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PORK AS A SOURCE OF HUMAN INFECTION WITH *TOXOPLASMA GONDII**

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SUMMARY: Toxoplasma gondii is parasitic coccidia capable of invading almost every species, including humans. In Serbia, undercooked pork is considered to be the main source of human infection. Serological screening of pigs for T. gondii antibodies is one of the measures, proposed by European Food Safety Authority, that should contribute to better controle of the parasite. Currently, most promising tests in this field are enzyme-linked immunosorbent assay and indirect immunofluorescence antibody test.

Key words: *Toxoplasma gondii, pork, enzyme-linked immunosorbent assay, indirect immunofluorescence antibody test.*

INTRODUCTION

Toxoplasma gondii is intracellular, parasitic coccidia from the family *Sarcocystidae*, phylum *Apicomplexa.* Despite its worldwide distribution and wide range of hosts, it represents the only species within the genus *Toxoplasma.* The discovery of the parasite occured at the first decade of the 20-th century, as a result of independent work of two groups of scientists: Charles Jules Henry Nicolle (1866-1936) and Louis Herbert Manceaux (1865-1934), from the Pasteur Institute in Tunis and Alfonso Splendore (1871-1953), from Brasil (Morissette i Ajioka, 2009). Initially, both groups identified the protozoan as *Leishmania.* However, Nicolle soon realised they discovered a new organism and named it *T. gondii*, based on its morphological characteristics (mod. L. *toxo* = arc or bow, *plasma* = life) and the host from which it was isolated (African rodent *Ctenodactylus gundi*). Soon, other scientists started to report *Toxoplasma*-like parasites in various species, including humans (Ferguson, 2009), but it was Abner Wolf and his colleagues which, in 1939., first isolated *T. gondii* from an infant girl deceased from encephalomyelitis and

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conclusively identified it as a human pathogen (Weis and Dubey, 2009). Further research confirmed their findings and pointed many scientists' interests towards better understanding of morphology, biology, genetics, virulence and pathogenicity of *T. gondii*, as well as development of new diagnostic techniques, therapeutics and preventive measures (Morissette i Ajioka, 2009).

TOXOPLASMA GONDII LIFE CYCLE AND HOSTS

To date, infection with *T. gondii* has been confirmed worldwide, in numerous mammalian and avian species, including arctic foxes from Svalbard (Prestrud et al., 2008). Most of these species, together with man and meat producing animals, act as intermediate hosts, in which parasite forms tissue cysts that contain slowly dividing forms – bradyzoites. Sexual phase of the life cycle may only be completed in the small intestine of *T. gondii*'s definite host, felids. Sexual phase results in the formation of oocysts, environmentaly resistant and orally infective forms. After ingestion of oocysts, gastric juices release sporozoites, which transform into tachyzoites (quickly dividing form, present in the acute phase of the disease and its remission) and disseminate through organism infecting all tissues, including placenta of the gravid uterus and, consequently, the foetus. In tissues, tachyzoites transform into bradyzoites and form tissue cysts which are capable of surviving in the affected host for years or even through its lifetime (Dubey and Hill, 2002). Although cysts can develop in virtually all tissues of the infected host, there seems to be greater predilection for muscle and neural tissue.

There are three main routes of transmission of *T. gondii* between the hosts: feco-oral (ingestion of food and water contaminated with oocysts from cat feces), congenital (transplacental passage of tachyzoites, from mother to foetus) and carnivorism (ingestion of meat which contains viable tissue cysts). In humans, congenital infection was first described, followed by foodborne cases, while the existence and role of oocysts were last elucidated (Ferguson, 2009). Cases of acquiring infection through blood transfusion, transplanted organs and consumption of unpasterized goat milk have also been described in the literature, while other routes, such as sexual transmission, are considered unlikely (Hill and Dubey, 2002).

IMPACT OF *TOXOPLASMA GONDII* ON HUMANS AND ANIMALS

Clinical toxoplasmosis may be considered relatively rare in humans, bearing in mind that almost one third of the overall human population is estimated to be infected. When it does occure, disease usually affects immunocompromised or congenitally infected individuals. Infected imunocompetent humans express clinical signs in only 10% to 20% of cases (Weiss et al., 2009) and in most of them infection is mild and self-limiting (Đurković-Đaković et al., 2013). However, despite the low incidence, toxoplasmosis is currenly considered to be the most significant parasitic foodborne pathogen and its consequences, estimated on approximately 620 DALY-s (disability-adjusted life-years per year) parallel those of salmonelosis (approximately 670 DALY-s) (Đurković-Đaković et al., 2013). Reasons for such high DALY scoring of toxoplasmosis lie in the severe consequences of congenital infection, such as neonatal death, hydrocephalus, psyhomotor or mental retardation, chorioretinitis, myocarditis, hepatitis, polymyozitis and possibility of the ocurrence of disseminated, potentially lethal, form of disease in imunocompromised

individuals (Weiss et al., 2009). According to recent studies, simillar simptoms are increasingly becoming evident in immunocompetent humans as well, while many authors find *Toxoplasma* to be responsible for various psychiatric and neurological disorders, especially schizophrenia (Mc Allister, 2005; Flegr, 2013).

Majority of animals infected with *T. gondii* also remain asymptomatic. Still, some individual animals and species (Australian marsupials, arctic foxes, european brown hare etc.) may develop acute, potentially lethal form of the disease or suffer from reproductive disorders, such as abortions or congenital malformation, as is frequently case with sheeps and goats. From the aspect of human health, however, more important is the risk of acquiring *Toxoplasma* infection after consumption of raw or undercooked meat of afected animals, which, according to the results of some European studies, occurs in 30% to 63% of the cases (OIE, 2008).

PIGS AS A SOURCE OF HUMAN INFECTION WITH *TOXOPLSMA GONDII*

Like the majority of other hosts, infected pigs usually act as asymptomatic carriers of *T. gondii*. If clinical signs do appear, they tend to be mild, with increased body temperature, loss of appetite and tachypnea. Other signs, like reproductive disorders, pneumonia, myocarditis, encephalitis, weakness of the hind limbs and mortalities are rare (EFSA 2007, Basso et al., 2013).

Together with sheeps and goats, pigs are considered to be the meat-producing animals that most frequently harbour infective cysts of *T. gondii* in their tissues. This is one of the reasons why consumption of raw or undercooked pork is recognised as an important risk factor for human infection (Tenter et al., 2000; Bobić et al., 2003; Klun et al., 2011).

In Serbia, pork is most frequently consumed meat (50% of the total meat consumption) and therefore believed to impose a greater risk to human health than mutton and beef, although *T. gondii*-specific atibodies were more frequently found in sheep (84.5%) and cattle (76.3%), than in pigs (28.9%) (Klun et al., 2006). Lack of programs for the slaughter inspection of pigs for *T. gondii* (routine methods of meat inspection are unable to detect microscopic cysts of the parasite) and Serbian tradition of home-curing of the meat, where no standardised concentrations of salt and spices are used and people often tend to check the taste of the raw product during its preparation, additionally increases the exising risk of acquiring *Toxoplasma* infection through pork meal.

Studies conducted in Europe during the last 30 years estimate that prevalence of *T. gondi* specific antibodies in pigs varies between the countries, from 0% to 64% (EFSA, 2007). The same studies report that, during the last ten years, some countries, such as Netherlands, Austria and Germany, significantly decreased the number of seropositive pigs or even completely eliminated infection from their farms, by implementing following biosicurity measures: (a) keeping producing animals indoors through their life-time, (b) keeping the sheds free of rodents, birds and insects, (c) feeding meat-producing animals on sterilised food, and (d) no pet animals in sheds and feed stores (Tenter et al., 2000). On the other hand, consumers' demands for organicaly produced meat are constantly increasing. Consequently, it is to be expected that the number of pigs reared in extensive systems will also increase in the future, exposing more pigs to the environment and *T. gondii* oocysts (EFSA, 2007). Reports from Switzerland support these expectations, since they experienced

an increase of 13% in prevalence of infected pigs after introduction of animal welfare regulations (Berger-Schoch et al., 2011).

CONTROL OF *TOXOPLASMA GONDII*

Currently, there is no commercialy available vaccine against *T. gondii* (except the one registered for sheep) and the efficiacy of traditional therapy is limmited to treating the acute form of the disease i.e. proliferative form of the parasite, without possibility of eliminating tissue cysts (Đurković-Đaković et al., 2013). Therefore, preventive measures, which hinder intake of the infective forms of *T. gondii*, still remain the best protection from toxoplasmosis. In the case of meatborne infections, European Food Safety Authority (EFSA 2007, EFSA 2011) suggested the following: improvement of biosecurity measures at the farm level, monitoring of human infections, monitoring of meat producing animals at the farm and abattoir level, implementation of prescribed hygienic practices and following recommended temperature regimes during cooking (67°C) and freezing (-12 °C) of meat destined for human consuption. In their recommendations for animal health monitoring techniques, EFSA (2007) gives priority to indirect (serological) techniques, over the methods intended for direct detection of the parasite in tissues (bioassay, PCR, histology and immunohistochemical staining). Although direct methodes proved to be highly specific, small size of the sample significantly reduces their sensitivity due to random distribution and often low density of parasite in tissues. Moreover, direct methods are generally time-consuming and require speciall equipment for their performance, which, together with earlier mentioned reduction of sensitivity, makes them less suitable for screening a large number of samples in contrast to indirect methods. On the other hand, serological methods (indirect immunofluorescence antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), modified agglutination test (MAT), Western blott (WB), latex agglutination (LA), indirect haemagglutination (IHA)) which are based on the detection of specific antibodies, are mainly highly sensitive, easy to perform, cheap, and therefore more suitable for the monitoring purposes. However, it should be emphasized that, currently, none of this serological technuiques is sufficiently sensitive or speciffic to confirm or exclude infection on the individual level with 100% security.

SEROLOGICAL TESTS

Serological methods are the key in the routine diagnosis of toxoplasmosis in pigs (Pardini et al., 2012). They have been traditionally used to screen pig sera, however, meat juices are slowly emerging as an equally interesting alternative matrix, from numerous reasons: (a) procedure of meat juice extraction from muscle is more simple than that of blood sampling and sera preparation; at the same time it is posible to automate the procedure and meet the requirements of large-scale analyses; (b) there will be situations when meat will be the only available sample; (c) after extraction of juices, meat could be reused for parasitological examinations. Among all available sampling sites, diafragm stands out as well vascularized muscle, easy to collect, with no exchange value, available from all fresh carcases, including the ones from abroad (Villena et al., 2012).

IFAT, MAT and ELISA are the main tests used for anti-*T. gondii* antibody detection in pig sera and meat juices. However, when compared with each other,

IFAT and ELISA stand out from this trio due to their superior specificity and sensitivity (Garcia et al., 2008). While some authors (Basso et al., 2013) found ELISAs to be more specific and sensitive than IFAT, others (Veronesi et al., 2012) report almost perfect agreement between these two tests.

Many authors (Garcia et al., 2006; Veronesi et al., 2012) use IFAT as a reference test i.e. a „gold standard" for detection of anti-*T. gondii* antibodies in pigs. IFAT produces titers comparable with the Sabin–Feldman dye test which is the longest established serological method, considered as a „gold standard" in humans, but is, at the same time, much safer than the dye test as it uses formalin-fixed (killed) tachyzoites (Hamzavi et al., 2007). Utillization of the whole tachyzoites also increases the specificity of IFAT, since the surface antigenes of the apicomplexan species are considered more specific than the intracellular ones (Björkman i Uggla, 1999). For this very reason IFAT has no affinity towards cross-reactions with other coccidia as it is the case with indirect ELISA tests which use solubile antigenes of *T. gondii.* Finally, IFAT has the ability of early detection of antibodies. In studies conducted on experimentally infected pigs IFAT detected specific antibodies in all examined animals as soon as 7^{th} day after experimental infection, while ELISA and MAT managed to do so at 14^{th} and 21^{st} day, respectively (Garcia et al., 2006). Attempts have been made (Pardini et al., 2012) to achieve earlier detection of antibodies with ELISA by using of native affinity purified TgSAG1 surface antigen, that also has potential to reduce number of cross-reactions; however, once more IFAT performed better on this field.

On the other hand, IFAT is considered subjective since the interpretation of results directly depends on the amount of experience and personal judgement of the researcher due to the visual reading of the results (EFSA, 2007). Furthermore, lack of the standardised cut-off values (values above which test is considered positive) which range from 1:16 to 1:200 (Dubey, 2008) additionaly complicates interpretation and comparison of results between different studies.

By contrast, reading of the results of ELISA is objective and does not require skilled personel. Unlike IFAT, it can be semi automated and therefore more suitable for examination of a large number of sera.

According to Italian authors (Ranucci et al., 2012; Veronesi et al., 2012) both IFAT and ELISA showed good performance in detecting *Toxoplasma*-specific antibodies in meat juices of naturally infected pigs. However, they emphasize that results obtained on meat juices could not be used as an alternative method to serum to guarantee the absence of the parasite from the pork, but could be applied for monitoring purposes (Ranucci et al., 2012). Finally, monitoring programs based on serological investigation of muscle fluids have already been successfully implemented for other foodborne pathogens, such as *Salmonella*, *Trichinella*, Aujeszky virus etc. (Villena i sar., 2012).

CONCLUSION

Undercooked pork meat represents significant risk for acquiring human infection with *T. gondi.* However, this risk could be significantly reduced by applying adequate controle measures. One of these measures is monitoring of the health status of herds and using obtained results for advising farmers with regard to improve the existing biosecurity measures on their farms and/or expose the meat from seropositive farms to the stricter processing conditions. Both IFAT and ELISA

have good potential as serological tests that could be used for monitoring the presence of anti-*T.gondii* antibodies in pigs, however, in order to implement any of them in the monitoring program, they first must be subjected to adequate standardization an harmonization.

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SVINJSKO MESO KAO IZVOR INFEKCIJE LJUDI SA *TOXOPLASMA GONDII*

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Izvod

Toxoplasma gondii je parazitska kokcidija koja je u stanju da inficira gotovo svaku vrstu, uključujući i čoveka. U Srbiji, termički nedovoljno obrađeno svinjsko meso smatra se glavnim izvorom infekcije za ljude. Serološko testiranje svinja na prisustvo antitela protiv *T. gondii* je jedna od mera, predloženih od strane Evropske Agencije za Bezbednost Hrane (EFSA), koja treba da doprinese poboljšanju kontrole ovog parazita. Trenutno, testovi koji najviše obećavaju na tom polju jesu imunoenzimski test i test indirektne imunofluorescencije.

Ključne reči: *Toxoplasma gondii, svinjsko meso, imunoenzimski test, test indirektne imunofluorescencije.*

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