that the French National Health and Nutrition Programme recommends that pregnant women should not consume these products outside the home (including aromatic herbs) if the sanitisation treatment used is unknown (PNNS, 2007). The *European Toxo Prevention Project* (EUROTOXO, 2006) establishes recommendations to prevent toxoplasmosis infections in pregnant women:

- Wash hands before handling food.
- Cook meat at a high temperature in order to kill *Toxoplasma*.
- Clean cooking surfaces and utensils after they have been in contact with raw or cured meat, poultry, fish and unwashed fruit and vegetables.
- Use gloves to remove domestic cat faeces or clean their beds and wash hands thoroughly after doing so.
- Use gloves to handle indoor or outdoor plants and wash hands thoroughly after doing so.

2.3 *Brucella* spp.

Brucellosis is a zoonotic disease caused by a number of species belonging to the *Brucella* genus. In humans, there are six identified pathogens and all of them have specific animal reservoirs: *B. melitensis* (goats and sheep), *B. abortus* (bovine), *B. suis* (pigs), *B. canis* (dogs) and *B. ceti* and *B. pinnipedialis* (marine mammals). *B. melitensis* is the most virulent species and is responsible for the majority of the notified cases in Mediterranean countries (Pappas et al., 2005). In 2012, 328 cases of brucellosis in

humans were confirmed in the European Union (0.07 cases per 100 000 inhabitants), which represented a drop of 2.4 % compared with the number of cases notified in 2011. Of these, the involved species of *Brucella* was identified in 99 cases, of which 83.8 % were due to *B. melitensis*, 10.1 % to *B. abortus*, 3.0 %, to *B. suis* and the remaining 3.1 % were due to other species of *Brucella* (EFSA/ECDC, 2014).

The number of cases of brucellosis varies greatly in terms of geography, with the most prevalent areas being countries in the Mediterranean, Latin America, western Asia and some parts of Africa (Corbel, 1997). In the European Union, the number of cases has dropped in a statistically significant level over the last 5 years and although it is considered to be a rare disease, it should be noted that it is very much a regional zoonoses. The drop in cases coincides with a drop in number of cattle, sheep or goats with *Brucella*, which indicates the efficiency of the health campaigns and eradication programmes in place in the European Union (Rodríguez et al., 2012). In a report on the *EpiSouth* project, part of the Network of Surveillance for Infectious Diseases of countries in the Mediterranean area and the Balkans, it identified brucellosis as one of the five most important zoonoses to be controlled (Vorou et al., 2008).

In Spain, the percentage of herds classified as free or official free from ovine and caprine brucellosis in 2011 was 94.87 %. At 31 December 2011, 98.53 % of the herds were negative in the last diagnostic test (MAGRAMA, 2013a). With regard to bovine brucellosis, the percentage of herds classified as free or officially free from brucellosis was 97.83 % and on 31 December 2011, 99.68 % of the herds were negative in the last diagnostic test (MAGRAMA, 2013b).

In the European Union, 67.7 % of the confirmed cases of brucellosis were notified by countries that did not yet have the classification of "officially free from brucellosis", among which were Spain, Greece and Portugal. Greece and Portugal had the highest number of notifications with 1.09 and 0.36 cases per 100 000 inhabitants, respectively. The majority of the confirmed cases in countries with the classification of "officially free from brucellosis" were for people that had contracted the disease outside the country. In Spain, in 2012 there were 62 confirmed cases (0.13 cases per 100 000 inhabitants) (EFSA/ECDC, 2014).

In Spain, brucellosis is a mandatory notifiable disease since 1943. Royal Decree 2210/95 creating the National Epidemiological Surveillance Network indicates that individualised information has to be obtained for cases of brucellosis, via the mandatory Disease Notification System (BOE, 1996). This information is complemented with the collection by other subsystems that form part of the Basic Surveillance System, such as the notification of outbreaks (Rodríguez et al., 2012).

The disease in humans begins suddenly or insidiously, with non-specific symptoms, similar to flu symptoms (fever, headache, weariness) and its duration can vary. However, sometimes, serious infections can occur, causing central nervous system complications and endocarditis. Also, it may last a long time, leading to chronic symptoms with recurring fever, joint pain, arthritis and fatigue. In 2012, in the European Union, almost four out of five cases of human brucellosis (taking into account those for which hospital information is available) included hospitalisation, although only one death was reported (EFSA/ECDC, 2014). Given that *Brucella* causes miscarriage and foetal malformations in animals, it has traditionally been considered a potentially abortive and teratogenic agent in humans, despite the absence of erythritol in the human placenta (Khan et al., 2001) (Doganay and Aygen, 2003) (Pappas et al., 2005) (Al-Tawfiq and Memish, 2013). Some authors communicated significantly more cases of

spontaneous miscarriages and intra-uterine deaths as a result of *Brucella* infections than those found in the general population of pregnant women (Khan et al., 2001). Gulsun et al. (2011) discovered that the number of premature births and underweight babies significantly increased in pregnant women with brucellosis. However, the relationship between the infection and the appearance of problems during pregnancy may be controversial for many authors, the number of spontaneous miscarriages, premature births and intrauterine infection is no higher than that observed in infections attributed to other microorganisms (Doganay and Aygem 2003). However, recent studies have shown the ability of these bacteria to grow in the extravillous trophoblasts, which are vital for the implantation of the zygote during the initial stages of pregnancy (Salcedo et al., 2013). Congenital brucellosis due to an infection transmitted by the mother during the last month of pregnancy or during childbirth appears within a few days or weeks of life of the child, acutely, subacutely or chronically mainly with digestive symptoms: Hepatitis with diffuse affectation of the liver and, more rarely, hepatic abscesses have also been reported.

In humans there are two main forms of transmission: 1) through food as a result of consuming products from infected animals, particularly raw milk and dairy products made with raw milk and 2) through direct transmission from touching infected animals or infected tissues, inhalation of infectious particles and accidental inoculation with live *Brucella* vaccines.

In Spain, between 1996 and 2011, there were 319 outbreaks of human brucellosis declared to the National Epidemiological Surveillance Network (food transmission and direct transmission). The outbreaks that were transmitted directly were the more prevalent ones and they occurred mainly in people related to the rural and farming scene or through touching or handling infected animals or products thereof (inhalation or direct contact), abattoir workers as well as laboratory staff through handling samples for diagnosis or from live vaccines. During the 1996-2011 period, the number of directly transmitted brucellosis outbreaks reached 233, representing 73 % of the total. Outbreaks through food transmission were related to the consumption of raw milk or dairy products (cheeses) from infected animals, made in an artisan manner and without suitable health controls, with 86 outbreaks being registered (27 % of the total). Of these, 84 % (72) were attributed to the consumption of cheese and 14 % (12) to the consumption of raw milk. In two outbreaks the relevant food could not be identified (Rodríguez et al., 2012).

As in Spain (Méndez Martínez et al., 2003) (Colmenero et al., 2011), the consumption of raw milk and dairy products made with raw milk is still the most common source of outbreaks through food transmission in European countries of the Mediterranean, such as: Bulgaria (Tzaneva et al., 2009), France (Mailles et al., 2012), Greece (Karagiannis et al., 2012), or Italy (Farina et al., 2008), among others.

In the European Union, Regulation (EC) No 853/2004 of the European Parliament and of the Council, of 29 April 2004, lays down specific hygiene rules for food of animal origin (EU, 2004). This rule, in its chapter on health requirements for the production of raw milk, includes specific provisions in relation to brucellosis. In this regard it establishes that raw milk must come from cows, buffalos, sheep or goats belonging to herds/flocks that have been declared free or officially free from brucellosis. However, if authorisation is obtained from the relevant authorities, raw milk from animals that do not meet the aforementioned requirements may be used, providing:

1. In the case of cows and buffaloes, they do not have a positive reaction to the brucellosis tests or show signs of this disease; the milk must be submitted to a thermal treatment until it shows a negative reaction to the phosphatase test.
2. In the case of ovine and caprine species, they must not have a positive reaction to the brucellosis tests or they must be vaccinated against brucellosis in the framework of an authorised elimination programme and not show signs of the disease, providing the milk is only used to prepare cheese with a maturing period of at least two months or it is submitted to a thermal treatment until it shows a negative reaction to the phosphatase test.

Compliance with regulations in force by farms and establishments for processing milk is the best way of reducing cases of foodborne human brucellosis.

Pregnant women should avoid consuming raw milk or homemade dairy products made with raw milk, particularly if the health classification of the source farms is unknown. Likewise, they should be especially careful with these types of products when they travel to countries where brucellosis is very prevalent.

2.4 Hepatitis E virus

In the European Union, enteric viruses were responsible for 14 % of all the outbreaks of toxoinfections in 2012, which is a 44.3 % increase in comparison to 2011 (EFSA/ECDC, 2014). The viruses involved were Norovirus and viruses causing enteric hepatitis (hepatitis A and E). Although hepatitis E is rare in Europe, it is considered an emerging infection as shown with the increase of sporadic cases in France (Colson et al., 2012) or more importantly, the outbreak in Italy in 2011 (Garbuglia et al., 2013). In Spain a number of cases of acute hepatitis E in tourists have been published, and three in natives of Spain, two in Seville and one in Madrid. The seroprevalence in blood donors in Madrid is 2.9 % and in Seville it is 4 % (Mateos and Tarragó, 2000).

The hepatitis E virus (HEV) causes an acute, benign and self-limiting infection. However, some cases of chronic hepatitis have been described particularly in patients with suppressed immune systems including those receiving organ transplants (Kamar et al., 2008) (Abravanel et al., 2014) (Fujiwara et al., 2014) (Pischke et al., 2014). HEV shows four main transmission routes (Khuroo, 2008): Consumption of contaminated water, consumption of raw or rare meat from infected animals, parenteral transmission through blood transfusions or organ transplants and mother-child vertical transmission. Although in western countries most cases are sporadic and isolated, in Asia and Africa there are large numbers of epidemic outbreaks associated with the consumption of contaminated water (Wong et al., 1980) (Aye et al., 1992) (Teshale et al., 2010a). Person-to-person transmission can lead to outbreaks (Teshale et al., 2010a), but it is rare. However, no outbreaks have been described due to the consumption of shellfish or vegetables contaminated through faeces.

There is only one serotype of HEV. Despite this, the differences in the RNA genome sequence allow for differentiating four genotypes, which include 24 subgenotypes (Bosch, 2011). Therefore, genotype 1 consists of isolated epidemic strains in Asia and Africa. Genotype 2 includes strains isolated in Mexico and Nigeria. Genotype 3 is the most genetically diverse and includes strains isolated in western

countries not considered endemic and lastly, genotype 4 includes strains isolated in China. Genotype 1 is subdivided into five subgenotypes (a-e), genotype 2 into two (a and b), genotype 3 into ten (a-j) and genotype 4 into seven (a-g).

HEV is a zoonotic agent that infects birds (Goens and Perdue, 2004), rats (He et al., 2002), wild boars (Sonoda et al., 2004), deer (Tei et al., 2003) and pigs (Meng et al., 1997) (Banks et al., 2004), among other animals. The strains that infect animals belong to genotypes 3 and 4 and are phylogenetically similar to human genotypes present in the geographic zone in which they are isolated. However, zoonoses has not been described for genotypes 1 and 2 (Teshale et al., 2010b).

Despite the fact that normally hepatitis E manifests as a benign infection, the severity in pregnant women during the third trimester can be much higher than in the rest of the population. Mortality rates are estimated to be between 15 % and 20 % as a result of fulminant hepatitis (Khuroo, 2008) (Kumar et al., 2004b). These cases of fulminant hepatitis are characterised by a faster development of the disease, with rapid encephalopathy, lower levels of bilirubin and high levels of disseminated coagulation (Khuroo and Kamili, 2003). There is evidence to suggest that the higher the viral replication, the greater the tendency of fulminant hepatitis (Kar et al., 2008) (Borkakoti et al., 2013). It has been suggested that high levels of oestrogen and progesterone during pregnancy would enable a greater viral replication, which combined with a low ratio of lymphocyte counts CD4+/CD8+ could be the reason for the onset of fulminant hepatitis (Jilani et al., 2007). However, this is a controversial issue and therefore it must be concluded that the factors that affect the fulminant development have not been entirely identified. It should be pointed out that fulminant cases are more common in endemic countries such as Pakistan (Shahzad et al., 2001), India (Kumar et al., 2004a) or Nepal (Shrestha, 2006). However, in Egypt, a country with a very high prevalence of anti-HEV antibody, there have never been any reported cases of fulminant hepatitis (Stoszek et al., 2006a, 2006b). This suggests that the development of fulminant hepatitis in pregnant women in the third trimester would not be so much the result of a greater probability, due to the greater number of infections, but to infections with more virulent genotypes. Therefore, it can be concluded that genotypes 1 and 2 may be more virulent than the 3 and 4 genotypes (Kar et al., 2008). In fact the severity of hepatitis E in western countries does not seem to differ greatly among the general population and that of pregnant women in the third trimester (Kar et al., 2008).

Other consequences of the infection in the third trimester of pregnancy are the greater number of miscarriages, more premature births, higher mortality rates among newborns and vertical mother-child transmission in those that survive (Khuroo et al., 1995). Once again it has been suggested that these are phenomena related to the mother's viral burden and the virus genotype (Khuroo, 2008).

From a food safety point of view, it is worth pointing out that cases of the disease due to the consumption of pork have been reported (Yazaki et al., 2003) (Bouwknegt et al., 2007) (Feagins et al., 2007) and to a lesser degree, game (Tei et al., 2003). In Germany a systematic case-control study was carried out and the consumption of offal and wild boar has been identified as a risk factor for the infection of hepatitis E (Wichmann et al., 2008). Another case-control study carried out on a small scale in France related the consumption of sausages made with raw pig's liver with the appearance of the disease (Colson et al., 2010), and other authors consider the risk of contracting the disease

through the consumption of these products as high (Berto et al., 2013). Of all the meats mentioned above, the most relevant one is that of pork, given its high consumption and also the fact that it has been reported that 11 % of pig's liver sold in butchers and meat shops in the United States is infected by HEV (Feagins et al., 2008). Studies on seroprevalence in pig farms in Spain showed that 30 % of adult pigs are positive and viral RNA was detected in 3 % and 6 % respectively, in line with what was detected in Italy and the Czech Republic (Di Bartolo et al., 2012). In all the cases the genotype that was detected was 3 (Di Bartolo et al., 2012). However, the anti-HEV seroprevalence in Spain, a country that consumes high amounts of raw pork, is less than 10 % of the population (Echevarría et al., in press). Also, the genotypes associated with zoonoses are fortunately less virulent.

With regard to the inactivation of HEV, there is not much information available given the low replication of the virus in cell cultures, which prevents infectivity determination studies from being developed.

A study carried out with genotype 1 and 2 of the HEV strains adapted to cell culture showed that they were less resistant to inactivation by cooking than the HAV (Emerson et al., 2005). As an alternative to the in vitro replication, infection models in pork have been used (Barnaud et al., 2012). This allowed for establishing that internal temperatures of meat of 71 °C are required during 20 minutes in order to completely inactivate the infectivity of the genotype 3 HEV. There are no inactivation studies with emerging technologies.

2.5 Other biological risks

In general terms and given the particular immunological condition of pregnant women, any infection can pose a risk, even when the relevant microorganism does not have a particular tropism for the placenta or the foetus. For example, maternal hyperthermia during the first term of pregnancy has been associated with defects in the neural tube (Graham et al., 1998) (Moretti et al., 2005) and cardiovascular alterations of the foetus (Tikkanen and Heinonen, 1991). Also, infestations by helminth parasites or intestinal protozoa infestations tend to be associated with nutritional deficiencies, particularly the onset or worsening of pregnancy anaemia, or to a greater risk of low birth weight (Rodríguez-García et al., 2002) (Obiezue et al., 2013).

However, as the list of foodborne microorganisms that could affect pregnant women is very long, this chapter will only address microorganisms that are mentioned in the bibliography as having a risk of transplacental transmission.

2.5.1 Enterohemorrhagic *Escherichia coli* (EHEC/VTEC)

Enterohemorrhagic or verotoxigenic *Escherichia coli* (EHEC/VTEC) is defined as the group of pathogen strains that produce Shiga toxins (Stxs) that cause hemorrhagic colitis and sometimes Haemolytic Uremic Syndrome (HUS) and Purple Thrombocytopenia Thrombosis (PTT). Although the number of infections by EHEC is much lower than those of other enteric pathogens, diseases caused by these microorganisms have much higher morbidity and mortality rates. 5-10 % of patients develop HUS, with a mortality rate of 3-5 %. HUS is the first cause of acute renal failure in small children and is associated with severe neurological complications (convulsions, coma, etc.) in 25 % of the cases and chronic renal failure in approximately 50 % of survivors (Lim et al., 2010).

The number of confirmed cases of EHEC has shown a progressive increase in the European Union since 2008. In 2011 the number of cases increased by 2.6 and complications to 4.5, with regard to 2010, due to a massive outbreak produced by a particularly virulent strain of serotype O104:H4 (EFSA/ECDC, 2013). In 2012, the number of reported cases was 5 671, 40 % less than in the previous year (EFSA/ECDC, 2014).

Around 380 different serotypes of *E. coli* producers of Shiga toxins in humans and animals have been isolated, but only a few were related to the manifestation of the disease in humans. *E. coli* O157:H7 is the most frequent isolated pathogen serotype (Nguyen and Sperandio, 2012) (EFSA/ECDC, 2014). Most strains of this serotype have the special feature of not fermenting D-sorbitol, not producing β -glucuronidase and an inability to grow at 44.5 °C. Other serotypes causing outbreaks or sporadic cases are O26:H11, O91:H21, O111:H8, O157:NM (Lim et al., 2010).

The Shiga 2 toxin (Stx2) is the main virulent factor known for enterohemorrhagic *Escherichia coli* (EHEC/STEC). The main studies about the effect of the Shiga toxin on foetuses have been carried out on rodents which were administered the purified toxin intraperitoneally. Burdet et al. (2009) showed that premature births in rats were the result of the action of the Stx2 in treated rats and indicated that EHEC infections could be an undetermined cause of foetal morbidity in humans. In previous studies, Yoshimura et al. (2000) concluded that Stx2 produced foetal mortality in the initial stages of pregnancy and affected the puerperal behaviour of the female if the infection occurred during the latter stages of the pregnancy. Its effects on other animal species are also known. For example Shiga toxin producing *E. coli* is a continuous cause of miscarriages in flocks of sheep (Sargison et al., 2007).

Various studies on humans, some of them epidemiological, indicate that these bacteria could have an effect on pregnancy or neonates. There is some controversy concerning whether or not exposure to *E. coli* O157:H7 increases the risk of suffering from high blood pressure during pregnancy (Moist et al., 2009) (Nevis et al., 2013).

Although the frequency of infection by *Escherichia coli* in neonates and newborns has not been determined, it has been shown that there could be a transplacental transmission of the bacteria (Sgro et al., 2011). Also Stritt et al. (2013) refer to the appearance of HUS due to a Shiga toxins producing *E. coli* O146:H28 strain in a neonate, through transmission during childbirth. Ulinski et al. (2005) saw that the appearance of a HUS in a newborn was the result of the transmission by the mother of a Shiga-like toxin producing *E. coli* strain. Also, in a prospective study of HUS in the USA, an infection by EHEC was detected in three women who had developed this syndrome after childbirth (Banatvala et al., 2001).

Most of the epidemiological information available about EHEC/STEC refers to the serotype O157:H7 since, due to its special biochemical characteristics (inability to ferment D-sorbitol and to grow at 44.5 °C) it is easily differentiated from other *E. coli* strains. The primary reservoir is the intestine of beef cattle and other ruminants. However, most bovine strains are not transferred to humans and do not exhibit virulence factors associated with human diseases. On a few occasions virulent EHEC strains have been isolated in pigs, poultry and wild and domestic animals such as seagulls, goats, sheep, horses, dogs or rodents (Rahal et al., 2012). *E. coli* O157:H7 has also been detected in amphibians, fish and insects and can colonise plants, through different adhesion mechanisms to those that determine intestinal adhesion. *E. coli* also survives in biofilms on the surface of equipment and

it has been proven that the incomplete elimination of these biofilms increases the development of greater resistance to acids and biocides (Ferens and Hovde, 2011).

EHEC can grow at temperatures ranging between 7 to 50 °C with an optimal temperature of 37 °C. Some ECEH can grow in acid foods, up to a pH of 4.4 and in foods with water activity (aw) of 0.95. The bacterium is heat sensitive and does not survive when foods are completely cooked (70 °C or higher in the entire product) (WHO, 2011).

The transmission to humans mainly occurs through inadequately processed contaminated water or food and to a lesser degree, through contact with infected manure, animals or people. Ground beef is a particularly efficient transmission vehicle for EHEC as a result of easy cross contamination, the dispersion of bacteria along the substrate and the poor efficiency of dry heat as a sterilising agent, while contaminating bacteria on the surface of the canal have little chance of surviving the exposure to heat. Vegetables are also an important transmission vehicle for EHEC, given that the bacteria can remain adhered to raw or processed products and can survive in unpasteurised fruit juices (Ferens and Hovde, 2011). Increased expression of virulence factors during cold storage of contaminated vegetables has been reported (Carey et al., 2009).

There is evidence to suggest that *E.coli* O157 can survive in cheese produced with unpasteurised milk, even after a 60 day maturing period and recent outbreaks of the disease associated with *E.coli* O157 have been documented as a result of consuming unpasteurised ripened cheese in the United States (CID/CNP, 2014).

The main infection outbreaks by EHEC are related to the consumption of rare beef, particularly hamburgers, undercooked meat products such as salami or sausages, unpasteurised milk, raw shoots, fresh spinach, tomatoes, lettuce and unpasteurised apple juice (Ferens and Hovde, 2011). On some occasions, contamination has been detected on the equipment (mincers) after the appearance of outbreaks from beef (Banatvala et al., 1996).

Outbreaks due to the consumption of contaminated water have also been documented and the microorganisms have been detected in recreational waters, in amounts that did not coincide with the levels of faecal bacterial indicators (Duris et al., 2009).

Person-to-person transmission of EHEC also seems to be important and is considered to be responsible for the appearance of secondary cases (up to 14 % according to some authors) through a holomiantic outbreak (Seto et al., 2007) (Gilbert et al., 2008). Therefore, washing hands correctly and good hygiene practices must form part of any prevention programme.

2.5.2 *Salmonella* spp.

Salmonella is the second most common producer of toxoinfections, with 92 916 confirmed cases in Europe in 2012, although these numbers are dropping continuously (EFSA/ECDC, 2014). In Spain 4 215 cases were notified in 2012. *Salmonella* Typhimurium was the most commonly declared serotype with 1 218 cases, followed by *S. Enteritidis* with 1 024 cases (ISCIII, 2013).

There are three species within the genus: *Salmonella enterica*, *Salmonella bongori* and *Salmonella subterranea*. These species are divided into seven subspecies (Chen et al., 2013). *S. enterica* species is divided into six subspecies: *enterica* (subspecies I), *arizonae* (subspecies IIIa), *diarizonae* (subspecies

IIIb), *houtenae* (subspecies IV), *indica* (subspecies VI) and *salamae* (subspecies II). Subspecies "V" is reserved for the *S. bongori* species. With regard to *Salmonella subterranea*, whether or not it is a species is a controversial issue among different authors (Grimont and Weill, 2007).

Today, both the World Health Organisation and reference laboratories use the scheme known as Kauffmann-White as the basis for classifying bacteria from the genus *Salmonella*. In this system, subspecies are divided into around 50 serogroups, defined according to major somatic O antigens and around 2 500 serotypes, characterised by a unique antigenic formula that includes O antigens, flagellar antigens or H1 and H2 antigens and, occasionally, capsular antigens (for Typhi, Paratyphi and Dublin serotypes).

Most potentially pathogenic *Salmonella* serotypes are encased in the *enterica* subspecies (Chen et al., 2013). This subspecies has maintained the classic denominations of many serotypes, which refers to the main host or to the place where the isolation was carried out initially for its identification.

Based exclusively on clinical manifestations, two types of strains can be differentiated: Those that produce enteric fever (*S. enterica*, subspecies *enterica*, serotype Typhi and serotype Paratyphi A) and the rest of the pathogenic serotypes that cause gastroenteritis (Sánchez-Vargas et al., 2011).

Enteric fever or typhoid fever is a serious systemic disease that manifests as high fever, general ill-feeling and profuse diarrhoea, which sometimes contains blood. Some patients present macules and papules on abdomen and chest. Other symptoms include hepatosplenomegalia, intense fatigue, weakness and lethargy. Mortality rates can reach 10-30 % for untreated cases and these are normally the result of peritonitis and intestinal perforation, encephalopathy, myocarditis and haemodynamic shock. If treated early, the mortality rate is lower than 1 %.

Gastroenteritis caused by other *Salmonella* serotypes are normally self-limiting, with average durations ranging between 3 and 7 days.

Systemic infection is a complication that appears in approximately 5 % of the cases and is more common in patients with weakened immune systems, particularly those with alterations in the cellular immune compartment (Chen et al., 2013). Secondary bacteraemia is associated with extra-intestinal manifestations such as meningitis, encephalopathy, endocarditis, pneumonia, abscesses, osteomyelitis, cellulitis or arthritis. Children aged between 0 and 6 years with bacteraemia have a 24 % risk of contracting meningitis (Sánchez-Vargas et al., 2011).

Salmonella bacteria are excreted in the faeces after the infection for a period of around 5 weeks, which is longer in children under the age of 5 years and in patients with alterations in the cellular immune compartment. Between 1 and 4 % of patients with enteric fever continue as chronic carriers, while in the case of gastroenteritis, the percentage of chronic carriers ranges between 0.1 and 1 % (Sánchez-Vargas et al., 2011).

Pregnant women do not appear to have a greater risk of acquiring the infection than the general population. However, maternal infection increases the risk of complications such as neonatal and maternal sepsis, chorioamnionitis, spontaneous miscarriage, premature birth and perinatal complications. This would appear to be related to the level of immaturity of the neonate immune system, somewhat related to the weight of the newborn child. Studies with murine models have also shown increased maternal mortality (Pejic-Karapetrovic et al., 2007) (Chattopadhyay et al., 2010).

Contrary to what occurs with *S. Typhi* infections, in which the vertical transmission and manifestation of neonatal typhoid fevers is relatively common (Vigliani and Bakardjiev, 2013), the vertical transmission in the case of *S. Paratyphi* is rare (Reed and Klugman, 1994). Raveendran et al. (2007) describe a case of neonatal enteric fever through vertical transmission by the mother to the foetus. In the mother, infections by *S. enterica* serotype Paratyphi A tend to be benign, but in neonates they are systematically fatal.

With regard to serotypes causing gastroenteritis, the associated bacteraemia can cause intrauterine sepsis (Scialli and Rarick, 1992). Transplacental transmission cases have been detected (Coughlin et al., 2003), as well as infections in neonates with different symptoms through maternal transmission of *Salmonella* spp. via the mother's milk (Cooke et al., 2009).

Salmonella has a great adaptation capacity, enabling it to survive in very different environments over months or even years. It can multiply in a wide range of temperatures, from 5 to 45 °C. Its optimal growth temperature ranges between 35 and 37 °C and its generation time at this temperature is around 22 minutes. It is able to survive in a wide range of pH, between 3.8 and 9.5, growing better at nearly neutral pH (6,5-7,5). Exposure to low pH has been shown to increase its acid tolerance. The value of the optimal water activity (*aw*) for its multiplication is 0.995 although it can grow at values ranging between 0.945 and 0.999 and is able to multiply in foods with *aw* values below 0.93. Its survival under refrigeration temperatures has been demonstrated and some strains are psychrophilic. Another point is its ability to acquire a certain resistance to heat after being exposed to sublethal temperatures (Li et al., 2013). Even so, in general terms it is accepted that *Salmonella* does not survive cooking temperatures (70° C or more during at least one minute).

Humans are the only reservoir for *S. Typhi* and *S. Paratyphi* and transmission is related to contact with fomites or consumption of food and water contaminated with faecal material from patients or carriers. The natural habitat of other *Salmonella* serotypes is the gastrointestinal tract of mammals, reptiles, birds and insects. It is also found in water, food or the environment which it reaches through faecal materials. Farm animals are the main reservoir in developed countries. The most common mode of transmission to humans is the faecal-oral route, by consuming foods of animal origin, but also through cross contamination, ingesting water or through contact with domestic or farm animals. Person-to-person transmission is also common (Sánchez-Vargas et al., 2011).

Foods more commonly involved in the transmission of *Salmonella* include milk and unpasteurised derivatives, raw or rare meat and poultry, raw eggs or lightly cooked eggs, raw shoots (alfalfa, soy, radishes), raw vegetables and any foods prepared with the aforementioned ingredients, including salads, desserts, sauces, etc. (Wattiau et al., 2011).

The main prevention measures for salmonellosis include good hygiene practices, including washing hands often with soap and hot water, particularly after using the toilet or after coming into contact with anybody with diarrhoea or fomites thereof (nappies, underwear). Adopting safe food handling practices is key to preventing transmission: Hands and surfaces must be washed thoroughly after handling meat, fish, poultry and other raw foods, fresh fruit and vegetables must be washed (Dean and Kendall, 2012) before eaten and foods such as unpasteurised milk and derivatives, raw or lightly cooked eggs, raw sprouts and raw or rare meat should be avoided.

2.5.3 *Campylobacter* spp.

Campylobacter is the main zoonotic agent worldwide. In Europe in 2012, 214 268 confirmed human cases were notified and this figure is increasing every year (EFSA/ECDC, 2014). In Spain it is also the leading cause of notified bacterial gastroenteritis: In 2012, 5 539 cases were declared, being *C. jejuni* the major species, with 4 497 cases (ISCIII, 2013).

Campylobacter infection is characterised by gastroenteritis that is indistinguishable from those produced by other enteric pathogens. In most cases it is self-limiting, although in patients with weakened immune systems it is much more serious. The disease caused by *C. foetus* tends to be more severe than that caused by *C. jejuni*. Sometimes, it involves gastrointestinal complications (proctitis, pancreatitis or colecistitis) or extraintestinal complications such as septic or reactive arthritis and Guillain-Barré syndrome. Although the risk of developing the latter disease after having suffered from campylobacteriosis is very low (1/1 000 patients), the rate of occurrence of the infection has led it to be considered a risk factor. Bacteraemia, which is more common in cases of infection by *C. foetus* than by *C. jejuni*, appears in 0.1-0.6 % of the cases (Nyati and Nyati, 2013).

Pregnant women are at no more risk of getting the disease than the general population and it is normally moderate and self-limiting. However, some authors indicate increased cases of high fever, systemic dissemination and septic shock (Simor and Ferro, 1990) in infected pregnant women. Although there is not much information available concerning sequelae or increased maternal mortality rates, there is at least one documented case of death through sepsis, 17 days after childbirth and 11 days after the death of the foetus. Infections during the second half of pregnancy would appear to have a better prognosis than earlier ones (McDonald and Gruslin, 2001). The Guillain-Barré syndrome during pregnancy does not affect foetal development, or increase the risk of miscarriage or perinatal mortality. However, in more serious cases, it can lead to premature labour, if the infection is caught during the last trimester of pregnancy (Smith, 2002).

Vertical transmission to the foetus following maternal bacteraemia has been illustrated (Smith, 2002), as well as the onset of neonatal infections after faecal contamination during labour (McDonald and Gruslin, 2001). Infections during early stages of pregnancy are characterised by bacteraemia, prolonged fever and pneumonitis but they are also associated with an increased number of miscarriages and premature births. In the case of neonates, the majority undergo moderate illness, although the infection leads to neonatal sepsis or to the onset of meningitis more often than in adults. The latter complications, which are more common in the case of *C. foetus*, can be fatal or lead to very serious neurological sequelae (Smith, 2002). In neonates the mortality rate by *Campylobacter* is 2.5 % (McDonald and Gruslin, 2001).

The disease is mainly transmitted through the consumption of contaminated poultry or cross contamination from these products. Also through the consumption of undercooked pork or lamb, unpasteurised milk and contaminated water. Although patients excrete *Campylobacter* in stools for at least 2 weeks after the disease, person-to-person transmission is unusual (Friedman et al., 2000).

In comparison with other foodborne bacterial pathogens, *C. jejuni* has a limited capacity to survive under environmental stress (Dasti et al., 2010). It is unable to grow at temperatures under 30 °C; it is sensitive to most disinfectants, high oxygen concentrations, desiccation and low pH. Its decimal

reduction time at 55 °C is 1 minute and its z value is 5 °C (Habib et al., 2013).

Main prevention measures include good personal hygiene and suitable food handling practices: Wash hands after using the toilet, particularly if suffering from diarrhoea; wash hands before eating, particularly after touching animals; avoid handling food if suffering from diarrhoea, until the illness has stopped, etc.

The principal route of transmission of *C. jejuni* infection to humans is through eating or handling poultry. Therefore, the most efficient preventive strategies are those that interrupt the transmission, such as cooking meat correctly and avoiding cross contamination from the surfaces used to cut or handle raw poultry or other meats.

Conclusions of the Scientific Committee

When addressing microbiological risks for pregnant women, it is important to bear in mind that, in Spain, the number of main foodborne diseases in this population group is low. Therefore, the problem of perinatal listeriosis or toxoplasmosis must be kept in perspective when talking to patients, pointing out that very few pregnant patients actually contract the disease. The number of other risk diseases for this population group, such as brucellosis or hepatitis E, is even lower. Also, the risk of pregnancy complications due to other toxoinfections, such as those caused by *Salmonella*, *Campylobacter* or verotoxigenic *E. coli* is also very low. However, this information is of little comfort to those who continue with their pregnancy and give birth to a disabled child or a stillborn baby.

A number of studies have found a consistent relationship between the application of preventive measures in the food industry and the reduction of the number of foodborne diseases, including listeriosis. These measures must be accompanied by educating individuals belonging to high-risk groups and training health agents in the early detection of suspicious cases. It would also be wise to include easily understandable information on the product labelling about the processing treatment to which the food has been subjected, in order to provide pregnant consumers with more information.

During the initial prenatal visit, it is important to explain to pregnant women what listeriosis is and how it is transmitted, to inform them of the pathogen's capacity to replicate in refrigerated food and possibly, in heat-treated products if they are not handled correctly. Also, it is important to discuss the existence of other possible microbiological risks, particularly *Toxoplasma* infection. By doing so, this specific vulnerable population group can be provided with general prevention rules that are easy to understand and to follow and will reduce the risks for this group. These rules should list foods that should be avoided, correct food handling practices and hygiene rules for avoiding cross contamination:

1. Foods to avoid during pregnancy

Pregnant women should be advised to avoid eating:

- Raw milk.
- Cheeses made with raw milk.
- Soft cheeses (Brie, Camembert).
- Fresh and soft cheeses, Burgos/Villalón style cheeses or Latin cheeses, Mozzarella and blue cheeses.
- Industrial grated cheeses and sliced cheeses, both industrial and from the delicatessen.

- Raw fruit and vegetables (including ready-packed salads and those eaten in establishments) that have not been washed and disinfected beforehand.
- Raw shoots (alfalfa, soy...).
- Unpasteurised juices.
- Partially cooked eggs.
- Foods that contain raw egg, including homemade sauces and mayonnaises, mousses, meringues and homemade cakes, tiramisu, homemade ice creams and eggnog.
- Raw meat, rare or medium meat.
- Smoked or marinated meat that is not going to be cooked.
- Cured raw meat products, such as sausages and delicatessen products (chorizo, salami, sausage...) cured ham, etc. Other sliced cold cuts.
- Refrigerated pates.
- Raw fish, common in sushi and sashimi dishes and ceviche.
- Refrigerated smoked or marinated fish that is not going to be cooked.
- Raw or undercooked bivalve molluscs: Oysters, mussels, etc.
- Ready-made sandwiches and other prepared foods that contain vegetables, egg, meat, cold cuts, fish and derivatives.
- Pre-cooked meals and ready-to-eat poultry should not be consumed cold. If these foods are included in the diet, they must only be eaten when heated to more than 75 °C.

Semi-cured and cured cheeses can be eaten when made with pasteurised milk, providing the rind is removed.

2. Some basic rules for the hygienic handling of foodstuffs:

- Ensure that foods are cooked completely at home: 71 °C must be reached for at least 1 minute (until the centre of the meat changes colour). A food thermometer should be used to ensure that food is cooked at the correct temperature.
- Foods should be stored in a separate refrigerator compartment, away from cheeses and raw foods. Foods should be stored for the shortest possible time, and in the case of commercial products, the expiry date on the labelling must be respected.
- Keep the refrigerator at the correct temperature, at or below 4 °C.
- Fruits and vegetables must be washed and disinfected. Specific products can be used for this, following the manufacturer's instructions. Products can also be disinfected by submerging them in water containing bleach suitable for drinking water (see labelling) for at least 10 minutes, at the rate of a heaping coffee spoon (1.2 to 2 ml) of bleach for each litre of water*. After the disinfecting stage, the product must always be rinsed with drinking water. This process must not be carried out before storing, but immediately before the product is going to be eaten.
- When using microwave ovens, users must read and follow the manufacturer's instructions carefully to ensure that foods are heated uniformly.

*Corrigendum (24-2-2016): a heaping coffee spoon (1.2 to 2 ml) of bleach for each litre of water instead of four drops.

- Reheated leftovers should not be eaten.
- Wash your hands with warm water and soap for at least 20 seconds, before and after handling foods, after touching any dirty materials (waste, animals) and particularly after using the toilet or coming into contact with any material contaminated with faeces (nappies, underwear...).
- Hands, kitchen surfaces and utensils that have been used should be washed thoroughly after handling meat, fish, poultry, unwashed fruit and vegetables and any other raw foods.
- Rinds should be removed from all cheeses.

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