The occurrence of selected zoonoses associated with consumption of meat and meat products

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Abstract

Zoonoses are diseases that can be transmitted from animals to man, either directly or via contaminated food. They are an extremely large group of human diseases with an extremely serious medical and economic impact, for which reason great attention is devoted to monitoring the incidence of zoonoses and their causative agents. The individual EU member states have national data collection systems, and the data collected are passed to the European Commission through the organisation EFSA, which conducts its annual assessment. The diseases with the largest number of proven cases in the Czech Republic have long included salmonellosis and campylobacteriosis. Listeriosis and diseases caused by verocytotoxigenic strains of *E. coli* are other serious diseases of bacterial origin. Trichinellosis is the most serious parasitic zoonosis with confirmed positive occurrence in the EU. This study aims to assess the results of monitoring of the selected significant alimentary diseases given above, including their causative agents, and to compare the number of reported cases of these diseases in the Czech Republic and the European Union in the period 2004–2012, including an evaluation of trends.

Campylobacteriosis, high-risk foodstuffs, listeriosis, monitoring of zoonoses, salmonellosis

Introduction

Selected results of monitoring in the Czech Republic

Salmonellosis

Human salmonellosis is caused by a number of serotypes of the genus *Salmonella*. The genus *Salmonella* is divided into two species – *Salmonella enterica* and *S. bongori*. *S. enterica* is further divided into six subspecies, though the majority of zoonotic salmonella belong to the subspecies *enterica*. This subspecies is further divided into more than 2 600 serovars, of which some are capable of causing disease in man. The five serovars monitored in livestock for slaughter are *S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Virchow and *S.* Hadar. *S.* Enteritidis and *S.* Typhimurium are the serotypes occurring most frequently in the EU and the Czech Republic. *S.* Enteritidis is found most commonly in contaminated eggs and poultry meat, while *S.* Typhimurium occurs in pork and beef meat. The digestive tract of a number of domestic and wild animals is a common reservoir of the genus *Salmonella*. Foodstuffs of plant and animal origin may be contaminated directly and indirectly by bacteria, including salmonella, from the digestive tract. Man may also be a source of a contamination. Salmonella is ubiquitous. It can be isolated from water, soil, plant surfaces, insects, and aquatic molluscs and crustaceans (Amagliani et al. 2012) and from a wide range of cold-blooded and hot-blooded vertebrates including household pets, livestock and wild animals (Paulsen et al. 2012; EFSA and ECDC 2013).

Domestic animals and poultry in particular, are considered the most important source of these bacteria for man. *Salmonella* spp. commonly inhabits the digestive tract of animals, though only certain serovars induce clinical illness in adult animals. Poultry do not generally show any signs of infection, while clinical symptoms are more frequent in cattle

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than in pigs (EFSA and ECDC 2013). Animals generally become asymptomatic carriers of salmonella and excrete bacilli into the environment, particularly under the influence of stress. Salmonella are highly resistant in the external environment and can survive for long periods in the animal rearing environment if decontamination is inadequate and act as a source of infection for other animals (Jay et al. 2005).

Salmonellosis generally clinically manifests itself as enteritis. Infection takes an oral route, most frequently by consumption of contaminated food or water, though transmission by direct contact with infected animals, including household pets, is also possible. The incubation period ranges from 6 hours to 3 days. A shorter incubation period is generally associated with a higher infectious dose or highly sensitive individuals. Clinical symptoms include watery diarrhoea, nausea or vomiting, abdominal pain and a slight fever. The symptoms generally disappear on their own after between 2 to 7 days, and only last more than a week in extraordinary cases (Hanes 2003). The infectious dose is generally 10^6 cells, though cases of the disease with an infectious dose of 10 – 100 cells or less have been recorded. The size of the infectious dose fluctuates depending on the virulence of the agent, the sensitivity of the given individual and the type of contaminated foodstuffs (Adams and Moss 2008). Death occurs in less than 1% of cases, generally as a consequence of other medical complications (EFSA and ECDC 2013).

In 2012, there were 91 034 reported cases of salmonellosis in EU member states, which represents a fall of 4.7% over 2011. A statistically significant fall in the number of reported cases of salmonellosis was seen in the period 2008–2012. This decline in the disease can be explained by the introduction of effective therapeutic programmes on poultry farms in all EU states. Salmonella occurs most frequently in raw poultry meat and also in minced meat and products made from minced meat, in meat products and in certain seafood.

Large numbers of cases of salmonellosis are traditionally reported in the Czech Republic (Plate I, Fig. 1). The incidence of salmonellosis in the Czech Republic is around 100 cases per 100 000 population. The most significant fall in the Czech Republic was recorded in the years 2010 and 2011, when 82 – 83 cases per 100 000 population were reported, while 100 cases per 100 000 population were reported in 2012. In comparison with the EU, where an average of 20.7 cases were reported in 2011 and 22.2 cases per 100 000 population in 2012, it is clear that the Czech Republic is one of the countries with the largest number of reported cases of salmonellosis in the EU (Plate I, Fig. 2).

In view of the seriousness of salmonellosis and the occurrence of salmonella in household pets and livestock animals, salmonella is given in EU Regulation No. 2073/2005 as one of the bacteria for which not only criteria of occurrence are stipulated in foodstuffs, but their occurrence is also evaluated in pig, cattle and poultry carcasses. Table 1 shows the percentage of positive findings of salmonella in samples taken at slaughterhouses, cutting plants and the retail. It is clear from the results that findings of salmonella on the surface of poultry carcasses at the slaughterhouse are relatively common in comparison with findings of salmonella on the surface of pig or cattle carcasses. Raw minced poultry meat and meat preparations made from poultry were among the foodstuffs that exceeded the permitted criteria most frequently in 2012, 2011 and preceding years. Other foodstuffs that exceeded the permitted criteria include minced meat (other than poultry) and meat preparations intended to be eaten cooked, but also cooked meat and cooked meat products. Salmonella was found in eggs and egg products only in isolated cases.

The incidence of salmonella in raw meat, meat preparations and meat products is wide-ranging. It goes without saying that the presence of salmonella in ready-to-eat meat products that have already been cooked or that are meant to be consumed raw represents the greatest risk to the consumer.

Table 1. The occurrence of salmonella in fresh meat at slaughterhouses, cutting plants and retail in the Czech Republic in [%] (EFSA 2014)

Type of fresh meat	Place of occurrence	2008	2009	2010	2011	2012
Poultry	Slaughterhouses	4.2	3.0	7.0	9.2	10.8
	Cutting plants	-	-	12.9	13.3	7.9
	Retail	-	1.7	-	10.0	-
Pigs	Slaughterhouses	0.6	0.2	0.4	0.4	0.7
Cattle	Slaughterhouses	0.2	0.3	0.3	0.4	0.2

The requirement for the absence of salmonella given in the legislation is part of the microbiological criteria relating to slaughter hygiene and the criteria valid for foodstuffs placed on the market in the case of fresh poultry meat, minced meat, mechanically separated meat, meat preparations, high-risk meat products and seafood – crustaceans, molluscs, echinoderms and tunicates (Commission Regulation (EC) No. 2073/2005, as amended). Determination of salmonella also comprises part of the determination of the effectiveness of cleaning and disinfecting procedures in facilities under veterinary supervision (Decree No. 289/2007 Coll. as amended).

Campylobacteriosis

Campylobacteriosis is one of the most common types of alimentary gastroenteritis around the world and is caused by thermotolerant *Campylobacter* spp. *Campylobacter jejuni* (*C. jejuni*), *C. coli* and *C. lari* are the species identified most frequently in human patients. The main reservoir of thermophilic *Campylobacter* is the digestive tract of wild animals and livestock, in particular poultry, pigs, cattle, sheep, dogs, cats and wild birds; water sources represent the most frequent environmental source. Campylobacter species are common contaminants of foodstuffs such as meat, raw milk, fish, fishery products, shellfish and drinking water. Cross contamination during household food preparation plays a significant role in transmission of the pathogen to food (Hu and Kopecko 2003).

Campylobacteriosis has been the most frequently reported food-borne zoonosis in the EU since 2005. There was a statistically significant ($P < 0.001$) upwards trend in the number of confirmed cases of campylobacteriosis in the period 2008 to 2011. There were 220 209 confirmed cases of campylobacteriosis in the EU in 2011, which represents an incidence of 50.28 cases per 100 000 population (EFSA 2013). Campylobacteriosis is also the most frequently reported bacterial alimentary disease in the Czech Republic, exceeding even salmonellosis (Plate I, Fig. 1). The total number of reported cases in the Czech Republic in 2012 was 18 412 which represents an incidence of 175 cases per 100 000 population. Along with Luxemburg (138 cases per 100 000 population), the Czech Republic is one of the countries with the largest number of confirmed cases of campylobacteriosis (NIPH 2014). A statistically significant upwards trend in the occurrence of this disease has been confirmed in practically every country in Europe, the single exception being Austria. However, it should be noted that any comparison of the occurrence of the disease in the EU member states is significantly influenced by factors such as the system of reporting, the quality of monitoring and the system of organisation of medical care in the individual countries.

The principal high-risk foods associated with campylobacteriosis are raw poultry, poultry meat cuts and poultry meat preparations, and to a lesser extent pork and beef meat

or crustaceans and other seafood. In addition to meat, raw unpasteurised milk, water and vegetables may also be contaminated by Campylobacter. Consumption of raw, unpasteurised or insufficiently thermally processed milk has been repeatedly confirmed as a significant risk in the last five years. Circumstances associated with changing lifestyles may also represent a problem; travel to high-risk areas, contact with animals and consumption of contaminated water (service water or surface water). Culinary processes such as grilling or roasting may also represent a risk, as may unhygienic handling of raw meat during household food preparation during which cross contamination may occur (EFSA 2013).

The frequent occurrence of *Campylobacter* spp. on the surface of chilled and frozen poultry and poultry meat cuts is confirmed by a number of studies conducted all over the world. In the Czech Republic, in view of the high seroprevalence on farms and the great prevalence of the pathogen in the digestive tract of poultry, secondary contamination of the surface of poultry carcasses may occur during carcass processing, which is reflected in the large number of positive finding of *Campylobacter* spp. in chilled (75%) and frozen (37%) broilers in retail (Bardoň et al. 2011). A similar situation was also confirmed by EU monitoring in 2011 in chilled poultry in Poland (82.7%), Spain (75.8%), Ireland (52.9%) and Hungary (41.3%); in contrast, the lowest occurrence demonstrated was in Belgium (17.1%). *C. jejuni* was confirmed as the dominant species in all countries (EFSA 2013).

The occurrence of *Campylobacter* spp. in pork meat in retail facilities falls within a range of 1–10% (Juntunen et al. 2010). In spite of the low numbers of positive findings, pork meat and pork preparations are considered a possible source largely because the digestive tract of pigs is colonised to a high degree by *C. coli* in particular, which is another proven agent of human campylobacteriosis. Its occurrence in poultry is less prevalent. Mdegela et al. (2011) state that the level of contamination of pig halves at slaughterhouses in Europe ranges from 2.9% (Poland) to 1% (Sweden), while Malakauskas et al. (2006) stated a figure as high as 64% positive findings in processed pig halves in Lithuania.

Listeriosis

The genus *Listeria* is made up of around 10 species, though only the species *Listeria monocytogenes* has been confirmed as a human pathogen. *L. monocytogenes* is considerably widespread in the environment, being found in surface waters and wastewaters. It is capable of surviving for long periods in soil, and also occurs in plant material, including silage. It has been isolated from the faeces of many mammalian and avian species and also from fish and marine bivalves and crustaceans. Listeria causes disease mainly in ruminants (cattle and sheep), it's most common manifestations being neurological symptoms and miscarriages, though Listeria may also cause mastitis in isolated cases. *L. monocytogenes* is also isolated to a lesser degree from the faeces of poultry and pigs, which are almost always asymptomatic carriers (Ivanek et al. 2006). The alimentary transmission of listeriosis, a disease caused in man by *L. monocytogenes*, has been known for only a few decades. This disease is not particularly common, though great attention has been devoted to this pathogenic microorganism and its presence and survival in ready-to-eat foods in particular in recent times in view of its serious manifestations and certain properties of *L. monocytogenes* (its growth in foodstuffs at refrigeration temperatures, its survival in the environment of food processing plants in the form of a biofilm).

L. monocytogenes may get into the food processing plant environment in view of the fact that it is commonly found in the environment and in the digestive tract of slaughter animals. This pathogen is often found in unprocessed and processed foods, including raw and pasteurised milk and dairy products (in particular cheeses), raw meat and meat products (Table 2), raw vegetables (particularly cut vegetable), fish products (cold-smoked fish) and delicatessen products.

The particularly high-risk foodstuffs in which *L. monocytogenes* can reproduce include, e.g., soft ripening cheeses (Farber and Peterkin 1991; Swaminathan and Gerner-

Table 2. Findings of *Listeria monocytogenes* in foodstuffs in the Czech Republic 2010–2012 (EFSA 2014)

Foodstuff	2010			2011			2012		
	Positive in 25g	≤ 100 CFU·g^{-1}	> 100 CFU·g^{-1}	Positive in 25g	≤ 100 CFU·g^{-1}	> 100 CFU·g^{-1}	Positive in 25g	> 100 CFU·g^{-1}	≤ 100 CFU·g^{-1}
Meat products	0.1	0	0	-	-	-	-	-	-
Soft and semi-hard cheeses									
- Processing	2.3	0	0	1.5	0.8	0.4	0	0	0
- Retail	0	0	0	-	0	0	0	0	0
Hard cheeses	0	-	-	0	-	-	0	-	-
Smoked fish products									
- Processing	-	-	7.7	2.4	0	6.4	0	0	0
- Retail	-	-	0	-	0	2.6	0	0	0
Other ready-to-eat products	1.3	-	0.8	-	-	-	-	-	-
Ready-to-eat poultry products	-	-	-	0.3	0	0	1.3	-	-
Ready-to-eat pork products	-	-	-	0.8	0	0.5	2.3	0	1.0
Fermented meat products	-	-	-	-	-	-	-	0	0

Smidt 2007 and EFSA 2008). These include the traditional Czech sour cottage cheese ("tvarůžky"), Romadur and soft blue cheeses of the Camembert type. Listeria cannot survive temperatures higher than 65 °C, though it can grow at temperatures of +2 to +4 °C. Secondary contamination of ready-to-eat foods that do not require further cooking and that have a relatively long shelf life is a frequent problem.

The foodstuffs posing the greatest risk are generally ready-to-eat products stored at refrigeration temperatures for long periods, which allows *L. monocytogenes* to grow to an infectious dose. Although the occurrence of Listeria in foodstuffs may be relatively high, it is not generally found in numbers approaching the infectious dose for the general population (EFSA 2008). Consideration has been given to this fact in the legislative requirements (Commission Regulation (EC) No. 2073/2005, as amended), which introduced a quantitative limit (100 CFU·g^{-1}) for ready-to-eat foodstuffs whose properties do not support the growth of *L. monocytogenes* (products with pH ≤ 4.4 or water activity ≤ 0.92; products with pH ≤ 5.0 and water activity ≤ 0.94; products with a shelf life of less than 5 days). Frozen products and foodstuffs that are cooked in their final packaging and are not, therefore, threatened with recontamination can also be included in the definition of foodstuffs not supporting the growth of *L. monocytogenes*. For foodstuffs in which Listeria could multiply the manufacturer must gather evidence in the form of challenge studies that this quantitative limit will not be exceeded during the product's shelf life. If such evidence does not exist, a stricter limit for the absence of Listeria in the sample applies to the foodstuff in question before it leaves the processing plant.

When the occurrence of *L. monocytogenes* in the food processing plant environment and foodstuffs is monitored, consideration should also be given to the occurrence of other species of Listeria (in particular *L. innocua*) in view of their similar growth properties and ecological niches (Tompkin 2002). The presence of *L. innocua* indicates the existence of suitable conditions for the presence of *L. monocytogenes* (e.g. inadequate sanitation, insufficient inactivation of Listeria during production processes, etc.). Human infection with *L. monocytogenes* occurs primarily by means of the consumption of contaminated foodstuffs, though transmission by direct contact with animals is possible in rare cases (the cutaneous form of listeriosis) and cases of nosocomial infection have also been recorded. Transplacental transmission is a specific case of its own.

The infectious dose of *L. monocytogenes* depends on the sensitivity of the individual and the virulence of the strain. Numbers of around 10^6 CFU·g^{-1} of consumed food have been reported

in healthy individuals, though the infectious dose is probably significantly lower in high-risk groups. The incubation period ranges from several days to several weeks or even months depending on the infectious dose, the virulence of the bacteria and the state of health of the patient. The risk of the disease is higher in groups of population at particular risk, including pregnant women, newborns and people with weakened immunity. Listeriosis, however, occasionally occurs in people who do not have any predisposition (Painter and Slutsker 2007). The mortality in cases of listeriosis is generally considerably high, amounting to 20 – 30%.

The occurrence of listeriosis in the Czech Republic is relatively low with around 30 cases annually. Years 2006 and 2007 were exceptions to this, with an epidemic of listeriosis caused by consumption of contaminated ripening cheeses and resulting in a larger number of reported cases (78 cases in 2006 and 51 cases in 2007). In 2012, 198 fatal cases of listeriosis were reported in the EU which was the largest number of fatal cases since 2006. The serotypes occurring most commonly in the EU in 2012 were serotype 1/2 (47%) and 4b (42%), followed by serotypes 1/2b, 1/2c and 3a. The types of foodstuffs failing to meet the limits stipulated by EU Regulation No. 2073/2005 were most frequently fish products (8% of all examined samples) and certain types of cheese. Listeria was found in raw cow's milk only in isolated cases. Similarly low findings were also published for fermented meat products. In the Czech Republic, the number of findings of Listeria in foodstuffs is extremely low. The greatest percentage of Listeria findings was in smoked fish products and certain types of cheese, similarly to the other EU states.

Verocytotoxigenic *Escherichia coli*

Verocytotoxigenic *E. coli* (VTEC) comprises one group of *E. coli* that is characterised by the ability to produce toxins known as Shiga-like toxins. Only certain strains of VTEC are capable of causing illness in man. The most commonly isolated serotypes include O157:H7 for which cattle and other ruminants are considered one of the principal reservoirs. More than 120 O serotypes of VTEC *E. coli* have been isolated from clinically healthy cattle, and of these more than 100 O serotypes have been isolated in man. Strains of *E. coli* may be present in varying quantities in the digestive tract of cattle. Cattle are, however, only a host to *E. coli* O157:H7 in the vast majority of cases without any clinical symptoms of disease (Kaspar et al. 2010). Clinical symptoms in the form of haemorrhagic diarrhoea are generally described only in calves; such calves may then become reservoirs of the pathogen (Duffy et al. 2006). The occurrence of VTEC in the digestive tract of cattle varies in individual countries and may amount to as much as 30%. In contrast, beef meat is not often contaminated with VTEC; confirmed findings do not exceed 5%. The occurrence of VTEC was monitored in the Czech Republic on the surface of 622 beef quarters, with only eight positive findings being proven (1.3%) (EFSA 2014). Vegetables and drinking water are other important sources of VTEC.

The majority of human cases of VTEC are isolated cases. Typical clinical symptoms of illness in man induced by VTEC include diarrhoea, haemorrhagic colitis, dehydration, abdominal pain and occasional vomiting. Fever occurs only in severe cases, though patients may have an elevated temperature. Haemolytic-uraemic syndrome (HUS), which causes acute kidney failure, is an extremely dangerous complication affecting around 10% of patients.

The number of reported cases of illness caused by VTEC is relatively low, with between 3 000 and 5 000 cases per year in the EU as a whole. Year 2011 was an exception, with 9 500 cases reported in connection with an epidemic in Germany and subsequently in other 15 countries caused by consumption of contaminated bean sprouts, in which as many as 3 800 people became ill. Cases of human VTEC illness have been reported in the Czech Republic since 2010, with the number of cases not exceeding 10 in a year. The serotypes occurring most frequently in the Czech Republic are, as in other EU countries, O157 and O26.

Trichinellosis

Trichinellosis is a zoonosis caused by parasitic roundworms of the genus *Trichinella*. This parasite has a wide range of hosts, including man, and is peculiar for the fact that its entire development cycle takes place in the host organism without the need for an intermediate host.

There are nine species and three genotypes in the genus *Trichinella*, though the majority of cases of illness in humans are caused by *T. spiralis* and *T. britovi*. A number of cases have also been caused by *T. pseudospiralis* and *T. nativa*. Man is most often infected by the consumption of raw or inadequately heat treated meat containing infectious *Trichinella larvae* (Plate II, Fig. 3). The commonest source of Trichinella for man is pork meat, though the meat of wild boar and other game species may also be a source. Horsemeat may also be a vehicle for Trichinella.

The clinical symptoms of trichinellosis occur in two phases. Nausea, diarrhoea, vomiting, fatigue and abdominal pain are typical symptoms in the first phase, though this phase is generally mild or may even be asymptomatic. Symptoms such as muscle pain, headache, fever, skin rash, swelling and joint pain appear in the second phase. Death may even occur in man depending on the number of larvae ingested and the site of encapsulation.

The number of cases of trichinellosis in man has been on the decline in the EU in recent years. While 670 cases of human trichinellosis were reported in 2008, there were 268 cases in 2011 and 378 cases in 2012. Cases of trichinellosis in man are extremely rare in the Czech Republic, and a long time has passed since the last positive finding was made. Serological proof of trichinellosis was found in one person in 2012. In contrast, dozens of cases of human trichinellosis are reported every year in countries such as Romania, Lithuania, Latvia and Italy. Trichinella is most often found in free-range or farmed wild pigs, domestic pigs and other species of game. Half of the positive findings of Trichinella in pigs in the whole of the EU in 2011 and 2012 were found in Romania. Findings in pigs in other EU countries usually come from farms with uncontrolled zoohygienic conditions and are extremely low in number. Findings of Trichinella in wild pigs are not usually frequent, as is the case for other species of game. In the Czech Republic, all pigs slaughtered at slaughterhouses are subject to compulsory examination for the presence of larvae of the genus *Trichinella* and no positive finding has been made in domestic pigs for a number of decades. Trichinella findings in wild pigs and other animals have also been isolated cases.

Conclusions

The basic priority laid down in both the legislation of the Czech Republic and European food law is the production of healthy foodstuffs of a high quality designed for human consumption. To ensure the food safety, however, we need not merely a good legal framework based on up-to-date scientific knowledge, but first and foremost a functioning system of detection and inspection. There has been compulsory monitoring and reporting of the occurrence of selected diseases in the individual EU member states since 2005. When judging and comparing the results of these national monitoring systems, it is essential to bear in mind that the systems in place for detecting, investigating, identifying and reporting these results have not as yet been entirely harmonised, and they copy the differing national systems to a large extent. For these reasons, even large differences in the number of confirmed cases of individual diseases or in the occurrence of agents of these diseases in examined samples need not always reflect the level of food safety in the individual member states, but rather reflect the differences in the sensitivity of national systems.

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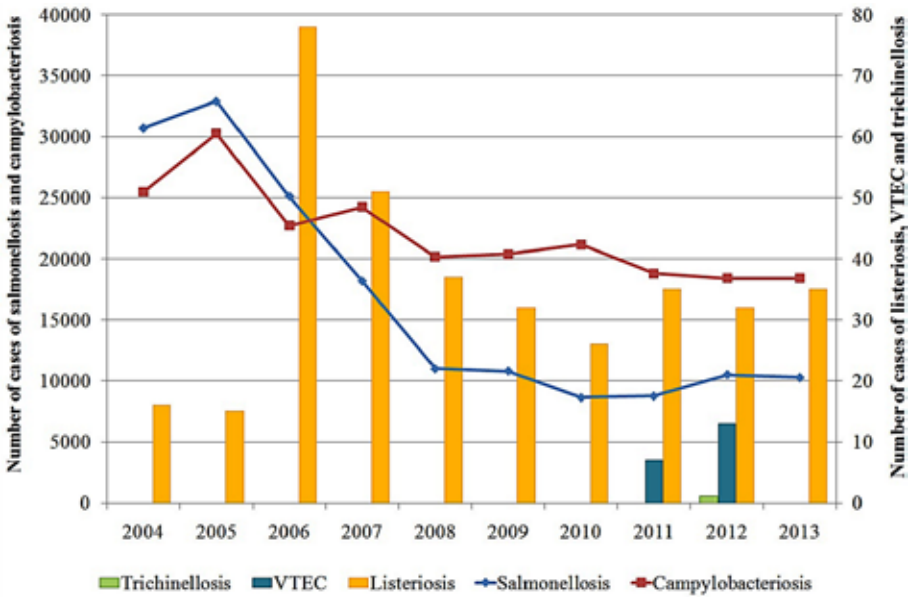

Fig. 1. Number of confirmed cases of selected alimentary diseases in the Czech Republic (NIPH 2014)

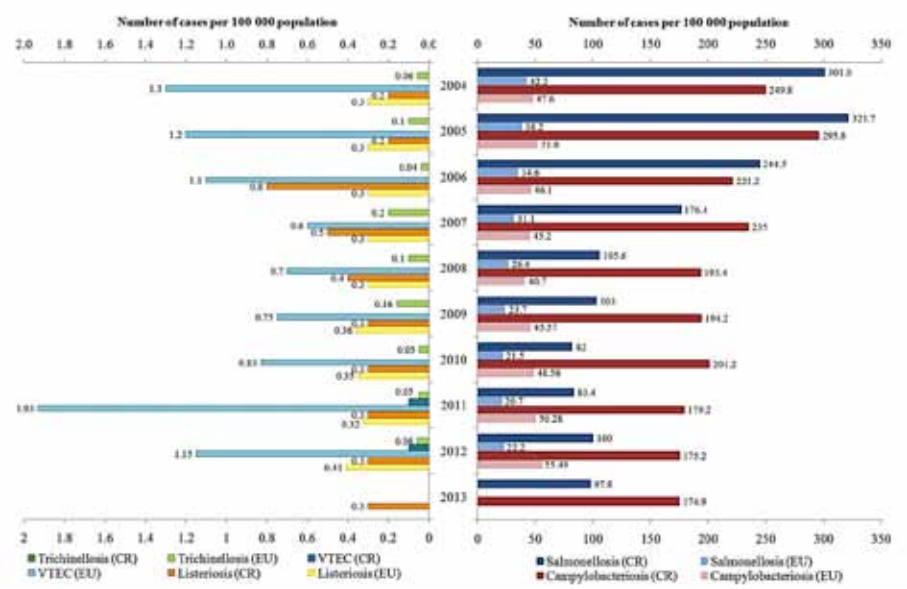

Fig. 2. Relative numbers of confirmed cases (per 100 000 population) of selected alimentary diseases (EFSA 2014)

Plate II

Fig. 3. *Trichinella spiralis* - the larvae (Svobodová I)