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Review paper

Yersinia enterocolitica and control measures for reducing risks in the pork production chain

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ABSTRACT

Yersiniosis caused by *Yersinia enterocolitica* is the third most common zoonosis transmitted from asymptomatic, healthy pigs to humans through raw or insufficiently cooked meat. The occurrence of *Y. enterocolitica* on a farm can vary, depending on different risk factors, including production system and biosecurity level. At the slaughterhouse, the contamination level of carcasses depends to a great extent on practices during lairage, along with the handling and processing of the head, tongue, tonsils, and rectum of slaughtered pigs. A comprehensive approach for further *Y. enterocolitica* farm/slaughterhouse categorization, improved hygiene practices, and mandatory surveillance for underestimated pathogens within the food chain is necessary for maintaining the One Health concept.

1. Essential characteristics of *Yersinia enterocolitica*

According to the latest taxonomic investigations, *Yersinia enterocolitica* belongs to the genus *Yersinia* and the family *Yersiniaceae* within the order *Enterobacterales* (*Schoch et al.*, 2020). *Y. enterocolitica* has two subspecies: *Yersinia enterocolitica* subsp. *enterocolitica* includes strains with the 16S rRNA type of American origin and *Yersinia enterocolitica* subsp. *palearctica* includes strains of European origin (BT1A, BT2, 3, 4, and 5) (*Neubauer et al.*, 2000). *Y. enterocolitica* is a heterogeneous group of strains classified into six biotypes (1A, 1B, 2, 3, 4, and 5) based on their phenotypic characteristics. Biotypes 1B, 2, 3, 4, and 5 are pathogenic to humans (EFSA, 2011), while biotype 1A is considered non-pathogenic (*Singh & Virdi*, 2004). Based on the chemical properties of the surface O antigen, *Y. enterocolitica* is divided into more than 48 serotypes. The most pathogenic serotypes for humans are biotype 4 (serotype O:3) and biotype 2 (serotype O:2) (*EFSA*, 2011; *Keet*, 1974). In many European countries, the most critical serotype of *Y. enterocolitica* is serotype O:3, followed by serotype O:9 (*Bottone*, 1997). *Y. enterocolitica* is an asporogenic, facultatively anaerobic, Gram-negative bacillus with morphological variations ranging from small cocco-bacilli with rounded ends to elongated bacilli. It is motile at 25°C, with peritrichously arranged flagella, and becomes non-motile when cultured at 37°C. Unlike other enteropathogenic bacteria, *Y. enterocolitica* is psychotropic and can grow from 0 to 44°C (*Keet*, 1974). As such, it can multiply at refrigerator temperatures and survive in frozen food for extended periods.

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Y. enterocolitica can grow on solid nutrient media such as blood agar with sheep blood, MacConkey, and Hektoen-enteric agar. However, on these media, *Enterobacteriaceae* also grow well simultaneously (*Bottone*, 2015). The standard method for the isolation and identification of *Y. enterocolitica* (*SRPS EN ISO 10273:2017*, 2017) involves the use of a selective solid medium, CIN agar (agar supplemented with antibiotics cefsulodin, irgasan, and novobiocin), which is incubated at 30°C for 24–48 hours. Characteristic *Y. enterocolitica* colonies on CIN agar are small (<1 mm) and smooth, with a red centre (due to mannitol fermentation) and a translucent zone (*SRPS EN ISO 10273:2017*, 2017). Because of their characteristic appearance on CIN agar, they are called bull's-eye colonies, allowing easy recognition (*Schiemann*, 1979).

2. *Yersinia enterocolitica* within the food production chain

Y. enterocolitica is widely distributed in nature and can be found in the intestinal tracts of various mammals, birds, cold-blooded, and aquatic species. Isolates from the environment are primarily avirulent and do not cause human disease. However, isolates originating from pigs may contain pathogenic serotypes. Additionally, it is stated that dogs, ruminants, rodents, and water from the environment can also be reservoirs of these pathogenic bio-serotypes (*Fredriksson-Ahomaa et al*., 2006a; 2006b). The main source of infection is considered to be healthy, asymptomatic pigs. Humans are most commonly infected by consuming raw or undercooked food and contaminated water (*EFSA and ECDC*, 2019). Pigs are considered a natural reservoir of *Y. enterocolitica*, which explains its presence in slaughterhouses and the association between pork consumption and the frequency of yersiniosis cases (*Bonardi et al.*, 2013; *Vilar et al.*, 2015). It is estimated that 77.3% of yersiniosis cases in humans are attributed to consuming contaminated pork (*Fosse et al*., 2008). The most common mode of transmission is through the consumption of undercooked pork and pork products (*EFSA and ECDC*, 2018).

Production systems (conventional and organic) can influence the presence of these bacteria, with higher prevalence on farms using conventional production methods than organic production (*Von Altrock et al.*, 2006). The prevalence can vary within a farm, indicating the influence of specific factors on the farm. It has been observed that the prevalence of *Y. enterocolitica* BT4/O:3 is higher on open farms that purchase piglets (fattening farms) from external sources or small farms (*Skjerve et al.*, 1998; *Arsić et al.*, 2022; *Arsić*, 2023). Depending on the applied biosecurity measures on the farm, it has been found that the prevalence is higher on farms with lower levels of biosecurity measures (*Zdolec and Kiš*, 2021; *Arsić et al.*, 2022).

The slaughter process of pigs is an open process, during which the slaughter of infected pigs can lead to contamination and cross-contamination of carcasses and organs of slaughtered pigs (*Fredriksson-Ahomaa et al*., 2006a; 2006b). As this pathogenic microorganism remains present in the pig meat production chain, contamination of carcasses and products is possible at the early stages, especially during the handling and processing of the head, tongue, and tonsils of slaughtered pigs (*Van Damme et al.*, 2015). In a study conducted in a slaughterhouse in Brazil, the presence of *Y. enterocolitica* BT4/O:3 was detected in 10% of tonsil samples (*Martins et al.*, 2021). *Van Damme et al.* (2013) found the presence of *Y. enterocolitica* BT4/O:3 in 11.4% of swab samples from carcass surfaces and 4.9% of ground pork samples.

3. Implementation of good hygiene practices to reduce contamination

The presence of *Y. enterocolitica* cannot be detected by conventional meat inspection methods, so control measures focus on preventing or reducing faecal and other contamination starting from the farm during transportation, lairage period, and slaughter operations. The application of these measures is ensured through the implementation of good hygiene practices and the analysis of risks and critical control points at all stages of production (*Blagojevic et al.*, 2021). Slaughtering techniques and hygiene can influence the percentage of contamination on slaughter products. As pigs are asymptomatic carriers of *Y. enterocolitica*, meat inspection on the slaughter line can pose a risk of further meat contamination (*Laukkanen et al.*, 2009), since there is a possibility of transmitting this bacterium further along the meat production chain during carcass cutting and lymph node examination. Particular attention should be given to removing tonsils from the throat to reduce contamination, as incomplete removal can lead to contamination from lymphatic tissue to adjacent muscle tissue (*Borch et al.*, 1996). Implementing good hygiene practices throughout the entire slaughter process, particularly in handling procedures such as the release of the rectum and simultaneous tying with a plastic bag, can significantly reduce carcass contamination (*Andersen*, 1988). It has been found that removing the

head without prior splitting together with the carcass and washing and sterilizing knives can significantly reduce the contamination of carcasses with pathogenic *Y. enterocolitica* strains (*Van Damme et al.*, 2015).

4. *Y. enterocolitica* farm and slaughterhouse categorization

In recent years, the risk-based meat safety system has been the subject of scientific research (*Buncic et al*., 2019). The strategy to reduce the prevalence of *Y. enterocolitica* infections includes gathering epidemiological data and exchanging information within the food chain system, as well as defining risk factors and implementing measures to reduce or eliminate them to achieve and determine the priority categorization of farms/slaughterhouses, aiming to enhance food safety and public health (*Blagojevic et al*., 2021; *Zdolec and Kiš,* 2021). The presence of *Y. enterocolitica* in asymptomatic carriers poses an additional challenge to meat safety systems and interferes with hazard control in meat production. Since detecting this hazard on each carcass is practically infeasible and economically unjustifiable, applying preventive and control measures on farms and slaughterhouses is proposed as the only effective and efficient control approach (*Blagojević and Antić*, 2014). Based on risk categorization data on farms, the veterinarian overseeing the slaughter process could make decisions regarding ante-mortem meat inspection and, accordingly, approve or prohibit slaughter or implement additional measures for risk control, such as traditional post-mortem meat examination methods, meat freezing, laboratory analyses, or the application of carcass decontamination techniques (hot water, steam). This approach ensures better meat safety compared to the application of existing standard techniques (*Blagojević and Antić*, 2014; *Zdolec and Kiš*, 2021). Serological methods at the farm level are recommended for *Y. enterocolitica* farm categorization (*Bonardi et al*., 2016).

5. Monitoring and surveillance of *Y. enterocolitica*

At the level of the European Union, there is currently no obligation to monitor and report *Y. enterocolitica* findings in pigs and pork products. However, due to the increasing incidence of yersiniosis in humans caused by pathogenic strains of *Y. enterocolitica*, the European Food Safety Authority (EFSA) proposes the implementation of monitoring and reporting of *Y. enterocolitica* findings (*EFSA*, 2009). In the Republic of Serbia, a year-long epidemiological study has been conducted on *Y. enterocolitica* in pigs on the slaughter line. The test results indicate a prevalence of 10.4%, and the main risk factors for *Y. enterocolitica* infection have been identified as open-type farms, prolonged stay of pigs in slaughterhouse depots, and the winter season (*Arsić et al*., 2022; *Arsić*, 2023). Risk factor analysis revealed a twofold increased risk of infection in pigs originating from fattening farms compared to farrow-to-finish farms ($p<0.001$) (*Arsić et al.*, 2022; *Arsić,* 2023). There is also an increased risk of infection associated with prolonged stay in slaughterhouse depots (>3h) compared to shorter stay (0–3h) at the slaughterhouse level ($p<0.035$) (*Arsić et al*., 2022; *Arsić*, 2023). Regarding seasons, there is an almost fourfold higher probability of pig infection during the winter season compared to other annual periods ($p<0.001$) (*Arsić et al*., 2022; *Arsić*, 2023).

6. Conclusion

The finding of a large number of pigs infected with *Y. enterocolitica*, the possibility of further cross-contamination during slaughter and meat processing, and the ability of the bacteria to multiply at low temperatures during storage represent a risk to public health. Therefore, it is crucial to pay special attention to the hygiene conditions during the slaughter and the handling of pig parts, such as the head, tonsils, tongue, and rectum. Understanding the sources and pathways of *Y. enterocolitica* contamination is crucial in preventing foodborne illnesses. In addition to measures applied at the slaughterhouse, reducing the initial contamination on the pig farms is essential. A comprehensive approach for further *Y. enterocolitica* farm/slaughterhouse categorization and improved hygiene practices, along with mandatory surveillance for underestimated pathogens within the food chain, are necessary for maintaining the One Health concept.

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