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Review

Yersinia enterocolitica - Isolation, Pathogenicity, and Prevalence in Farms for Slaughtered Pigs

Maya Angelovska, Maya M. Zaharieva, Hristo Najdenski*

The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Abstract

Yersinia enterocolitica is a significant zoonotic pathogen that poses a considerable threat to both animal and human health. This review paper aims to summarize and critically evaluate current knowledge on the isolation, pathogenicity, and prevalence of *Y. enterocolitica* in farms dedicated to the production of slaughtered pigs. Isolation of *Y. enterocolitica* from various sources, including porcine tonsils, feces, and mesenteric lymph nodes, is essential for understanding prevalence and transmission dynamics. The different isolation methods and their advantages and limitations in detecting the pathogen are explored, highlighting the challenges associated with its detection. We examine the role of specific virulence factors, in the ability of *Y. enterocolitica* to colonize and cause disease in humans. Additionally, this review provides a comprehensive analysis of factors influencing the prevalence of *Y. enterocolitica* in farms for slaughtered pigs. The review highlights the need for standardized surveillance strategies to accurately assess the prevalence of *Y. enterocolitica* in swine populations, ensuring effective control and prevention measures. In conclusion, the findings presented here underscore the importance of *Y. enterocolitica* as an emergent pathogen as well as implementing comprehensive surveillance programs, adopting effective control measures, and promoting awareness among stakeholders to mitigate the risk associated with *Y. enterocolitica* infection in both animals and humans.

Резюме

Yersinia enterocolitica е значим зоонозен патоген, представляващ сериозна заплаха за здравето на животните и хората. Тази обзорна статия има за цел да обобщи и критично оцени съвременните познания отнасяши се до изолирането, патогенността и разпространението на *Y. enterocolitica* във ферми, за свине за клане. Изолирането на *Y. enterocolitica* от различни източници, включително свински тонзили, изпражнения и мезентериални лимфни възли е от съществено значение за събиране на информация касаеща разпространението и динамиката на предаване на патогена. Обхванати са основните методи за доказване заедно с техните предимства и ограничения като се подчертават предизвикателствата, свързани с неговото откриване. Ние изследваме ролята на специфичните вирулентни фактори имащи отношение към способността на *Y. enterocolitica* да колонизира и да причинява заболяване при хората. Освен това, този обзор предоставя анализ на по-значимите фактори, влияещи върху разпространението на *Y. enterocolitica* между свинете за клане. Подчертава се необходимостта от стандартизирани процедури за определяне и точна оценка на разпространението на *Y. enterocolitica* в популациите от свине, с цел да се осигури ефективен контрол и мерки за превенция. В заключение, представените данни подчертават значението на *Y. enterocolitica* като важен патоген, както и необходимостта от изготвяне на програми за наблюдение, с цел да се създадат ефективни мерки за контрол и намаляване на риска, свързан с инфекцията от *Y. enterocolitica* както при животни, така и при хората.

Taxonomy

The genus *Yersinia* comprises twenty six Gram-negative bacterial species (Le Guern *et al.,* 2020). Based on the phylogenetic analyses and the conserved molecular characteristics identification, *Yersinia* is taxonomically arranged in the family *Yersiniaceae*, order *Enterobacterales*, class *Gam-*

* Corresponding author: hnajdenski@gmail.com

maproteobacteria, phylum *Proteobacteria*, kingdom *Bacteria* (Adeolu *et al.,* 2016; Parte *et al*., 2020; Schoch *et al.,* 2020). Three of them occupy a particularly significant place as causative agents of diseases in humans and animals. *Yersinia pestis* is the causative agent of plaque, a vector-borne disease (Barbieri, 2021), while *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are well-known gastrointestinal pathogens, associated with food-borne disease - yersiniosis (European Food Safety Authority and European Centre for Disease Prevention and Control, 2022). Two of the species are pathogenic only for animals: *Yersinia ruckeri*, the causative agent of red mouth disease in salmonids and *Yersinia entomophaga,* responsable for the disease among grass grubs *Costelytra zealandica* (Hurst *et al.,* 2011). In a recent study by (Savin *et al.,* 2014) the pathogenic properties of *Yersinia wautersii* were identified, establishing it as the fourth *Yersinia* species capable of causing harm to humans. Approximately twenty strains within the genus *Yersinia* have been identified as non-pathogenic. When it comes to enteric pathogens, *Y. enterocolitica* and *Y. pseudotuberculosis*, although they cause similar infections, exhibit differences in terms of their ecological and epidemiological niches. Despite their evolutionary relationship being distant, both pathogens contribute to the overall understanding of this group of diseases (Wren, 2003; Reuter *et al.,* 2014). After all, the evolution of the *Y. enterocolitica* and *Y. pseudotuberculosis*/*Y. pestis* might not be totally parallel (Reuter *et al.,* 2014; Tan *et al.,* 2016). The *Y. pseudotuberculosis*-*Y. pestis* evolutionary linkage diverged from *Y. enterocolitica* between 41 and 186 million years ago (Achtman *et al.,* 1999). *Y. enterocolitica sensu stricto* has been classified into two distinct subspecies based on DNA-DNA hybridization and differences in the *16S rRNA* gene. These subspecies, namely *Y. enterocolitica subsp. enterocolitica* and *Y. enterocolitica subsp. palearctica*, exhibit separate geographic distributions. The former is predominantly found in strains originating from America, while the latter is associated with strains from Europe (Neubauer *et al.,* 2000). Moreover, a comprehensive comparison of *Y. enterocolitica* strains using DNA microarray analyses revealed the existence of three distinct clades within the species (Howard *et al.,* 2006). This finding sheds light on the heterogeneity observed within *Y. enterocolitica*. Based on their biochemical characteristics, *Y. enterocolitica* strains are categorized into six biotypes: 1A, 1B, 2, 3, 4, and 5 (Swaminathan *et al.,* 1982; Wauters *et al.,* 1987). These biotypes display variations in their geographic distribution, ecological preferences, and pathogenic potential. The pathogenicity of *Y. enterocolitica* is assessed through a mouse infectious model, which identifies biotype 1A as non-pathogenic, biotype 1B as highly pathogenic, and strains belonging to biotypes 2, 3, 4, and 5 as having relatively low pathogenic potential (Aulisio *et al.,* 1983). However, recent studies have reported an emerging pathogenic potential in *Y. enterocolitica 1A*, attributed to its putative virulence factors and involvement in certain infections (Tuompo *et al.,* 2017; Morka *et al.,* 2021; Platt-Samoraj, 2022). Furthermore, more than 70 serotypes have been identified based on variations in the structure of the somatic O-antigen. Some serotypes (O:3, O:5,27, O:8, and O:9) are frequently isolated from both humans and animals (Virdi and Sachdeva, 2005; Marimon *et al.,* 2017; Zdolec *et al.,* 2022a; Angelovska et al., 2023; Yue et al., 2023). Determining the biotype and serotype is particularly crucial for conducting epidemiological studies. In terms of geographic distribution, *Y. enterocolitica* 1A is the most widespread biotype, primarily found in the environment and occasionally isolated from healthy animals and humans (von Altrock *et al.,* 2015; Syczyło *et al.,* 2018; Lucero-Estrada *et al.,* 2020). Historically, highly pathogenic *Y. enterocolitica 1B* and serotype O:8 were commonly associated with the Americas, particularly the United States. However, due to global movement, these strains have now spread to European countries, causing yersiniosis cases in humans (Rastawicki *et al.,* 2013; Morka *et al.,* 2018; Savin *et al.,* 2018). Recently, *Y. enterocolitica* 4/O:3 has gained significant importance as numerous studies have identified it as the most frequently isolated bioserotype in yersiniosis cases and outbreaks worldwide (Rosner *et al.,* 2010; Karlsson *et al.,* 2021; European Food Safety Authority and European Centre for Disease Prevention and Control, 2022). The reason for the dominance of the 4/O:3 bioserotype is not fully understood; however, it is speculated that possessing alternative putative virulence and fitness factors contributes to its successful adaptation and dissemination (Batzilla *et al.,* 2011; Valentin-Weigand *et al.,* 2014).

Epidemiology

Prevalence in humans

Pathogenic *Y. enterocolitica* bioserotypes 1A, 2 - 5, have been recognised as the causative agents of yersinosis, isolated from different sources (Karlsson *et al.,* 2021; Yue *et al.,* 2023). For 2021, yersiniosis is the third most commonly reported bacterial zoo-

nosis in The European Union, with the pathogen being detected in food-borne outbreaks reported by twelve European countries: Belgium, Denmark, Estonia, Finland, France, Germany, Lithuania, the Netherlands, Norway, Slovakia, Spain, Sweden (European Food Safety Authority and European Centre for Disease Prevention and Control, 2022). According to the organisation, a total of 6789 human cases of illness are reported, with 508 hospitalisations, of which 125 cases of illness were related to the 21 foodborne outbreaks. There is a difference in number of cases among countries. For 2021 Germany was on the top accounting for the highest number of cases (1,912), followed by France (1,451) On the other hand, the notification rate of confirmed yersiniosis cases per 100,000 populations in Denmark was highest, followed by Finland (7.8 and 6.0 respectively), with an overall 1.9 cases per 100,000 population calculated for the EU. It is known that for three of the food-borne outbreaks, the serotype involved was O:3 (European Food Safety Authority and European Centre for Disease Prevention and Control, 2022). In USA (Centers for Disease Control and Prevention (CDC) 2016) estimates that *Y. enterocolitica* causes almost 117,000 illnesses, 640 hospitalizations, and 35 deaths every year.

Transmission

Due to the complex epidemiology of these pathogens, there is still a lack of complete understanding regarding the reservoirs and routes of transmission for these infections. The transmission of *Y. enterocolitica* is associated with multiple pathways. As a zoonotic pathogen with widespread distribution in nature, the consumption of contaminated food or water is the primary mode of transmission (Bottone, 2015). Raw or undercooked meat and untreated water are considered the primary risk factors for human yersiniosis (Guillier *et al.,* 2021). *Yersinia enterocolitica* possesses certain biological properties that enable it to thrive at low temperatures, even below 4°C (Rakin *et al.,* 2015), This characteristic allows the bacteria to persist and multiply easily in refrigerated environments and modified atmospheres (Nesbakken *et al.,* 2008; Fredriksson-Ahomaa *et al.,* 2012). Consequently, the seasonal distribution of pathogenic *Y. enterocolitica* isolation occurs predominantly during the colder months (Van Damme *et al.,* 2015; Arsić *et al.,* 2022; Angelovska *et al.,* 2023). However, the pathogen can still be isolated throughout the year, as it can be transmitted through food items consumed consistently year-round, such as meat and meat products (Rosner *et al.,* 2010). Furthermore, *Y. enterocolitica* can also be spread through other sources such as milk, poultry meat, and ready-to-eat salads like spinach (Espenhain *et al.,* 2019; Gruber *et al.,* 2021; Karlsson *et al.,* 2021). Raw vegetables have the potential to become contaminated through contact with soil, water, and fertilizers. Moreover, direct contact between humans and animals, as well as contact with water and soil, can serve as additional means of pathogen transmission (Boqvist *et al.,* 2009; Yue *et al.,* 2023). Although person-to-person transmission of *Y. enterocolitica* is rare (Moriki *et al.,* 2010) animals, especially pigs, are the most common source of the pathogen. The high genetic similarity between porcine and human isolates confirms the relevance of pigs in the epidemiology of human yersiniosis (Morka *et al.,* 2021). Healthy pigs are the main reservoir of *Y. enterocolitica* infection, particularly the 4/O:3 serotype in humans (Morka *et al.,* 2021; Yue *et al.,* 2023). Pigs can asymptomatically carry the *Y. enterocolitica* bacteria, which are primarily isolated from their tonsils and submaxillary lymph nodes (Fois *et al.,* 2018; Angelovska *et al.,* 2023), although they can also be found in feces or intestinal contents (Yue *et al.,* 2023).

Prevalence at slaughter level

Slaughtered pigs have long been recognized as a reservoir for pathogenic *Y. enterocolitica* strains, consistently identified in their tonsils and fecal samples (Mazzette *et al.,* 2015; Van Damme *et al.,* 2015; Fois *et al.,* 2018; Terentjeva *et al.,* 2022; Zdolec *et al.,* 2022a; Angelovska *et al.,* 2023). Consequently, the presence of infected tissues and intestinal contents in slaughterhouses poses a risk for the potential contamination of pig carcasses during slaughtering (Martins *et al.,* 2018). The reported prevalence of pathogenic *Y. enterocolitica* among healthy slaughtered pigs in European countries ranged widely, from as low as 6% to as high as 95% (Martinez *et al.,* 2011; Van Damme *et al.,* 2015; Ibañez *et al.,* 2016; Sacchini *et al.,* 2018; Arsić *et al.,* 2022; Terentjeva *et al.,* 2022; Zdolec *et al.,* 2022a; Angelovska *et al.,* 2023). The discrepancies in these percentage values arise from variances in pathogen isolation methodologies, the particular pig farms from which the animals originate, and the heterogeneous conditions prevailing in distinct categories of slaughterhouses. The isolation methods employed for the detection of the pathogen and their influence on the detection rate are outlined in the following text. Pigs raised in fattening farms had over twice the likelihood of infection compared to pigs raised in farrow-to-finish farms, and the risk

of contamination has been significantly elevated when keeping pigs within a slaughterhouse for over three hours (Arsić *et al.,* 2022). Regarding shedding the bacteria to the carcass other factors are detail reviewed by (Zdolec *et al.,* 2022b). Among them, the duration of pigs' confinement in the lairage facility and their exposure, whether through direct or indirect contact, to other groups of pigs prior to slaughter, have a significant influence on the bacterial load found on carcasses as well as the occurrence of pathogens within lymphoid tissues, leading to lower prevalence when the contact between pigs is prevented (Zdolec *et al.,* 2022a). In order to prevent the occurrence of pathogenic *Y. enterocolitica*, on the surfaces of slaughtered pigs' organs and carcasses, it is imperative to implement precautionary steps. Regarding that, combining the head removal with the carcass splitting has been associated with higher *Y. enterocolitica* contamination, due to the possibility of the splitting machine being contaminated by tonsils, leading to the contamination of subsequent carcasses (Van Damme *et al.,* 2015). To minimize cross-contamination these authors suggest keeping the tongue and the tonsils inside the head during evisceration as well as constant cleaning of the knives. Contamination of the pig meat, especially the head of the pig should not be underestimated, so future heat-treated cooking practices should be considered (Fredriksson-Ahomaa *et al.,* 2012). Regarding determination, biotype 4 is mostly isolated from pigs on the slaughterhouse level (Morka *et al.,* 2018; Angelovska *et al.,* 2023; Yue *et al.,* 2023).

Prevalence on pig farm level

Pathogenic *Y. enterocolitica* strains are commonly found in domestic pigs on farms, with a various herd prevalence observed in pigs across Europe (Nesbakken *et al.,* 2006; Nowak *et al.,* 2006; Råsbäck *et al.,* 2018; Koskinen *et al.,* 2019). And the bioserotype 4/O:3 is the most commonly isolated from farm animals (Råsbäck *et al.,* 2018). However, the occurrence of *Y. enterocolitica* in swine exhibits variability across different farms, indicating the existence of underlying factors that influence its prevalence within farm settings. The management system is regarded as a pivotal factor in the regulation of pathogenic *Y. enterocolitica* transmission within pig farms. Furthermore, in farrow-to-finish farms, the practice of mixing pigs from different groups has been widely acknowledged as a significant risk factor for the transmission of pathogenic *Y. enterocolitica*. It has been observed that when pigs are relocated to facilities that do not implement the all-in/all-out system, the infection disseminates rapidly throughout the entire pig population (Virtanen *et al.,* 2014; Koskinen *et al.,* 2019). Opposite to farrow-to-finish farms where there is no piglet suppliers, in fattening farms the number of piglet suppliers has been recognized as a risk factor (Virtanen *et al.,* 2014; Vanantwerpen *et al.,* 2015). The likelihood of purchasing infected pigs and subsequently spreading pathogenic *Yersina* spp. within the pen escalates as the number of piglets procured from diverse suppliers increases (Virtanen *et al.,* 2012). Other risk factors for the dissemination of the pathogen included the presence of semi-slatted floors in the fattening pig units (Vanantwerpen *et al.,* 2015). Protective factors identified in various studies are related to hygienic measurements (Vanantwerpen *et al.,* 2015). In experiments examining piglet colonization with various serotypes, Schiemann (1988) suggested that colostrum is also a protective factor. He reported higher levels of colonization by *Y. enterocolitica* in piglets born through Cesarean section without access to colostrum showed compared to normally born piglets receiving colostrum. However, it is important to note that seropositivity alone cannot be considered an accurate indicator of prevalence. The occurrence of antibodies in specific piglets at birth is likely attributed to the transfer of maternal antibodies via colostrum and when piglets get older, levels of maternal antibodies appear to decrease (Koskinen *et al.,* 2019). Initially, the pathogen can be detected in the tonsils, where persist, then in feces, before antibodies can be identified through serology. The occurrence of pathogenic *Y. enterocolitica* in the fattening pig population is influenced also by the age of the animals. The pathogen shedding in feces is more common among piglets younger than 30 days old, but decreases as the pigs grow and approach the age for slaughter. This pattern is also reflected in the sample source (Nesbakken *et al.,* 2006; Virtanen *et al.,* 2012). In Germany conventional housing systems have shown an increased number of positive pigs (29% vs. 18%) with a twice as many tonsils being positive for *Y. enterocolitica* (22% vs. 11%) compared to organic housing systems (Nowak *et al.,* 2006).

Prevalence in other animals and food

Yersinia enterocolitica is widely prevalent among various domestic and wild animals. Examples include wild boars (Arrausi-Subiza *et al.,* 2015; Bancerz-Kisiel *et al.,* 2015), small rodents (Platt-Samoraj *et al.,* 2020), and domestic animals such as sheep (Yue *et al.,* 2023), dogs and cats (Byun *et al.,* 2011; Stamm *et al.,* 2013; Nasser *et al.,* 2023).

Wild animals play a significant role in the epidemiology of *Yersinia* infection, serving as important reservoirs of enteropathogenic *Yersinia* (Nikolova *et al.,* 2001; Arrausi-Subiza *et al.,* 2015; Bancerz-Kisiel *et al.,* 2015). The circumstances under which wild animals are killed and eviscerated have not been recognized as having a significant impact on the spread of pathogenic bacteria in the carcasses (Peruzy *et al.,* 2022). However, most pathogenic strains are isolated from wild animals during the colder periods (Nikolova *et al.,* 2001; Arrausi-Subiza *et al.,* 2015). Wild boars (*Sus scrofa*) are particularly examined for the presence of *Y. enterocolitica*, with the bacteria frequently being isolated from their tonsils and feces (Fredriksson-Ahomaa *et al.,* 2009; Bancerz-Kisiel *et al.,* 2015). The detection of bacteria in wild boar meat and carcasses is often a result of cross-contamination from bacteria present in their feces or tonsils (Bancerz-Kisiel *et al.,* 2016; Sannö *et al.,* 2018). However, notable differences exist in terms of virulence potential between isolates obtained from wild boars and those from slaughter pigs (Fredriksson-Ahomaa *et al.,* 2011). Wild boars predominantly exhibit biotype 1A (Bancerz-Kisiel *et al.,* 2016; Bonardi *et al.,* 2020; Morka *et al.,* 2021), while porcine isolates have been primarily of biotype 4/O:3 (Nikolova *et al.,* 2001; Fredriksson-Ahomaa *et al.,* 2009; Rodas *et al.,* 2014; Bancerz-Kisiel *et al.,* 2015). This implies that wild boars may carry a distinct *Yersinia* population, which can complicate their identification using conventional methods (Morka *et al.,* 2018). The presence of pathogenic bacteria among wild boars predisposes to transmission through the consumption of their meat, posing a public health risk (Peruzy *et al.,* 2022). Furthermore, the spread of pathogenic bacteria in the natural environment also poses a potential hazard for domestic animals (Peruzy *et al.,* 2022). Regarding food sources, *Y. enterocolitica* has been isolated from fresh vegetables and dairy products (Karlsson *et al.,* 2021); (Darwish *et al.,* 2015) with the former containing pathogens and the latter nonpathogens (Piras *et al.,* 2021; Mancini *et al.,* 2022). However, studies on the prevalence of pathogenic *Y. enterocolitica* in fruits and vegetables are limited, possibly due to the lower detection rate resulting from the sensitivity of culture detection methods (Verbikova *et al.,* 2018). However, the use of qPCR methods or a combination of culture and molecular methods has been shown to improve the detection rate of the pathogen in various types (Määttä *et al.,* 2013; Verbikova *et al.,* 2018). In terms of raw milk, the prevalence of pathogenic *Y. enterocolitica* in various milk types is relatively low, indicating a minimal risk for consumers. However, pathogenic *Y. enterocolitica* strains, specifically belonging to bioserotypes 4/O:3, have been identified in sheep milk (Alavi *et al.,* 2018), and strains 1B/O:8 and 2/O:5,27 have been found in cow milk (Jamali *et al.,* 2015; Bonardi *et al.,* 2018). Darwish *et al.* (2015) conducted a study using conventional, phenotypic, and PCR methods to analyze different types of raw milk, revealing a significant occurrence of pathogenic strains. The authors emphasized the importance of employing all the aforementioned methods to enhance the detection rate of pathogenic *Y. enterocolitica* in raw milk, an approach that is also applicable to fruits and vegetables. (Darwish *et al.,* 2015). On the other hand, pasteurized milk does not support the growth of *Y. enterocolitica* due to the absence of competing microflora (Gruber *et al.,* 2021). However, if pasteurization is inadequately performed or if there is initial low-level contamination present, dissemination of *Y. enterocolitica* through the milk can still occur.

Pathogenesis and Disease

Human yersiniosis is a gastrointestinal disease with different symptoms commonly reported as low-grade fever, abdominal pain, nausea, and diarrhea, which depend on the age and health status of the individuals (Šumilo *et al.,* 2023). In healthy adults, usually, the infection goes asymptomatically and onsets by itself, without needing any treatment (Rosner *et al.,* 2010). Recently, significant findings have emerged regarding the shifting epidemiology of yersiniosis in England. Notably, there has been a decline in the occurrence of cases among young children, while a concerning rise has been observed among individuals aged 65 and older (Šumilo *et al.,* 2023). These trends could suggest a notable change in the epidemiological landscape of yersiniosis. Nevertheless, yersiniosis is not devoid of significant complications, including but not limited to septicemia and abscess formation. Notably, a higher incidence of such complications has been observed in specific vulnerable populations, namely children under the age of five, elderly individuals, and those with compromised immune systems. (Rodio *et al.,* 2018; Liu *et al.,* 2021; Norrito *et al.,* 2021). Appendectomies and post-infectious complications like reactive arthritis and erythema nodosum are also reported in yersiniosis patients suggesting that they can be attributed to infections with *Y. enterocolitica* (Fernandes *et al.,* 2020; Takeda *et al.,* 2023). Also, there is an evident correlation between *Y. enterocolitca* epidemiology on the one side and prolonged

diseases like Crohn's disease and chronic infective colitis as a complication supposed to be associated to *Y. enterocolitica* involvement (Honda *et al.,* 2017; Norrito *et al.,* 2021; Fang *et al.,* 2023). The course of the disease is more severe when the infection has occurred with O:8 serotype and the hospitalization rate is higher when the infection is due to this serotype (Rosner *et al.,* 2010). After all, the severity of the *Y. enterocolitica* infection depends on both the pathogen serotype and the health condition of the infected person.

Virulence markers of pathogenic *Y. enterocolitica*

During the transmission process to various hosts, *Y. enterocolitica* needs to overcome environmental challenges, such as different temperatures or pH. To establish a successful infectious cycle, the bacteria express a number of virulence factors, several being temperature-dependent (Atkinson and Williams, 2016; Bancerz-Kisiel *et al.,* 2018; Morka *et al.,* 2021). Not all strains of *Y. enterocolitica* are pathogenic to humans; only those that carry a 70 kb plasmid known as plasmid of *Yersinia* virulence (pYV) exhibit pathogenicity. pYV, which is thermosensitive, contains genes that are transcribed in the presence of calcium at 37°C but are absent when the bacteria are cultured at the same temperature. A significant virulence factor encoded by the plasmid is the *Yersinia* Ysc-Yop Type Three Secretion System (Ysc-Yop T3SS). This system consists of a needle-like structure known as the *Yersinia* outer protein secretion apparatus (Ysc) and *Yersinia* outer proteins (Yop-s). Ysc interacts with eukaryotic cells outside the bacteria and injects Yop-s directly into the cell cytosol. Yop-s act as effector molecules necessary for a successful infection cycle. Among them, YopE, YopT, YopO, and YopH play a role in paralyzing cellular functions by disrupting cytoskeletal components, while YopP and YopM enable bacteria to evade immune system responses by inhibiting macrophage cytotoxicity (Mares *et al.,* 2021). Another plasmid-encoded virulence factor is *Yersinia* adhesin A (YadA), which facilitates bacterial adhesion to cell surfaces and mediates Yop injection into leukocytes (Mühlenkamp *et al.,* 2015; Deuschle *et al.,* 2016). Additional proteins that promote bacterial adhesion and invasion include attachment-invasion locus protein Ail, invasin InvA, and mucoid *Yersinia* factor MyfA, all encoded on the chromosome (Morka *et al.,* 2021). Furthermore, pathogenic *Y. enterocolitica* strains isolated from humans with yersiniosis are capable of producing *Yersinia* stable enterotoxin YstA, which is known for its role in causing diarrhea in yersiniosis. This toxin is typically detected only in pathogenic strains (Peruzy *et al.,* 2017). Lastly, lipopolysaccharide (LPS) located on the outer membrane is the major immunogenic component of *Y. enterocolitica*. It plays a crucial role in the pathogen's resistance to innate immune system responses (Skurnik and Bengoechea, 2009). Complete LPS expression in pathogenic *Y. enterocolitica* is essential for the bacteria to maintain full virulence (Najdenski *et al.,* 2006; Białas *et al.,* 2012). The expression of virulence markers is regulated through a complex network involving various chromosomally or plasmid-encoded regulatory proteins, as reviewed by Bancerz-Kisiel *et al.* (2018). Some of these proteins function in the initial stages of infection and regulate early virulence genes. One such protein is VirF, a transcriptional activator of the *Yersinia* virulence regulon that activates *yop* and *yadA* genes and is frequently found in pathogenic strains (Pegoraro *et al.,* 2021; Terentjeva *et al.,* 2022). Two factors regulate the synthesis of the InvA protein: the transcriptional regulator RovA, which stimulates the expression of the *invA* gene (Bancerz-Kisiel *et al.,*2018), and the *Yersinia* modulator YmoA, which inhibits it (Platt-Samoraj *et al.,* 2006). The main post-transcriptional regulator in *Y. enterocolitica* is the chaperone Hfq, which modulates the expression of surface virulence factors, particularly adhesins that facilitate the interaction of *Y. enterocolitica* with host cells, such as Ail, InvA, YadA, Myf, and LPS (Kakoschke *et al.,* 2016). The highly pathogenic *Y. enterocolitica* 1B biotype is the only known *Y. enterocolitica* variant that carries an additional chromosomal mobile genetic element called the High Pathogenicity Island (HPI). This island contains genes for yersiniabactin (*ybt*) and the *Yersinia* secretion apparatus Type 3 secretion system (*ysaT3SS*) (Carniel, 1999; Schubert *et al.,* 2004).

Isolation and identification

The identification of pathogens in biological material is primarily performed using conventional microbiological, immunological, and histopathological methods, including the enrichment followed by isolation on selective nutrient media, biochemical tests, and serological tests. In recent years, these methods have been complemented by molecular techniques for the detection of nucleic acids or proteins. Nucleic acid-based methods are specifically designed to recognize highly variable regions of the genome, displaying significant specificity across different species, including subspecies of pathogens. Consequently, molecular methods enable the

straightforward differentiation of pathogen genomes from those of their hosts. Because of the role of an emerging zoonotic pathogen with an influence on the food industry and the livestock it is necessary to establish and evaluate fast and accurate detection of *Y. enterocolitica* from biological and natural samples. The concentration of *Y. enterocolitica* in both biological and natural samples consistently falls beneath the detectable threshold, necessitating additional pre-concentration steps to employ culture-based methods for diagnostic purposes. Discrepancies in the prevalence of pathogenic *Y. enterocolitica*, reported in the articles, could potentially arise from variations in the employed isolation methods (Morka *et al.,* 2018). At present, there is no single method that can guarantee the recovery of all pathogenic serotypes. However, conventional microbiological methods are still the golden standard for the isolation and identification of *Y. enterocolitica* from different sources, especially from food (ISO, 2003). Two types of isolation procedures are mostly performed: cold enrichment and selective enrichment (Petsios *et al.,* 2016). The isolation of pathogenic *Y. enterocolitica* commonly relies on cold enrichment methods, performed at 4 to 10°C that require extended periods (7–21 days) (Van Damme *et al.,* 2013; Råsbäck *et al.,* 2018). This approach is applied due to the biological characteristics of *Y. enterocolitica*, which thrive at low temperatures. It also provides selectivity, effectively suppressing the growth of background microflora. On the other hand, cultivation at low temperatures is preferred by pYV, which is thermolabile and can be easily lost during repeated cultivation (Bhaduri and Smith, 2011). Thus, the detection of plasmid-harbored virulent determinants is always challenging. Recent discoveries may challenge earlier conclusions, suggesting that the absence of the pYV poses negligible risks when retrieving *Yersinia* species under conditions favoring bacterial growth at 37°C (Zhang and On, 2022). Various media have been employed for the cold enrichment isolation of *Y. enterocolitica*. These include phosphate-buffered saline (PBS), tryptic soy broth (TSB), and phosphate-buffered saline supplemented with 0.15% bile salts, as well as either 1% sorbitol or 1% mannitol. (PSB) or (PMB) (Van Damme *et al.,* 2013) (Arrausi-Subiza *et al.,* 2015) (Råsbäck *et al.,* 2018). According to Van Damme *et al.* (2013), cold enrichment is more effective than direct plating and selective enrichment for the recovery of *Y. enterocolitica* from tonsils, feces, and carcass (Van Damme *et al.,* 2013). However, cold enrichment is preferred for samples containing pathogenic *Y. enterocolitica* at low concentrations. For selective enrichment, the incubation time is reduced in favor of increased temperature. Peptone sorbitol bile (PSB) broth and irgasan ticarcillin and potassium chloride (ITC) broth are both utilized ((ISO, 2003; Van Damme et al., 2010; Morka *et al*., 2018). Alkali treatment following enrichment has been shown to significantly reduce background flora and enhance the recovery of pathogenic *Y. enterocolitica* from tonsils (Van Damme *et al.,* 2010; Zdolec *et al.,* 2022a). Several selective media have been employed after the enrichment step. MacConkey agar is a well-known differential medium for *Enterobacteriales* isolates, based on the differentiation of lactose utilization. *Y. enterocolitica* forms colorless colonies due to their inability to utilize lactose. *Salmonella–Shigella* deoxycholate calcium chloride (SSDC) agar has also been used either after enrichment or for direct plating (Van Damme *et al.,* 2010). However, the most commonly used selective medium developed for *Yersinia* is Cefsulodin–Irgasan–Novobiocin (CIN) agar (Schiemann, 1979). The addition of antibiotics inhibits strains belonging to *Enterobacteriaceae*. *Y. enterocolitica* forms small (≤1mm) and smooth characteristic colonies with a deep red center (as a result of mannitol fermentation), which are surrounded by a translucent zone referred to as "bull's-eye" colonies (ISO, 2003). Higher recovery of *Y. enterocolitica* is reported when applying shortened enrichment steps followed by plating on optimal selective media as CIN agar (Van Damme *et al.,* 2010; Råsbäck *et al.,* 2018). Nevertheless, the high expenses, extensive time requirements, and inadequate sensitivity of conventional techniques impede their application in the routine identification of *Y. enterocolitica*. Isolated presumptive colonies are further identified by biochemical and molecular methods. Presumptive isolates can be identified at the species level using a variety of biochemical tests. Among the frequently employed tests are the Kligler agar, Christensen urea tests and phenylalanine deaminase test. In general, *Yersinia* demonstrates positive catalase activity, negative oxidase activity, positive urease activity, the ability to ferment glucose and negative phenylalanine deaminase activity. For rapid species identification commercial panels like Vitec, API, and subtyping by MALDI-TOF are performed (Määttä *et al.,* 2013; Morka *et al.,* 2018; Råsbäck *et al.,* 2018; Zdolec *et al.,* 2022a). The API20E system demonstrates limitations in accurately identifying *Y. enterocolitica* strains that do not exhibit sucrose fermentation. Challenges arise

in correctly identifying *Yersinia spp*. through biochemical tests in certain cases (Fredriksson-Ahomaa *et al.,* 2018). The determination of pathogenicity in isolated strains relies on the utilization of phenotyping and biotyping schemes. Various authors have proposed different biotyping schemes, such as those suggested by Wauters *et al.* (1987) and Swaminathan *et al.* (1982). Essential diagnostic tests employed in biotyping encompass the assessment of esculin/salicin hydrolysis, tween esterase/lipase activity, pyrazinamidase activity, indole activity, as well as the metabolic utilization of xylose and trehalose (ISO, 2003). These tests collectively provide crucial insights into the distinctive biotype of the strains involved in epidemiological and microbiological studies. In addition, serotypes are defined by using commercially available sera. Conversely, the assessment of pathogenic potential relies on the detection of virulent genes using Polymerase Chain Reaction (PCR), which is a widely utilized approach. However different target genes are object of amplification, as well as different detection systems (Bancerz-Kisiel *et al.,* 2018; Morka *et al.,* 2021;Terentjeva *et al.,* 2022). The relatively low rates of isolating pathogenic *Y. enterocolitica* in natural samples may be also attributed to the limited sensitivity of traditional culture techniques. Thus, the utilization of conventional microbiological techniques, which involve an enrichment step in addition to a PCR-based confirmation method, serves to minimize the risk of false positive outcomes resulting from the presence of non-viable cells (Fredriksson-Ahomaa and Korkeala, 2003; Mazzette *et al.,* 2015). Standardized reference methods have been developed to facilitate the detection of pathogenic *Y. enterocolitica*. Currently, the detection of pathogenic *Y. enterocolitica* from food and environmental samples in the food production chain is performed according to the International Standard Organisation protocol (ISO, 2003). Aside from that, new fast, and not so expensive approaches with higher sensitivity and specificity are developed. Loop-mediated isothermal reaction (LAMP) is a novel molecular method by which deoxyribonucleic acid (DNA) is amplified in a short time, under isothermal conditions (Notomi *et al.,* 2000). LAMP is characterized by great specificity for the target sequence and a low detection limit, requiring only a few copies of DNA. The LAMP reaction mix contains four to six primers (two outer, two inner primers, and optional two loop primers) accurately selected, *Bacillus stearothermophilus* DNA polymerase (*Bst* polymerase) with unique strand displacement activity, not dependent on temperature changes. Confirmation of LAMP products is facilitated by using agarose gel electrophoresis, measuring turbidity or fluorescence, and by colorimetric methods with naked-eye monitoring (Park, 2022).

Conclusion

The prevalence of pathogenic *Y. enterocolitica* in pigs is of considerable concern for human health. Through extensive research and surveillance, it has become evident that pigs serve as a major reservoir for this pathogenic bacterium, which can be transmitted to humans through various routes. *Y. enterocolitica* bioserotype 4/O:3 is the most commonly isolated from farm animals. Further research is warranted to explore the genetic diversity, virulence factors, and antimicrobial resistance profiles of *Y. enterocolitica* strains in pig populations. This knowledge will aid in identifying high-risk strains and developing targeted interventions to mitigate the transmission of this pathogen. It is imperative to implement rigorous on-farm management practices, including hygiene protocols, biosecurity measures, and appropriate antimicrobial stewardship to reduce the transmission of pathogenic *Y. enterocolitica* from pigs to humans. Collaborative efforts between public health agencies, veterinarians, farmers, and the food industry are pivotal for the reduction the prevalence of pathogenic bioserotypes of *Y. enterocolitica* in both pigs and humans and the successful control of *Y. enterocolitica* infections in humans.

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