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YERSINIA ENTEROCOLITICA- SOURCES AND PATHWAY OF CONTAMINATION PIG CARCASS

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SUMMARY: Y. enterocolitica is a pathogenic microorganism with the possibility of meat and carcasses contamination of pigs which affects human health. This microorganism is at the highest percentage found in the tonsils of pigs and due to poor hygiene practice and inadequate manipulation with knives, can penetrate the carcass and become a source of infection for humans. In EFSA opinion on pork hazards is evaluated comprehensive system of maintaining food safety and quality, and rating hazards relevant to pig carcasses, particular during cooling, including Y. enterocolitica.

Key words: *Yersinia enterocolitica, pigs, meat, sources, tonsils.*

INTRODUCTION

Yersinia enterocolitica is in the group of foodborne pathogens that causes severe infections in humans. The infection is most frequently associated with pork but studies show that other types of meat can be sources of infection. Pigs are potential reservoirs of *Y. enterocolicica* which is pathogenic for humans. *Y. enterocolitica* is found in tonsils and lymph nodes of pigs but can be also isolated from feces (Thibodeau et al., 1999; Nesbakken et al., 2003). However, the presence of *Y. enterocoticica* in tonsils is six times higher than in feces (Nesbakken et al., 2003). According to data from EFSA (2006), yersiniosis in people, caused by foodborne infections, is at third place in Europe, behind salmonellosis and campylobacteriosis. The disease in humans is characterized by diarrhea, ileitis, arthritis and septicemia. According to the EFSA, among *Yersinia spp.* a special significance in foodborne infections due to consumption of animal products will be related to *Salmonella spp., Toxoplasma spp. and Trichinella spp.*

Review paper / *Pregledni rad*

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Taxonomy and phenotypic characteristics

Genus Yersinia belongs to the family of Enterobacteraceae and has 12 known species of this genus. From these 12 species only three are considered as pathogens: *Yersinia pestis*, especially important as the cause of the plague in people, *Yersinia pseudotuberculosis,* associated with zoonotic infection and *Yersinia enterololitica* (Mills, 2004). In addition to the three pathogenic species there are nine non-pathogenic. In the species there are a wide variety in biochemical features. Such variations inluenced distribution of *Y. enterocolitica* in six biotypes. Wautters (1991) has shown the difference between pathogenic biotypes (1B, 2, 3.4, 5) and non-pathogenic biotypes (biotype 1A) (Table 1.). Biotyping of strains is the most important step of testing *Y. enterocolitica. Y. enterocolitica* can be separated into serotypes by using the O-antigen. Over 76 different serotypes are known up to date. In Europe, the most common is biotype 4.

Table 1. Biotype of Y. enterocolitica

Biotype	Serotype (s)	Human virulence	Frequency in Europe
4	O:3	Pathogenic	++++
2	O:9,O:5,27	Pathogenic	++
3	O:3, O:5,27	Pathogenic	+
1B	O:8, O:21, O:13	Highly Pathogenic	0
5	O:3, O:2,3 O:1,2, 3	Pathogenic	0
1A	Numerous	Non-pathogenic	++++

Biotypes 1B, 2, 3, 4 and 5 are pathogenic for humans and animals. Biotype 1A includes a large number of serotypes found in food, environment and can be also found as supporting flora in the digestive tract of animals and humans. These serotypes don't have virulence characteristics. Biotype 1B involves highly pathogenic serotypes O: 8, O: 21 IO: 136. Serotypes O: 4, O: 18 and O: 20 were significantly rare in Europe. Biotype 2 includes two serotype O: 9 and 0:5,27 that are pathogenic for humans. Biotype 3 includes serotypes O: 1, 2, and 3, which were isolated from rodents. Biotype 4 contains only one serotype, O: 3, which is the most prevalent in the world and isolated from pigs.

Characteristics of Y. enterocolitica

Yersinia enterocolitica is the Gram-negative microorganism, rod-forms with a diameter 0.5 to 0.8 μm and length of 1 to 3.5 μm. *Y. enterocolitica* belongs to sporogenic and non-capsular microorganisms. At a temperature of 35-37 °C is immobile, but at a temperature from 22 to 25 °C possesses flagellas and is mobile. *Y. enterocolitica* is psychrotrophic microorganism. It has possibility to grow from -2 to 42 °C (Bercovier and Mollaret, 1984). The optimum air temperature rise of 28 to 29 °C (Bercovier et Mollaret, 1984). *Y. enterocolitica* has the ability to replicate in food, especially meat, at a temperature below 0 °C (Lee et al., 1981; Sterrn et al., 1980). In food with neutral pH kept at a temperature of 5 °C, there is a possibility that the number of *Y. enterocolitica* with 10 cfu/ml increased to 107 cfu/ml for a period of five days (Bhaduri et al., 1997). The minimum pH for growth of *Y. enterocolitica* is between 4.2 and 4.4. *Y. enterocolitica* has the ability to grow at pH values below 4.2 or above 9. The presence of organic acids reduces the growth ability of *Y.enterocolitica. Yersinia enterocolitica* is particularly significant because of their ability to replicate at temperatures of refrigerators, vacuum packaging or in packaging with modified atmosphere (Bercovier and Mollaret,

1984). *Y.enterocolitica* can survive in frozen foods at longer periods. In pork in vacuum packaging, stored between 2 and 7 °C, after five weeks of storage, *Y.enterocolitica* has the ability to grow (Hayashidani et al., 2008). In pork meat during storage at 4 °C in modified atmosphere with a 100% CO_2 *Y. enterocolitica* is suppressed (Bodnaruk et Draughon, 1998). However, Strotmann et al. suggest that different concentrations of CO_2 have no effect on growth of *Y. enterocolitica*. *Y. enterocolitica* grows in the packaging with modified atmosphere with the different concentrations of O_2 and CO_2. Also, in a modified atmosphere, where the ratio of O_2 and CO_2 is 50:50%, it is in pork meat noticed the growth of this microorganism (Strotmann et al., 2007). Its replication is not affect by associated microflora of meat, stored at 10 °C (Nissen et al., 2001). The high concentrations of O_2 in modified atmosphere of minced pork, shows an inhibitory effect at 4 ° C on the growth of *Y. enterocolitica* (Pin et al., 2000).

Today for isolation of *Y. enterocolitica* is used the standard procedure described in ISO standard (10273).

Incedinca disease in humans

According to data from WHO, the incidence of infections with *Y. enterocolitica* increases. The incidence of yersiniosis in European countries is on the decline, with 9533 cases confirmed in 2005 year and 6776 cases in 2010 year, so it is a significant cause of foodborne diseases. Situation in Serbia is unknown but according to unofficial data, in 2010 was recorded 108 cases (Malašević, 2012). The incidence of occurrence is shown in Table 2. The largest number of cases of yersiniosis was recorded in the United States where the number of patients in 1997 year was 87,000 and the incidence of disease 33.4. In Japan, the number of cases of yersiniosis, according to data from 2001 was 4, which is the smallest incedence of yersiniosis (less than 0.01).

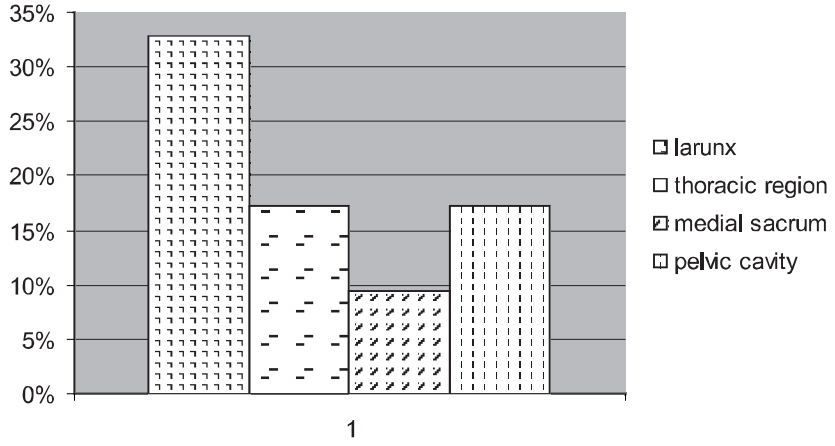
Table 2. Number of registered cases of humans yersiniosis (Nesbakken, 2005)

Country	Cases	Incidence (per 100.000 population)
Australia	73 (2000)	0,6
Austria	94 (1998)	1,2
Belgium	8291 (1994)	8,5
Denmark	7113 (2001)	8,7
Finland	647 (2003)	12,4
Greece	10 (1998)	0,1
Japan	4 (2001)	< 0,01
Norway	862 (2003)	1,9
Špain	425 (1998)	1,1
Šweden	7142 (2003)	8,0
Šwitzerland	51 (1998)	0,7
United Kingdom	27 (2000)	0,05
SAD	87,000 (1997)	33,4
Serbia	108 (2010)	0,9

Sources and pathways of infection

Yersinia enterocolitica is widespread in the environment. It is isolated from the

water (wells, springs), pigs, dogs, cats, ruminants, rodents, molluscs and other invertebrates. However, primarily as a source of pathogenic strains are pigs. There is a connection between the tonsils and the contamination of pig carcasses. Among the tonsils as the most common source of these pathogens (Fondrevez et al., 2010), also other sources were observed (lymph nodes, intestines, liver and heart). As a psychrotrophic microorganism, *Y. enterocolitica* can be replicated in the cold chain of the meat production process as well as in refrigerators at home. Pets, especially dogs, can be either a host or transmission source of infection for children. There is a direct transmission from human to human, which is possible with fecal-oral route and over germ-carriers. However, the first source of infection for humans is raw pork and pork products. An important source of cross-contamination is the content of intestine and tonsils. Observing slaughter line, Van Damme De Zutter (2011) suggest that the most contaminated are medial part of the larunx with this pathogen (32.8%), thoracic region (17.2%), medial sacrum (9.4%) and pelvic cavity (17.2%). These results are shown in graph 1.



Graph. 1. Participation *Y.enterocolitica* in some regions

Participation of positive samples in examined pigs was 57.2% in tonsils and 20% in rectum. Prevalence of *Y.enterocolitica* in sows on the farma is often very low (Gurtler et al., 2005). During breeding, pigs are colonized after the first two months of life (Nesbakken et al., 2005). Initially, this pathogen is present in faeces and tonsils, and later its number significantly increases in tonsils and does not change until slaughter (Nesbakken et al., 2006). Among the tonsils and feces, *Y. enterocolitica* can be isolated from mandibular and mesenteric lymph nodes (Nesbakken et al., 2003). A study in France showed that in slaughterhouse was found 19.8% positive pigs originated from 80% of positive herds (Fondrevez et al., 2010). Risk to the pork safety in terms of this pathogen in slaughterhouses primarily depends on the process hygiene in slaughterhouses, staff practice and their hygiene. In terms of process steps in slaughterhouses, technical aspects of the individual steps may be different between slaughterhouses, but their order is usually similar. It starts with lairage, stunning, slaughter, bleeding, scalding, singeing, washing, evisceration, carcass cutting and cooling. In Europe,the pigs are carriers of

pathogenic strains of *Y. enterocolitica* for people, especially strain biotype 4 (serotype O: 3) and less biotype 2. There are reports that even more than 80% of swine herds can be positive, with a high percentage of positive pigs at slaughter. During the process of slaughter, pig carcasses can be easily contaminated with this pathogen through fecal contamination and from the oral cavity. Particularly strains of biotype 4 (serotype O: 3) are often found on pig carcasses surfaces after slaughter and processing, but before chilling. Slaughter technique and hygiene of premises, equipment and staff who are responsible for this work have a great impact on the incidence of contamination. Fecal contamination can be significantly reduced by technique of rectum binding immediately after evisceration (Andersen, 1988). Oral cavity and tonsils are at high percentage contaminated with *Y. enterocolitica*, so removing the tonsils or cutting carcasses may cause spread of the pathogen that is in this part of carcass. Very often is the muscle M. *digastric* contaminated with *Y. enterocolitica*.

During cutting, further process and distribution of fresh pork and carcass waste is also possible further contamination with *Y. enterocolitica*. However, according to literature data, *Y. enterocolitica* is rarely isolated from chilled pork at retail stores, unless they are cooled tongues as products. *Yersinia enterololitica* has the potential to replicate during storage of meat and meat products. However, the ability to survive, especially at low temperatures and at normal pH is low.

Risk factors

Pigs and pork products are a potential source of *Y. enterocolitica* for humans (Norrung et al., 2009). Genetic characterization showed that the strains of *Y. enterocolitica* that caused the disease of people were identical to the strains that were isolated in the tonsils of healthy pigs (Fredriksson-Ahomaa et al., 2001, 2006). The most common *Y. enterocolitica* is isolated on carcasses and chilled pork and pork products. According to EFSA (2012), a total of 4.1% of pork samples were positive in the European Union in 2010. The main risk factor for the presence of *Y. enterocolitica* in pork is the slaughter of pigs that are carriers of the pathogen. Cross-contamination of carcasses and entrails is possible during slaughter and processing (Fredriksson-Ahomaa et al., 2001). Proper techological operation and hygiene during slaughter have a significant impact on the microbial contamination of pig carcasses and entrails. The major measures affecting the reduction of contamination are: compling with the principles of good manufacturing practices and standard operating procedures (ligation of rectum, handling head and tongue).

Prevention and control in various stages pork meat production

Yersinia enterocolitica is in gastro-intestines, tonsils and skin of pigs that are source of contamination of pig carcasses at slaughter (Nesbakken et al., 2003). The pork is primary source of infection for people. Pigs are asymptomatic carriers of the pathogen. *Yersinia enterocolitica* spreads by objects within farm and along the entire production chain of pork through cross-contamination. This results in the contamination of pig carcasses and human exposure to this pathogen. Control of *Y. enterocolitica* on farms and at slaughterhouses is very important because it is considered that pathogen carrier pigs are the main risk of the presence of *Y. enterocolitica* in pork (Fredriksson-Ahomaa et al., 2001). This pathogen is detected by laboratory testing of pig carcasses. Growing pigs without mixing with other animals from different farms or with different

age groups reduce the occurrence of this pathogen. Also, cleaning and desinfection of objects before inserting the pigs is of particular importance. In order to reduce the spread of infection is necessary to provide a special ventilation system in buildings where pigs are kept, use of hygiene barriers when entering the farm, the use of clean straw as bedding, identification and removal of seropositive animals from the herd, maintaining good hygiene and housing conditions of pigs, protection of other diseases, the use of clean drinking water free from pathogens, prevention of faecal contamination of water and food.

After slaughter, carcass inspection is necessary, which aims to protect human health. However, foodborne pathogens can not visually be detected, including *Y. enterocolitica*. Major foodborne pathogens, including *Y. enterocolitica* can be controlled, in order to reduce the possibility of cross-contamination of carcasses. Cross-contamination is common in the process of palpation and cutting the carcass. That is why process hygiene, sterilization of knives and staff training are very important. Efficient refrigeration and maintenance of the cold chain are primarily the most important measures to reduce the replication of this pathogen. Control of *Y. enterocolitica* in the processing, distribution, retail and consumers is based on similar measures applied in most other foodborne pathogens. Cooling of meat has limited efficiency in control of *Y. enterocolitica* and therefore previous phases in meat production are very important in providing the better microbiological status. Raw meat should be stored separately from other foods. Cross-contamination from raw meat to meat that has been treated with heat regime should be prevented in institutions where it is processed, butcher shops and retail outlets. Knives, equipment and machines used for cutting and processing of raw meat and meat products must be regularly washed and disinfected before they are used for other foods. Also all surfaces that have been in contact with raw meat should be washed and disinfected before next use. Consumption and under-consumption of thermally-treated meat should be kept to a minimum because in these situations the prevalence of foodborne diseases including yersiniosis is much higher.

Examination and monitoring of Y. enterocolitica in slaughter pigs

Distribution and prevalence of *Y. enterocolitica* vary geographically. Pigs are the main carriers of human pathogens *Y. enterocolitica*, especially biotype 4 (serotype 3). Among pigs and other reservoirs have a significant role in the epidemiology of yersiniosis in people. Data suggests that ruminants, particularly cattle may be reservoirs of biotype 2 (serotype ¬ O: 9 and O: 5,27). The presence of *Y. enterocolitica* can be detected in several ways. Cultural methods includes sampling of tonsils and faeces and carcass swabs. When testing large numbers of animals can be used ELISA for screening identification of infected herds and then *Y. enterocolitica* may be confirmed by the cultural method. Serological tests are possible from serum or meat juice during slaughter. Bacteriological examination of feces and tonsils are the long-term, economically non profitable compared to serological testing. On the other hand, serology relies on delayed reaction and positive response does not mean that animal excretes the pathogen (Nesbakken et al., 2006). In pigs for slaughter, prevalence of *Y. enterocolitica* is higher in tonsils than in feces. It is considered that the highest level of pig carcass contamination with this pathogen is at a slaughterhouse. It is also expected high contamination of internal organs, tongue, liver and heart. However, these organs are thermic processed before consuming and *Y. enterocolitica* will be eliminated by this way. In the chain of

production, the number of positive samples with *Y. enterocolitica* decreases. Fresh meat enables replication and survival of pathogenic *Y. enterocolitica*, especially in the case of cross-contamination. For meat products cross-contamination is possible, only in case of contamination in the production or distribution of these products.

REFERENCES:

ANDERSEN, K.: Contamination of freshly slaughtered pig carcasses with human pathogenic Yersinia enterocolitica. International Journal of Food Microbiology, VII:192-202, 1988.

ANONYMOUS.: Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica. ISO 10273. Geneva, Switzerland.2003. International Organization for Standardization.

BERCOVIER, H. and MOLLARET H.: Genus XIV. Yersinia, Krieg N.R., Bergey manual of systematic bacteriology, Baltimore, 1:498-506, 1984.

BHADURI, S., COTTRELL, B. and PICKARD, A. R.: Use of single procedure for selective enrichment, isolation, and identification of plasmid-bearing virulent Yersinia enterocolitica ofvarious serotypes from pork samples. Appl. Environ. Microbiol., 63:1657-1660, 1997.

BODNARUK, P.W and DRAUGHON F.A.: Effect of packaging atmosphere and pH on the virulence and growth of Yersinia enterocolitica on pork stored at 4° C. Food Microbiology, XIV:129-136, 1998.

EFSA .: The community summary report on trends and sources of zoonoses and zoonotic agents in the European Union in 2007. EFSA Journal, 223:8–13, 2009.

EFSA.: The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistence and foodborne outbreaks in the European Union in 2005.The EFSA Journal 94, 5:223, 2005.

EFSA.: The European Union Summary Report in Trends ans Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010. Efsa Journal, X.:2597, 2012.

FONDREVEZ, M., LABBE,A., HOUARD, E., FRAVALO, P., MADEC, F. And DENIS, M., A simplified method for detecting patthogenic Yersinia enterocolotica in slaughtered pig tonsils. Journal of Microbiological Methods, 83:244-249, 2010.

FREDRIKSSON-AHOMAA, M., BUCHER, M., HANK, C., STOLLE, A. and KORKEALA, H.: High prevalence of Yersinia enterocolitica 4/O:3 on pig offal in Southern Germany: A slaughtering technique problem. Systematic and Applied Microbiology, 21:457–463, 2001.

FREDRIKSSON-AHOMAA, M., KORTE, T., and KORKEALA, H.: Contamination of carcasses, offals and the environment with yadA-positive Yersinia enterocolitica in a pig slaughterhouse. Journal of Food Protection, 63:31–35, 2000.

FREDRIKSSON-AHOMAA, M., STOLLE, A. and KORKEALA, H.: Molecular epidemiology of Yersinia enterocolitica infections. FEMS Immunology and Medical Microbiology, 47:315–329, 2006.

FUKUSHIMA, H., KAWASE, J., Etoh, Y.: Simultaneous screening of 24 target genes of foodborne pathogens in 35 foodborne outbreaks using multiplex Real-Time SYBR Green PCR analysis. Int. J. Microbiology, X :18, 2010.

GURTLER, M., ALTER, T., KASIMIR, S., LINNEBUR, M. and FEHLHABER, K.: Prevalence of Yersinia enterocolitica in fattening pigs. Journal of Food Protection,

68:850-854, 2005.
HAYASHIDA, H., IWATA, T., YAMAGUCHI, S., HARA-KUDO, Y., OKATAMNI, T.A., WATANABE, M., LEE,K., KUMAGAI, S.: Survival of pathogenic Yersinia enterocolitica in vacuum-packed or non-vacuum-packed pork at low temperature. Biocontrol Sci., 13:139-44, 2008.
LEE, W.H., VANDERZANT, C., STERN, N.: The ocurrence of Yersinia enterocolitica in food. Yersinia enterocolitica. Boca Raton, Fla, 161-171, 1981.
MALAŠEVIĆ, M.: Ocena rizika i opcije za redukciju rizika od Yersinai enterocolitica u mesu svinja (Master rad), Poljoprivredni fakultet, Novi Sad, 2012.
MILLS, J.: Yersinia enterocolitica. Enciclopaedia of meat sciences. Elsevier, Oxford, II:841-820, 2004.
NESBAKKEN, T., IVERSEN, T., ECKNER, K. And LIUM, B.: Testing of patogenic Yersinia enterocolitica in pig herds based on the natural dynamic of infection. Int. J. Food Microbiology, 111:99-104, 2006.
NESBAKKEN, T.: Yersinia enterocolitica, Foodborne pathogens. Microbiology and molecular biology, Norwich, UK, Caister Academic, 228-249, 2005.
NESBAKKEN,T., ECKNER, K., HOIDAL, H.K., ROTTERUD, O.J.: Occurrence of *Yersinia enterocolitica* and *Campylobacter spp.* in slaughter pigs and consequences for meat inspection, slaughreting and dressing procedures. Int.J. Food Microbiol., 80:231-240, 2003.
NISSEN, H., MAUGESTEN, T. and LEA, P.: Survival and growth of Escherichia coli O157:H7, Yersinia enterocolitica and Salmonella enteritidis on decontaminated and untreated meat. Meat Sci., 57:291-298, 2001.
NORRUNG, B., ANDERSEN, J.K. and BUNCIC, S.: Main concerns of pathogenic microorganisms in meat. U:Todra F. (Ed). Safety of meat and processed meat (Food microbiology and food safety). Springer, New York, USA, pp. 3-30, 2009.
PIN, C., BARANYI, J. and GARCIA DE FERNANDO, G.: Predictive model for the growth of Yersinia enterocolitica under modified atmosphere. J. Appl. Microbiol., 88:521-530, 2000.
STERN, N.J., PIERSON M.D., KOTULA,E.W.: Effects of pH and sodium chloride on Yersinia enterocolitica growth at room at refrigeration temperature. J. Food Sci., 45:64-67, 1980.
STROTMANN, T., MUEFFLING,V., KLEIN, G. and NOWAK, B.: Effect of different concentration of carbon dioxide and oxygen on the growth of pathogenic Yersinia enterocolitica 4/O:3 in ground pork packaged under modified atmospheres. Journal of Food Protection, 71:845-849, 2008.
THOBODWAU, V., FROST, E.H., CHENIER, S., QUESSY,S.: Present of Yersinia enterocolitica in tissues of orally-inoculated pigs and the tonsil and feces of pigs at slaughter. Can.J.Vet. Res., 63:96-100, 1999.
VAN DAMME, L., HABIB, I., DE ZUTTER.: Yersinia enterocolitica in slaughter pig tonsils: enumeration and detection by enrichment versus direct plating culture. Food Microbiol., 27:158-161, 2010.

YERSINIA ENTEROCOLITICA- IZVORI I PUTEVI KONTAMINACIJE TRUPOVA SVINJA

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Izvod

Yersinia enterocolitica pripada patogenim mikroorganizmima koji mogu da kontaminiraju trupove i meso svinja što utiče na zdravlje ljudi. Ovaj mikroorganizam se nalazi najčešće u tonzilama svinja za klanje a usled loše higijenske prakse i neadekvatne manipulacije noževima, može dospeti na trup i tako postati izvor infekcije za ljude. EFSA je u svom mišljenju o opasnostima iz svinjskog mesa koje se moraju kontrolisati sveobuhvatnim sistemom osiguranja bezbednosti, ocenila kvalitativno i rangirala relevantne opasnosti na trupovima svinja i momentu nakon hlađenja trupova, uključujući i *Y. enterocolitica*.

Ključne reči: yersinia enterocolitica, svinje, meso, izvori infekcije, tonzile.

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