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LABORATORY DIAGNOSTICS OF YERSINIOSIS

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Abstract. *The article deals with the analysis of the current state of diagnostics of intestinal yersiniosis affecting animals and the importance of studying this bacterial zoonosis. It is based on domestic and foreign scientific literature and provides information on the spread of yersiniosis and laboratory diagnostics of this disease. Yersiniosis infections caused by Yersinia pseudotuberculosis and Yersinia enterocolitica, which are pathogenic for humans and animals, belong to the most common acute intestinal diseases worldwide. The kinship of the causative agents of pseudotuberculosis and intestinal yersiniosis leads to the similarity of clinical forms. Until recently, intestinal yersiniosis and pseudotuberculosis were considered as quite rare diseases and did not draw any increased attention of specialists. For some time, however, the cases of yersiniosis have been drawing more and more attention of veterinary and medical workers, which is due to the prevalence of Yersinia in nature (soil, water, sewage, plants, dust), an increase in the number of people suffering from yersiniosis, and suboptimal methods of laboratory diagnosis of these diseases. Non-compliance with sanitary and hygienic norms and rules concerning the harvesting, transportation, and storage of food products (milk, meat, vegetables, root crops, fruits) or food for animals increases the risk of Yersinia being accumulated and infecting humans and animals. Presently, the epizootological-epidemiological feature of the causative agents of these diseases is the change in the populations of circulating Y. enterocolitica - in particular, the decrease of the share of serotypes that used to be dominating in the past decades and the rise of new sero- and biotypes that were previously considered non-pathogenic. Given the various clinical manifestations of intestinal yersiniosis, its ability to affect numerous organs and systems as well as its ability to act "in disguise" as various other diseases, the diagnostics of this disease is complicated and requires much effort.*

Taking into account the high potential danger of Yersinia bacteria to humans due to the contamination of food products of animal and plant origin, the laboratory diagnostics of Yersinia remains a highly relevant issue.

Key words: bacterial zoonoses, yersiniosis, laboratory diagnostics, bacteriosis, zoonosis.

The genus *Yersinia* belongs to the family *Yersiniaceae* comprising 17 types of bacteria: *Yersinia perstis, Y. pseudotuberculosis, Y. enterocolitica, Y. aldovae, Y. bercovieri, Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. mollaretii, Y. rohdei, Y. ruckeri, Y. aleksiciae, Y. similis, Y. massiliensis, Y. nurmii, Y. pekkanenii, Y. entomophaga*. Out of 17 Gram-negative bacteria of the genus *Yersinia,* only three types are harmful to humans and animals: *Y. enterocolitica*, *Y. Pseudotuberculosis,* and

Y. pestis. Y. pseudotuberculosis separated from *Y. enterocolitica* 41 to 186 million years ago, whereas *Y. pestis* separated from *Y. pseudotuberculosis* over the last 1500 to 20000 years, which means that these two types have a higher genetical relatedness. It comes along with the fact that both *Y. pseudotuberculosis* and *Y. enterocolitica* cause intestinal toxic infections, though their DNA-level relatedness is much lower compared to the relation between *Y. pestis* and *Y. pseudotuberculosis* (Abreu-Goodger, and Merino, 2005).

The genus *Yersinia* constitutes Gram-negative bipolar coccobacilli. As representatives of *Enterobacteriaceae*, they feature fermentative metabolism. *Y. pestis* produces antiphagocytic slime. When isolated, the bacterium is in motion, but it becomes non-motile, once it gets into the organism of a mammal. Presently, 21 serotypes of *Y. pseudotuberculosis* are known based on the O antigen. *Yersinia pestis* are also known to stem from *Yersinia pseudotuberculosis* of O1b serotype as a result of intragenomic transformations and deletions. Further selection with recombinant forms staying in the organisms of various eukaryotes resulted in the development of an utterly contagious and virulent pathogen. Outside warm-blooded macroorganisms, however, some of its features exposed to various factors often become subject to reversible and irreversible changes that bring the *Y. pestis* and *Y. pseudotuberculosis* phenotypes closer to each other. Still, these types need to be clearly differentiated, which is relevant for the nidus of infection of the mentioned microorganisms. The range of differential specifications referred to in the official guidelines is rather limited and mostly contains the data of microbiological and immunologic tests. Possible individual strain deviations from the classic phenotype caused by mutations can affect the reliability of the differentiation and requires the expansion of the range of the related tests involving the most reliable ones, which also include the ones serving to detect the type-related specific features of the genomic structure (; Adeolu, et al., 2016; Wade, 2019).

It shall be noted that both *Y. pestis* and *Y. pseudotuberculosis* use an absolutely different transmission mechanism; their range of the virulence factors results in varying clinical manifestations of the disease.

Yersinia pestis is a Gram-negative bacterium representing the family of enterobacteria. It causes bubonic plague as well as can cause pneumonic plague and septicemic plague. All three diseases account for the high morbidity in the most tragic epidemics in human history, including the Plague of Justinian (541-542), Black Death (1347—1353), and the third pandemics with *Y. pseudotuberculosis* spreding worldwide (1894–1921). *Yersinia pseudotuberculosis* causes pseudotuberculosis – an acute infectious zoonotic disease coming along with a fever and intoxication as well as affecting the small intestine, the lever and, in many cases, featuring the scarlet fever rash (Ushkalov, 2023). The main contagion mechanism is alimentary (Achtman, et al., 2004).

Yersinia pseudotuberculosis can be hosted by animals like dogs, cats, cattle, horses, rabbits, deer, turkeys, ducks, etc. It can also be found in soil, plants, insects, and amoebas in the natural environment. *Y. pseudotuberculosis* normally occurs following the consumption of contaminated food or water, which results in the bacterium colonizing the gastrointestinal tract. (Lambrecht, et al., 2013; Ushkalov, et al., 2020).

Yersinia enterocolitica causes yersiniosis of the gastrointestinal tract. It gets into the gastrointestinal tract and affects the bottom sections of the small intestine, mesenteric lymphatic knots, and, sometimes, the appendix. The pathogen releases an endotoxin that is spread hematogenously. The infection is spread in the entire organism along with the development of bacteremia. The disease can lead to a number of complications caused to a significant extent by the development of the infection allergy (Fredriksson-Ahomaa, 2023).

Yersinia ruckeri belongs to the family *Enterobacteriaceae* and causes enteric redmouth disease or yersiniosis, general septicemia affecting mostly salmon fish. Since

its first description in Hagerman Valley in Idaho in the 1950s, *Y. ruckeri* has been found to be widely spread in many species of salmon fish in Northern America. A short while ago the disease was reported and the bacterium was found in host fish worldwide, including Europe, Northern and South America, Australia, New Zealand, and South Africa. The *Y. ruckeri* infection leads to bacterial septicemia without any explicitly visible signs. In most cases, however, it is manifested through exophthalmos and retinal hemorrhage in infected fish. Enteric redmouth disease was manifested through a clearly visible purpura in the mouth and throat of rainbow trout *Oncorhynchus mykiss* (Evenhuis, et al., 2009).

The purpose of our research was to describe the biologic specifications of pathogenic *Yersinia* and to analyse the methods of laboratory diagnostics of yersiniosis.

Materials and methods. The following regulations of Ukraine and international regulations were analysed: ISO 10273:2003 “Microbiology of food and animal feeding stuffs - Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica”; Commission regulation (EC) No 2073/2005 “On microbiological criteria for foodstuffs” of 15 November 2005; Council directive 92/46/EEC “Laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products” of 16 June 1992; Federal Law of the Russian Federation “On the quality and safety of food products” of 02 January 2000; Methodological guidelines on the laboratory diagnostics of yersiniosis and detection of its pathogen in meat, milk, and plant-based fodder (3 October 2005); Methodological guidelines on epidemiologic surveillance and prevention of pseudotuberculosis and intestinal yersiniosis (1 March 2005); Sanitary Rules 3.1.094-95 “Prevention and fighting on contagious diseases common for humans and animals - yersiniosis”; Law of Ukraine “On the key principles and requirements concerning the safety and quality of food products” No. 771/97-VR of 26 October 2023; Law of Ukraine “On the safety and quality of food products” of 23 November 1997; Law of Ukraine “On steps to ensure the sanitary and epidemiologic safety of the population” of 24 February 1994; Methodological recommendations of 21 December 2012 on the laboratory diagnostics of intestinal yersiniosis in animals, detection of *Yersinia enterocolitica* in food products, fodder, and in the environment; National standard of Ukraine “Microbiology of food products and fodder. Horizontal method of detection of conditioned pathogenic *Yersinia enterocolitica*” (DSTU ISO 10273:2007) approved as per the Order of the State Committee for Technical Regulation and Consumer Policy of Ukraine of 01 January 2009; Instructions and methodological guidelines on the diagnostics, treatment and prevention of plague of 14 September 1976.

Results and discussion. Intestinal yersiniosis and pseudotuberculosis have been drawing more attention over the last years, which is due to the spread of these diseases and the environmemtasl relevance of their pathogens. Until recently, both intestinal yersiniosis and pseudotuberculosis were considered as rare diseases and remained outside the spotlight of specialists. However, the situation started changing in the first half of the 20th century, which served as a proof for the ubiquity of their pathogens. The pathogen of intestinal yersiniosis was first reported in the USA where approximately 15 strains of the bacteria were detected in humans in the period from 1923 to 1957. However, they were originally classified as atypical variants of the pseudotuberculosis microbe and were not acknowledged as the cause of the disease (Mollaret, 1995).

Presently, this disease is reported in all EU states and ranks as the third most common infection after campylobacteriosis and salmonellosis (table 1).

In 2020, campylobacteriosis was the most common infection, the status that it had had since. It accounted for more than 60% of all reported infection cases in 2020. It is followed by other bacterial diseases including salmonellosis, yersiniosis, and Shiga toxin-producing *E. coli* (STEC infections). The severity of these disease was subject to descriptive analysis based on the number of hospitalisations and the outcomes of the

registered infection cases. According to table 1, yersiniosis ranks third after campylobacteriosis and salmonellosis, which means that its pathogen ranks high among the pathogens of bacterial zoonotic dieseases and poses a threat for humans as well.

Yersiniosis was first officially reported on the territory of Ukraine in 1986. As per the reference literature, intestinal yersiniosis was found in all regions of the country. Presently, the territory of Ukraine can be divided in three zones: the first one has a low occurrence rate (0.01 to 0.11% of cases per 100,000 residents) and includes Volyn, Sumy, Mykolaiv, Ternopil, Khmelnytskyi, Chernivtsi, Kirovohrad, Cherkasy, Poltava, Zhytomyr, Zakarpattia, Ivano-Frankivsk, Lviv, Chernihiv regions; the infection rate in the second zone can achieve 0.58% of cases per 100,000 residents – this zone includes Vinnytsia, Dnipropetrovsk, Donetsk, Kyiv, Luhansk, Rivne, Kherson regions as well as the city of Kyiv; the third zone with the highest infection rate (starting from 0.59% of cases per 100,000 residents) includes Kharkiv, Zaporizhia, and Odessa regions (Truba, 2021).

Morphology of *Yersinia spp*. Gram-negative species. Strains of *Y. pestis* exhibit bipolarly staining (Leffler or Romanovsky-Gimse), they are non-motile and capsular. *Yersinia* can also become bipolarly stained when being cultivated in liquids or when extracted from the organisms of other species. They do not exhibit spores and capsules. *Y. pseudotuberculosis* posess 3-5 and *Y. enterocolitica* – an unlimited number of peritrichial flagellums. In smears from broth cultures, the cells can be present aced in short chains (Babkin, 1990).

Culture specifications: facultative anaerobic organisms. Optimal cultivation temperature - 28 – 29^{0}C, can grow at 2 to 40 ^{0}C. Motile (except for *Y. pestis*) at 22 – 28 ^{0}C, non-motile at 37 ^{0}C; *Y. enterocolitica* is more explicitly motile. In liquid media, *Y. pestis* cultures produce flake-formed precipitation and a thin surface film; on dense media they form round colonies with the bulged loose corn-structured core and flat rims formed as a fragile web structure (Diepold and Armitage, 2015).

At lower temperatures (4 – 8 ^{0}C), the cultures grow at a slower pace as smooth structures. At temperatures above 28 ^{0}C *Y. pseudotuberculosis* dissociates into the rough (R) form demonstrating the polymorphism of colonies by size, form, and colour. The dissociation of *Y. enterocolitica* under these conditions has a low level. When extracted from the organism of an infected human or from animals, the Yersinia of the culture *Y. pseudotuberculosis* and *Y. enterocolitica* are present in the S-form. However, newly extracted cultures of *Y. pseudotuberculosis* can sometimes be identified that are present in the R-form or in transitional (SR or RS) forms. Such forms can be often found when cultivating *Y. pseudotuberculosis* on friendly growth media, especially at temperatures above 28 ^{0}C. They grow on standard agar as larger randomly formed colonies with uneven rims. When placed in a broth, they produce turbidity and precipitation or precipitation and a surface loose film with a transparent liquid above the precipitation. The generation of rough forms usually comes along with the change or even loss of the S-antigen. Such cultures often demonstrate non-specific spontaneous agglutination. Yersinia cultures grow well on deoxycholate agar as well as on the MacConkey, Endo, Serov media and with bromothymol blue (BTB). The Ploskirev medium and bismuth sulfite agar act as inhibitors for Yersinia, which is why its colonies need more time for incubation (at least 5 days). Yersinia bacteria can grow on poor media (low acid agar, phosphate-buffered saline, and others). They produce smooth turbidity in liquid media (MPB, Hottinger Broth). When placed on dense media (MPA, Hottinger Agar, Endo), Yersinia bacteria produce round semi-transparent smooth colonies sized 0.1 to 1.0 mm in 24 – 48 hours (37 ^{0}C). Placed on the dence Serov medium, *Y. pseudotuberculosis* grow as matt colonies with uneven rims and the bulged core sized 0.2 to 2.0 mm. When taken with a loop, they move on the agar surface. *Y. enterocolitica* colonies of the O3 serovar have a similar morphology on this medium. Colonies of Yersinias of other serovars normally have a polymorphic structure and differ by size, form, and density. When placed on the BTS

medium, Yersinias build round smooth colonies within 24 – 48 hours that are blue and sized 0.1 – 0.2 mm (in 24 hours) and up to 2.0 mm (in 48 hours). These colonies have scalloped rims and the bulged core on the dark-blue background of the medium. A light blue rim can sometimes be seen, especially in the case with *Y. pseudotuberculosis*. When taken with a loop, the colonies move on the agar surface (Babkin, 1990).

Table 1

Absolute numbers of confirmed zoonosis cases among humans in 2020 – 2019 and the absolute and relative (%) difference in reported zoonosis cases per 100,000 residents in EU countries in 2020 (European Food Safety Authority Report, 2020).

Zoonosis	EU level [a]	Cases (number)		Reported cases		
		2020	Difference in 2020–2019	2020	Difference in 2020–2019	
					Absolute difference (%)	Relative difference (%)
Campillobacteriosis	EU	120,946	–99,693	40.3	–20.3	–33.4
	EU -27		–40,975		–13.7	–25.4
Salmonellosis	EU	52,702	–35,206	13.7	–5.8	–29.7
	EU -27		–25,488		–6.7	–32.8
Yersiniosis	EU	5,668	–1,299	1.8	0.10	6.0
	EU -27		–1,136		–0.27	–13.4
STEC infections	EU	4,446	–3,355	1.5	–0.43	–22.4
	EU -27		–1,768		–0.33	–18.2
Listeriosis	EU	1,876	–745	0.42	–0.03	–7.1
	EU -27		–591		–0.07	–14.2
Tularemia	EU	641	–639	0.15	–0.11	–42.5
	EU -27		–639		–0.15	–50.0
Q fever	EU	523	–428	0.12	–0.07	–36.7
	EU -27		–419		–0.10	–44.6
Echinococcosis	EU	488	–278	0.14	–0.03	–16.2
	EU -27		–275		–0.06	–28.4
West Nile fever [b]	EU	322	–68	0.07	–0.01	–12.9
	EU -27		–68		–0.02	–24.4
Brucellosis	EU	128	–182	0.03	–0.03	–52.6
	EU -27		–158		–0.04	–55.3
Trichinosis	EU	117	20	0.03	0.01	39.1
	EU -27		20		< 0.01	20.4
Tuberculosis	EU	88	64	0.02	–0.01	–32.0
	EU -27		29		–0.01	–24.9

Notes: (a): in 2019, data were collected for the United Kingdom as well, since it was an EU member. Since 1 February 2020, the UK has been a non-EU state. To calculate the difference in 2020/2019, the UK data for 2019 were included in the EU-wide calculation, while the ECDC did not collect the UK data in 2020 (“EU-27”).
(b): for West Nile fever, the total number of cases was considered (including alleged and confirmed ones).

Y. pestis do not produce carbohydrates and gas alcohols through fermentation; they ferment glucose, mannitol, mannose, xylose, arabinose (table 2). Most strains do not ferment sucrose and lactose, though sucrose- and lactose-positive strains can sometimes occur; they do not recover nitrates into nitrites, nor do they possess ureatic activity and produce indole.

When place on the Hugh-Leifson medium, the *Y. pseudotuberculosis* and *Y. enterocolitica* cultures ferment and oxidize glucose. They do not possess cytochrome c oxidase, do not produce hydrogen sulfide (on iron-containing media), do not liquefy gelatine. They do not possess phenylalanine deaminase, lysine decarboxylase, and arginine dihydrolase; nor do they dispose of Simmons citrate.

Y. pseudotuberculosis ferments the following substances along with acid-building: glucose, galactose, L-arabinose, levulose, mannose, xylose, rhamnose, maltose, trehalose, mannitol, salicin. It reduces nitrates, methylene blue; catalase, urease, and methylene red reactions are positive; Voges-Proskauer is negative (at 26 – 28 ^{0}C and 37 ^{0}C); it does not ferment lactose, sucrose, raffinose, cellobiose, dulcite, inositol, sorbit, inulin, D-arabinose.

Y. enterocolitica ferments the following substances along with acid-building: glucose, sucrose, mannitol, maltose, sorbit, trehalose; it possesses urease and ornithine decarboxylase; positive reaction with methylene red, Voges-Proskauer is positive at 26 – 28 ^{0}C and negative at 37 ^{0}C; it does not ferment lactose, rhamnose, raffinose (lactose- and raffinose-positive variants are available, mostly referring to serovars 05 and 07, 08). Based on biochemical properties (fermentation of xylose, salicin or aesculine, trehalose; production of indole), there are 5 biovars of *Y. enterocolitica* (tables 2 – 5).

The closely related types include *Y. frederiksenii, Y. intermedia, Y. kristensenii. Y. pseudotuberculosis* differ from *Y. enterocolitica* in terms of sucrose and rhamnose. Extended biochemical tests – fermentation of xylose, sorbit, salicin, and indole – allow to determin the biological type of *Y. enterocolitica*. Tables 2 – 5 list the main biochemical tests allowing to differentiate Yersinia from other similar microorganisms as well as to identify the species and biological type of *Y. enterocolitica.*

Y. pestis has a group of protein-polysaccharide and lipo-polysaccharide antigens: thermally stable somatic O-antigen and thermally unstable capsulary antigen, including V- and W-antigens to which the virulence of the bacteria is related. Other factors contributing to the high virulence of *Y. pestis* include plasmocoagulase, fibrinolysin, endotoxin, capsule. *Y. pestis* demonstrates a high level of cytotoxic, antiphagocytic, and adhesive activity encoded by plasmid genes. The structure of antigens is complicated and includes 30 known antigens; the cell structures have antigen properties and produce proteins; most important for diagnostics are the O-antigen – LPS of the outer membrane (has common determinants with enterobacteria), the phylum-specific capsular antigen, and the “mouse” toxin (Sören, et al., 2004).

Y. pseudotuberculosis has a flagellum (H) and two somatic (O) antigens – S i R, virulence antigens – V, W, as well as other antigens in the cell’s outer membrance and cytoplasm, with many of them only identifiable at 37 ^{0}C in vivo. The H-antigen is thermally unstable and has no diagnostic relevance. 8 serovars of *Y. pseudotuberculosis* can be identified based on the S-antigen. Most strains extracted from humans, animals, and the environment belong to serovar I (60-90%), some of them belong to serovar III (8-32%), the fewest belong to serovars II, IV, V (2 – 8%). O-antigens have a varying structure and consist of several components that determine antigen links both between the strains of different serovars within the phylum and with other enterobacteria. The R-antigen is common with the R-antigen of *Y. pestis*. Common antigens have also been identified with the salmonellas of the B, D groups and shigellas. The antigen links of *Y. pseudotuberculosis* and most serovars of *Y. enterocolitica* are weak and can only be detected using through immunodiffusion. Much more significant antigen links can be

observed between *Y. pseudotuberculosis* of serovar I with the cultures *Y. enterocolitica* of serovars 08, 018, 021 (table 6) (Sören, et al., 2004).

Yersinia enterocolitica have flagellum (H) and somatic (O) antigens. They also have virulence antigens related to the antigens of *Y. pseudotuberculosis*, which is due to the fact that the cells of both causative agents contain virulence plasmid with the molecular mass equal to 40-48 megadalton. The H-antigens have a varying structure, they are destroyed when boiled, demonstrate activity in the agglutination reaction at the cell cultivation temperature equal to 26 -28 °C. Most strains extracted from humans, animals, and the environment on different territories belong to serovar 03 (30-60%), some of them belong to serovars 05 and 27 (10-15%), 07 and 8 (5 -10%), and 09 (1-30%); the fewest belong to other serovars. Some strains cannot be classified based on available type-specific serums.

The O- and H-antigens contain specifc and cross-reacting antigens determining species-specific and common antigen links for enterobacteria. They are available as part of the species and present in PA in all serovars in the titers up to 1: 100 and higher. The most clearly identifiable are antigen links in the Yersinias of serovar 09 and brucellas. *Y. enterocolitica* of serovars 08, 014, 018, 021 have common antigens with the salmonellas of O-serogroups 27; 43; 55 i 66. 1,2 and cross reactions between these species can make it harder to gain laboratory analysis results (Fukushima, et al., 2001).

The outer membrane of pathogenic Yersinias contain proteins (outer membrane proteins, OMP) of the virulence plasmid pYV 42-48 MDa. B3M are factors contributing to the protection of the causative agent agains the macroorganism's immune system (Kudryashev, et al., 2015).

Yersinia pestis has a group of protein-polysaccharid and lipo-polysaccharid antigens: thermally stable somatic O-antigen and thermally unstable capsular antigens, including V- and W-antigens related to the virulence of the bacteria. Other factors contributing to the high virulence level of *Y. pestis* include plasmocoagulase, fibrinolysin, endotoxin, capsula bacteriana. *Y. pestis* demonstrates a high level of cytotoxic, antiphagocytic, and adhesive activity encoded with plasmid genes. The antigen structure is rather complicated, with 30 known antigens; cell structures have antigen properties and produce proteins; most important for diagnostics are the O-antigen – LPS of the outer membrane (has common determinants with enterobacteria), the phylum-specific capsular antigen, and the "mouse" toxin (Sören, et al., 2004).

Table 2

Key biochemical specifications of the bacteria genus *Yersinia* compared with some other bacteria of the family *Enterobacteriaceae*

Test/substrate	*Y.pestis*	*Y. pseudotuberculo*	*Y. enterocolitica* Biotypes						Y. frederiksenii	Y. intermedia	Y. kristensenii	Y. ruckeri	Y. aldovae	Y. rohdei	Y. mollaretti	Y. bercovieri	Hafnia	Proteus
			I															
			IA	IB	II	III	IV	V										
Maltose		+	+	+	+	+	+	+	+	+	+	+	-	-	+/-	+	+	+*
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-**
Sucrose	-	-	+	+	+	+	+	-	+	+	-	-	-/+	+	+	+	-	B
Rhamnose	+	+	-	-	-	-	-	-	(+)	(+)	-	-	-	-	-	-	+	-
Sorbitol	-	-	+	+	+	+	+	-	+	+	+	+/-	+	+	+	+	-	-
Urea	-	+	+	+	+	+	+	+	+	+	+	-	+	+/-	+	+	-	+
Voges–Proskauer 22-24 ^{0}C	-	-	+	+	+	+	+	+	+	+	-	-\+	+	-	-	-	+\-	-
Motility 22-24 ^{0}C	-	+	+	+	+	+	+	+	+	+	+	+\-	+	+	+	+	+	+
Indole	-	-	+	+	+	-	-	-	+	+	+\-	-	-	-	-	-	-	B
Xylose		+	+	+	+	+	-	-	+	+	+	-	+/-	+	+/-	+	+	+***
Salicin (24 hours)		-	+	-	-	-	-	-	+	+	-	-	-	-	-\+	-\+	-	B
Trehalose		+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	B
Simmons citrate	-	-	-	-	-	-	-	-	-/+	-	-	(+)	-	-	-	-	+	B
Phenylalanine		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Ornithine decarboxylase		-	+	+	+	+	+	-	+	+	+	+	+	+\-	+	+	-	- *
Aesculin hydrolysis (24		+	+\-	-	-	-	-	-	+	+	-	-	+	-	-\+	+\-		

Table 3

Key biochemical specifications of the bacteria genus *Yersinia*

Test or substrate		*Y. pestis*	*Y. pseudotuberculosis*	*Y. enterocolitica* Biotypes I 1A	I 1B	II	III	IV	V	*Y. frederiksenii*	*Y. intermedia*	*Y. kristensenii*	*Y. ruckeri*	*Y. aldovae*	*Y. rohdei*	*Y. mollaretti*	*Y. bercovieri*	*Y. aleksiciae*	*Y. similis*
1		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Urea hydrolysis		-	+	+	+	+	+	+	+	[+]	[+]	[+]	-	[+]	B	[-]	B	+	+
Indole production		-	-	+	+	B	-	-	-	+	+	B	-	-	-		-	+	-
Motility (22 ± 2) °C	(22 ± 2) °C	-	+	+	+	+	+	+	+	+	+	+	B	+	+	+	+	+	+
	(37 ± 1) °C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Voges–Proskauer	(26 ± 2) °C	-	-	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-
	(37 ± 1) °C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenylalanine deaminase		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ornithine decarboxylase		-	-	+	+	+	+	+	B	+	+	+	+	B	[-]	[+]	[+]	+	-
Lysine decarboxylase		-	-	-	-	-	-	-	-	-	-	-	B	-	-	-	-	+	-
Lipase (Twin-80)		-	-	+	+	-	-	-	-	B	B	B	B	-	-	-	-	-	-
Simmons citrate		-	-	-	-	-	-	-	-	B	+	-	+	+	-	-	-	-	-
Aesculin hydrolysis		B	+	[+]	-	-	-	-	-	[+]	+	-	-	-	-	-	[-]	-	+
Acid production from:																			
D-ксилози		+	+	+	+	+	+	-	B	+	+	[+]	-	B	B	B	+	+	+
Maltose		[+]	+	+	+	+	+	+	+	+	+	+	+	-	-	B	+	+	+
Melibiose		[-]	B	-	-	-	-	-	-	-	[+]	-	-	-	B	-	-	-	-
L-rhamnose		-	B	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+
Raffinose		-	[-]	-	-	-	-	-	-	B	B	-	-	-	B	-	-	-	-
Salicin		B	[-]	+	-	-	-	-	-	+	+	[-]	-	-	-	[-]	[-]	-	-
Sucrose		-	-	+	+	+	+	+	B	+	+	-	-	[-]	+	+	+	-	-
D-sorbitol		B	-	+	+	+	+	+	-	+	+	+	B	B	+	+	+	+	-
Trehalose		+	+	+	+	+	+	+	-	+	+	+	+	[+]	+	+	+	+	+

Notes: “+” – positive reaction among 90% and more strains; “[+]” – positive reaction among 76 - 89 % of the strains; “B” – positive reaction among 26 - 75% of the strains: “[-]” – positive reaction among 11 -25% of the strains: “-“ – negative reaction among 90% and more strains

Table 4

Key differential features of *Y. enterocolitica* and *Y. pseudotuberculosis* (at 37 ^{0}C)

TESTS	*Y. enterocolitica*	*Y. pseudotuberculosis*
Sucrose	+	-
Rhamnose	-	P
Sorbit	+	-
Ornithine decarboxylase	+	-
Indole	P	-
Voges- 22 - 25 °C	+	-
Proskauer 37 - 38 °C	-	-

Notes: “+” – positive reaction; “-“ – negative reaction; “P” – different values for different strains

Table 5

Differentiation of enteropathogenic Yersinias

Name	*Y. enterocolitica*		*Y. pseudotuberculosis*
TESTS	RESULTS		
Oxydase	-		-
Glucose	K		K
Simmons citrate	-		-
Urea	+		+
Sucrose	+		-
Salicin	-		-/+
Sorbit	+		-
Rhamnose	-		+
Glycerin	+		+/-
Maltose	+		+
TYPE	*Y. enterocolitica*		
Biovar	II	III	IV
Indole	+	-	-
Xylose	+	+	+

Note: “+” – positive reaction; “-“ – negative reaction; “K” - acid

Y. pseudotuberculosis has a flagellum (H) and two somatic (O) antigens – S i R, virulence antigens – V, W, as well as other antigens in the cell’s outer membrance and cytoplasm, with many of them only identifiable at 37 ^{0}C in vivo. The H-antigen is thermally unstable and has no diagnostic relevance. 8 serovars of *Y. pseudotuberculosis* can be identified based on the S-antigen. Most strains extracted from humans, animals, and the environment belong to serovar I (60-90%), some of them belong to serovar III (8-32%), the fewest belong to serovars II, IV, V (2 – 8%). O-antigens have a varying structure and consist of several components that determine antigen links both between the strains of different serovars within the phylum and with other enterobacteria. The R-antigen is common with the R-antigen of *Y. pestis*. Common antigens have also been identified with the salmonellas of the B, D groups and shigellas. The antigen links of *Y. pseudotuberculosis* and most serovars of *Y. enterocolitica* are weak and can only be detected using through immunodiffusion. Much more significant antigen links can be observed between *Y. pseudotuberculosis* of serovar I with the cultures *Y. enterocolitica* of serovars 08, 018, 021 (table 6).

Yersinia enterocolitica have flagellum (H) and somatic (O) antigens. They also have virulence antigens related to the antigens of *Y. pseudotuberculosis*, which is due to the fact that the cells of both causative agents contain virulence plasmid with the molecular mass equal to 40-48 megadalton. The H-antigens have a varying structure,

they are destroyed when boiled, demonstrate activity in the agglutination reaction at the cell cultivation temperature equal to 26 -28 °C. Most strains extracted from humans, animals, and the environment on different territories belong to serovar 03 (30-60%), some of them belong to serovars 05 and 27 (10-15%), 07 and 8 (5 -10%), and 09 (1-30%); the fewest belong to other serovars. Some strains cannot be classified based on available type-specific serums. The O- and H-antigens contain specifc and cross-reacting antigens determining species-specific and common antigen links for enterobacteria. They are available as part of the species and present in PA in all serovars in the titers up to 1: 100 and higher. The most clearly identifiable are antigen links in the Yersinias of serovar 09 and brucellas. *Y. enterocolitica* of serovars 08, 014, 018, 021 have common antigens with the salmonellas of O-serogroups 27; 43; 55 i 66. 1,2 and cross reactions between these species can make it harder to gain laboratory analysis results (Fukushima, et al., 2001).

Table 6

Serotypes of *Y. pseudotuberculosis* and *Y. enterocolitica*

Y. pseudotuberculosis	*Y. enterocolitica*
O:1a, O:1в, O:1c, O:2a, O:2в, O:2c, O:3, O:4a, O:4в, O:5a, O:5в, O:6, O:7, O:8, O:9, O:10, O:11, O:12, O:13, O:14, O:15	O:8; O:4; O:13a; O:18; O:20; O:21; O:9; O:5,27; O:1,23; O:5,27; O:3; O:2,3 O:5; O:6,30; O:6.31;O:7,8; O:10; O:13,7; O:14; O:16; O:19,8;O:22; O:36; O:41,42; O:41,43; O:46; O:63;O:64; O:65; O:66; O:72

The outer membrane of pathogenic Yersinias has been found to contain proteins (outer membrane proteins, OMP) encoded with both chromosome genes and genes located on the virulence plasmid pYV 42-48 MDa. The OMPs contribute to the protection of the causative agent against the macroorganism's immune system (Kudryashev, Diepold, Amstutz, 2015).

Pathogenicity factors of *Y. pestis*:

- adhesive - dusts, outer membrane structures;
- invasive – fibrinolysin, neuraminidase, pesticin, aminopeptidases;
- antiphagocytic - capsula, pH6-antigen, V- and W-antigens, superoxide dismutase;
- toxins - endotoxin (released when a cell dies), "mouse" toxin (protein-based, typical AB-structure; blocks the functions of the lever and heart cell mitochondria, causes blood clots).

Y. pseudotuberculosis and some of the *Y. enterocolitica* representatives share significant common features, including their invasive nature and ability to intracellular replication, which is in line with the spread of the infection process. Most of the *Y. enterocolitica* strains extracted from infected hosts feature adhesion, buildup of colonies on the surface of the intestinal epithelium, and enterotoxigenity along with the production of a high amount of thermally stable enterotoxin. Pathogenic *Y. pseudotuberculosis* and *Y. enterocolitica* have a wide range of pathogenicity factors determined by chromosome and plasmid genes. The ability of *Y. pseudotuberculosis* to adhesion and invasion into the cells of the incestinal epithelium depends on the expression of the chromosome inv-gene that encodes the protein of the outer membrane of the invasion with the molecular mass equal to 108 kDa. The systemic dissemination of the microbe in the host's organism is based on a system known as yersiniabactin that absorbs iron ions. The chromosome segment containing yersiniabactin genes is known as the high pathogenicity island (HPI). HPIs have been found to contain strains cirtulating in Europe, Australia, North America. They normally cause the gastrointestinal manifestations of the pseudotuberculosis infection (Tan, et al., 2016).

Y. pseudotuberculosis synthesizes *Yersinia pseudotuberculosis*-derived mitogen (YPM) responsible for the activation of polyclonal T-lymphocytes and hyperproduction of inflammatory cytokines. 98% of strains extracted from infected hosts in the Russian Far East, Japan, Korea contain a superantigen causing the systemic infection of organs and tissues (Pease, 1979).

The pathogenic *Y. enterocolitica* also have OMPs contributing to their adhesive and invasive properties. One of them is an invasion with the molecular mass equal to 92 kDa that is encoded with the chromosome inv-gene. Another chromosome ail-gene encodes the adhesive/invasive protein Ail 17 kDa. The HPI genes are not found in the non-pathogenic *Y. enterocolitica* of the biotype 1A and in the low-pathogenic strains of the biotypes 2-5, but they can be detected in all highly pathogenic strains of the biotype 1B. The enterotoxigenicity of the Y. enterocolitica of the biotypes 1B and 2-4 is related to the expression of the chromosome ystA-gene. The non-pathogenic representatives of the biotype 1A have been found to contain the ystB-gene. The yersinia virulence plasmid pYV 42-48 MDa is present in the strains *Y. pseudotuberculosis* and *Y. enterocolitica* that belong to the serotypes O:3 (biotype 4); O:5,27 (biotype 2 and 3); O:8 (biotype 1B); O:9 (biotype 2) and are the most widespread causative agents (table 7) (Pease, 1979).

Table 7

Bio- and serotypes *Y. enterocolitica* and their pathogenicity links

Pathogenic properties	Biotype	Serotype
Available	1B 2 3 4 5	O:8; O:4; O:13a; O:18; O:20; O:21; O:9; O:5,27; O:1,2,3; O:5,27; O:3; O:2,3
Non available	1A	O:5; O:6,30; O:6,31; O:7,8; O:10; O:13,7; O:14; O:16; O:18; O:19,8; O:22; O:36; O:41,42; O:41,43; O:46; O:63; O:64; O:65; O:66; O:72

The virulence plasmid encodes the outer membrane protein Yada (which is autotransporter adhesin Yersinia adhesin) as well as the outer membrane proteins Yops and their type III secretion machine Ysc (Yop secretion), which constitute a set of factors contributing to the neutralization of the macroorganism's immune response. The type III secretion machine Ysc enables the Yersinia to inject synthesized proteins into the cytoplasm of the target cell when in close contact with the cell without penetrating it; it includes at least 28 proteins. The plasmid with the molecular mass equal to 82 MDa has only been found in *Y. pseudotuberculosis* of serotype 1. It has been found that strains having the plasmid pVM 82 MDa leads to the more severe development of pseudotuberculosis (Raducheva, et al., 1989).

Table 8 contains summarized data on the key virulence factors of the bacteria *Y. pseudotuberculosis, Y. pestis, Y. enterocolitica*. The table is based on research works and demonstrates that *Y. pseudotuberculosis, Y. pestis, Y. enterocolitica* have relatedness in terms of their properties, in this case – in terms of their virulence factors. For comparison, the table also contains the virulence factors of *Y. ruckeri*, which causes the enteric redmouth disease in various species of salmonids.

The laboratory diagnostics of Yersiniosis comprises a number of components, includes 12 stages (table 9), and lasts 14 days. The Yersiniosis diagnostics includes bacteriologic, serological, immune, and genetic methods.

Table 8

Summarized data on the key virulence factors of *Yersinia* (Ushkalov, 2023).

Virulence factors	Species of *Yersinia*			
	Y. pseudotuberculosis	*Y. pestis*	*Y. enterocolitica*	*Y. ruckeri*
T3SS Yops	+	+	+	-
LcrV	+	+	+	-
Invasin	+	+*	+	+
Ail	+	+	+	-
YadA	+	+*	+	-
YadB	+	+	+	-
YadC	+	+	+	-
Pla	+	+	+	-
pH	+	+	+	-
YhlA	-	-	-	+
Azocasein protease	-	-	-	+
Yrp1	-	-	-	+
YraS	-	-	-	+

Note. *- pseudogenes.

Table 9

Key stages of laboratory research (except for *Yersinia pestis*) based on the standard methodology

Laboratory research stages	Required steps
Stage I (preparation of samples, microbiological culture)	Cold enrichment of the substance: the culture injected in a peptone-potassium medium, in a buffer saline solution or in 1% peptone water (accumulation medium) is placed in a cool storage camera (6 ± 2) °C and kept until the first positive culture appear (days 2-3, 5-7 or 10-15) (Koz'ko, et al., 2019).
Stage II (days 2-3)	Culture on dense differential diagnostical mediums (Holovko, 2007).
Stage III (days 4-5)	Selection of colonies typical for *Y. pseudotuberculosis* and *Y. enterocolitica* to place them on a differential medium with urea (Olkenytsky medium, USS or Russel medium I and II). The same colony is placed on canted agar sticks (nutrient agar or Hottinger agar) to accumulate bacterial mass. Incubation takes place on the Olkenytsky, USS, Russel medium I at (37 ± 1)°C, on nutrient or Hottinger agar, on the Russel medium II at (26 ± 2) °C. (Volkov, and Kuprienko, 2007).
Stage IV (days 5-6)	Identification and differentiation of urease-positive cultures from a test tube (or a cup) on nutrient agar that were sown at stage II of the research (day 2-3 of the cold enrichment): · microscopy of the Gram-stained smear; · cytochrome oxidase test; · fermental activity on the Giss medium (26 ± 2) °C: mannitol, sucrose, rhamnose, raffinose, sorbitol, melibiose, salicin, xylose (Dovidnik snfekciynuh zahvoruvan`, 1998)
Stage V (days 5-7)	Culture from the accumulation media (days 5-7 of the cold enrichment) on solid differential and diagnostical media after the preliminary alkali-based processing.

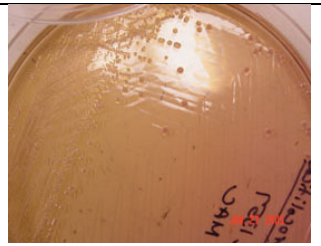
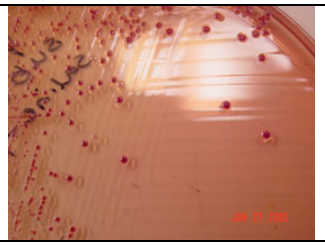
	Registration of the results of the biochemical identification and differentiation of *Y. pseudotuberculosis and Y. enterocolitica* conducted at stage IV of the research (days 2-3 of the cold enrichment), identification of the biotype of *Y. enterocolitica* (Golubovska, et al., 2022). Agglutination reaction with virulent Yersinias on glass with serum. Final positive response.
Stage VI (days 7-8)	Review of the cultures on differential diagnostical media from stage V of the research (days 5-7 of the cold enrichment) Selection of the colonies typical for *Y. pseudotuberculosis* and *Y. enterocolitica* for a differential medium with urea (Olkenytsky medium, USS or Russel medium I and II) and nutrient (Hottinger) agar (Simulik, 2013).
Stage VII (days 8 - 10)	Identification and differentiation of the urea-positive cultures from stage VI of the research (days 5-7 of the cold enrichment) based on the tests from stage IV of the research.
Stage VIII (days 9-11)	Registration of the results of the biochemical identification and differentiation of *Y. pseudotuberculosis* and *Y. enterocolitica* conducted at stage VII of the research (days 5-7 of the cold enrichment); identification of the biotype of Y. enterocolitica. Agglutination reaction with virulent Yersinias on glass with serum (CBI). Final positive response (Skurnik, et al., 1984).
Stage IX (day 10)	Culture from accumulation media on differential and diagnostical media after the alkali-based processing (day 10 of the cold enrichment).
Stage X (day 12)	Review of the cultures on differential and diagnostical media from stage IX of the research (day 10 of the cold enrichment), selection of the colonies typical for *Y. pseudotuberculosis* and *Y. enterocolitica* for a differential medium with urea (Olkenytsky medium, USS or Russel medium I and II) and nutrient (Hottinger) agar (Skurnik, et al., 1984).
Stage XI (day 13)	Identification and differentiation of the urea-positive isolate from stage X of the research (day 10 of the cold enrichment) based on the biochemical tests from stage IV of the research (Golubovska, et al., 2022).
Stage XII (day 14)	Registration of the results of the biochemical identification and differentiation of *Y. pseudotuberculosis* and *Y. enterocolitica* conducted at stage XI of the research (day 10 of the cold enrichment); Final positive response. The extracted culture can be transferred to a specialized laboratory for further identification at any stage of the research.

The FDA bacteriology analytical guide (BAM) contains the recommended laboratory procedure for the microbiological analysis of food products and cosmetics. Table 10 represents the procedure for extracting *Yersinia* from food, water and samples from the environment.

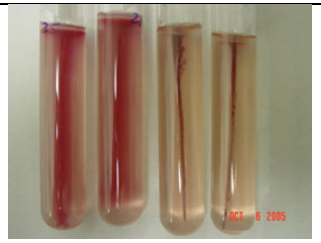
Some chromogenic cultivation media for Yersinias. Culture media including ferment substrates related to thermally resistant, water-soluble, indolile chromophores (14) are now widely used in clinical laboratories. Splitting the substrate with specific ferments in the target microorganism releases chromophore and thus stains growing microbial colonies. Thus, the use of chromogenic agars can make subcultures unnecessary and enable the possible identification of a selected microorganism. This approach cuts the time required for microbial diagnostics, reduces the analytical workload, and helps save costs compared with the traditional methods for the isolation and identification of bacterial causative agents (Renaud, et al., 2013).

Table 10

Simplified procedure for extracting *Yersinia* from food, water, and samples from the environment as per the FDA bacteriology analytical guide (BAM) (Vigant, and Feng, 2017).

Stage of the research	*Required steps*	*Visual results*	*Description*
Enrichment	*Weigh 25 g of the aseptical sample in 225 ml of PSBB. Homogenize for 30 seconds and proceed with incubation at 10°C for 10 days.* On day 10, remove the broth for enrichment from the incubator and stir it thoroughly. Place one full enrichment loop in 0.1 ml of 0.5% KON in the 0.5% saline solution and stir it for 2-3 sec (4). Move one loop to the MacConkey plate and one loop to the CIN plate consecutively. Place additional 0.1 ml for enrichment into 1 ml of the 0.5% saline solution and stir for 5-10 seconds prior to making streaks, as mentioned above. Proceed with the incubation of the cups with agar at 30°C for 1-2 day.	*Not available*	
Extraction of Yersinias	Make a visual check of the CIN plates after 1 day of incubation. Select smaller (1-2 mm in diameter) colonies with the intensively red-stained centre and clear borders encircled with a clear colourless zone with intact borders.	*Not available*	
	Make a visual check of the cups with MacConkey agar after 1-2 days of incubation. Separate red or slime colonies. Pick small (diameter: 1-2 mm) and smooth, colourless or pale pink colonies.		*MacConkey:* lactose-negative colonies, smooth, colourless or pale pink. Diameter: 1-2 mm
	Inoculate each selected colony into a plate with Christensen Urea Agar and a plate with bile esculin agar using an inoculation needle. Proceed with incubation for 48 hours. The isolates that are marked by the presence of alkali and acid, have neither gas nor H_2S and are also urea-positive are to be considered as probable *Yersinia*. Ignore the cultures producing H2S and/or any gas on lysine		*CIN:* dark red centre encircled with a transparent colourless zone. Diameter: 1-2 mm

	iron agar (LIA) or have a negative reaction to urease. Pick typical isolates that cannot hydrolyse (blacken) esculin.			*LIA:* *Y. enterocolitica* (left) = KA – – Salmonella (right) = KK + –
				Christensen Urea Agar: *Y. enterocolitica* - pink (urea-positive) *E. coli* - colourless (urease-negative)
				Y. enterocolitica (except for biotype 1A) esculin-negative (black colour missing). *Ent. faecalis* – esculin-positive (black colour).
Identification	Use the culture on LIA to place a streak culture on one TSAYE cup and proceed with incubation at the room temperature. Use the growth on the TSAYE cup to check the culture's purity as well as to conduct an oxidase test and Gram-staining. From the colonies on the TSAYE seed an agar-based medium containing egg yolk, for instance, anaerobic egg yolk agar (AEY) for the lipase reaction (days 2-5, incubation in aerobic conditions at the room temperature). In addition, inoculate the next biochemical test media and incubate everything at the room temperature for 3 days (except for one motility testing medium and one MR-VP broth that shall be incubated at 35-37°C for 24 hours).			*Motility testing medium with TTC:* Y. ent. motile at 25°C (2 left test tubes) and non-motile at 35°C (2 right test tubes).

	-Basal decarboxylase medium (Falkow) supplemented with 0.5% of lysine, arginine or ornithine; covered with sterile mineral oil - Phenylalanine deaminase agar -Motility testing medium (semi-solid), 22-26°C and 35-37°C -Tryptone broth -Indole test - MR-VP broth, RT for autoagglutination test, followed by the VP test (48 hours); 35-37°C for autoagglutination reaction -Bromocresol purple broth with 0.5% of the following carbohydrates sterilized with a filter: mannitol, sorbit, celobiosa, adonit, inositol, sucrose, rhamnose, raffinose, melibiose, salicin, trehalose, xylose -Simmons citrate agar -Veal broth Use the API 20E or Vitek GNI system for the biochemical identification of *Yersinia*. Follow the manufacturer's manual. These systems are normally reliable for the identification of Yersinia at the genus level, but are in general not reliable enough for identification at the species level (3, 32). Use standard biochemical tests for the species-building and biotyping of probably virulent isolates. The biochemical tests that are essential for the species-building within the genus *Yersinia* include the fermentation of sucrose, rhamnose, raffinose, and melibiose as well as the use of citrate. The biochemical tests that are essential for the biotyping include the fermentation of salicin, xylose, and trehalose along with the VP reaction, lipase, esculinase, β-D-glucose oxidase, and pyrazinamidase - Pyrazinamidase agar (48 hours) - β-D-glucose oxidase test (30°C, 24 hours) - Lipase test. Colonies can demonstrate lipase activity when growing on agar-based media containing egg yolk (like anaerobic agar with egg yolk). The positive reaction is displayed by oily, rainbow-coloured, pearl colonies encircled with a ring of fallout and an external clarity zone.		
Interpretation	Yersinias are oxidase-negative, Gram-negative bacteria. Use tables 1 and 2 to identify the species and biotype of the *Yersinia* isolates. Only *Y. enterocolitica* strains of biotypes 1B, 2, 3, 4, and 5 are presently known to be pathogenic.	-	-

	These biotypes as well as biotype 6 *Y. enterocolitica* and *Y. kristensenii* do not hydrolyze esculin in a fast way (within 24 hours), nor do they ferment salicin. However, *Y. enterocolitica* biotype 6 and *Y. kristensenii* are rather rare; their distinctive feature is the inability to ferment sucrose, they pyrazinamidase-positive. Keep the *Y. enterocolitica* isolates belonging to biotypes 1B, 2, 3, 4, and 5 for further pathogenicity tests.		
Pathogenicity test	Autoagglutination reaction. A test tube containing MR-VP and incubated at the room temperature for 24 hours shall become slightly turbid due to the growth of bacteria. At 35-37°C, MR-VP shall demonstrate agglutination of bacteria at the walls and/or at the bottom of the test tube along with a clear liquid over the turbid zone. The isolates providing such results are probably positive to the virulence plasmid. Any other agglutination pattern at these two temperaturs shall be considered as negative. **Reaction to the low calcium level. Virulence test for the agar-based Congo red**. Inoculate the testing organism into the BHI broth. Proceed with incubation in the night time at 25-27°C. Proceed with one-tenth dilutions in saline solution to obtain 1,000 cells/ml. Distribute 0.1 ml of the respective solution into each of the two cups containing Congo Red. Proceed with incubation at 35°C for the first cup and at 25°C for the second cup respectively. Make a visual check in 24 and 48 hours. Probably, Y. enterocolitica containing plasmid will be present as round bulged red non-transparent dotted colonies. *Y. enterocolitica* without plasmid will be present as big-sized randomly-formed smooth semi-transparent colonies. The intraintestinal infection of adult mice that previously received iron dextran and desferrioxamine B. The positive result of any pathogenicity test in vitro (H, 1-3 and higher) is a sufficient proof of pathogenicity. These results can be verified with a biological test using intravenous injections for adult mice (previously treated with iron dextran, siderophore binding iron, desferrioxamine) to introduce the infection.		When growing in the MRVP broth at 25°C, the pathogenic *Y. enterocolitica* demonstrates a diffuse development (left test tube); at 35°C, however, the cells agglutinate and sink to the bottom (right test tubed).
			Plasmid containing *Y.enterocolitica* colonies that are partially bulged, red, non-transparent. Plasmid-free colonies, big-sized, random form, smooth and semi-transparent.

CHROMagar Yersinia (CAY) is a new chromohgenic medium for the probable detection of the virulent *Yersinia enterocolitica* in stool samples. Based on the comparative analysis of 1494 consecutive defecations by hospitalized patients, it was found that CAY was as sensitive as the reference medium (agar Cefculodin-Irgasan-Novobiocin). At the same time, CAY was found to be much more specific and to have a very low level of faulty positive results. CAY reduces the workload (and, accordingly, the costs) for the analysis of stool samples, which is why it can be recommended for regular laboratory use (Renaud, et al., 2013).

The pathogenic *Yersinia enterocolitica* become purple-stained after 48 hours of incubation at 28 ^{0}C, while the non-pathogenic strains of the species and random isolates of other *Enterobacteriaceae* only grow as blue metallic colonies after 24 hours of incubation at the equal temperature.

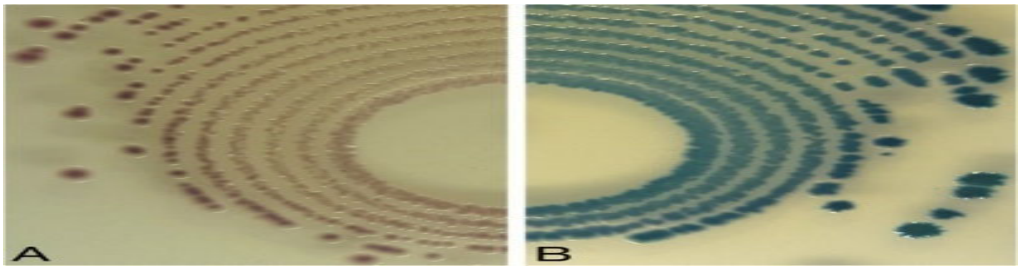


Figure 1. Colonies of *Y. enterocolitica* on CHROMagar Yersinia after the 48-hour incubation at 28°C. Growth of the pathogenic isolate (A; purple colonies) and the non-pathogenic isolate (B; blue metallic colonies) (Renaud, et al., 2013).

The genus *Yersinia* includes other enteropathogenic species (*Y. pseudotuberculosis*) as well as other species that can be rather rarely detected in humans' excrement but are normally considered non-pathogenic *(Y. frederiksenii, Y. kristensenii, Y. intermedia, Y. mollaretii, Y. aldovae ma Y. bercovieri*). Neither of the 19 examined strains of *Y. pseudotuberculosis* grew on CAY. In the same way, *Y. kristensenii (n= 5*), Y. aldovae *(n= 4), Y. intermedia (n= 3),* and *Y. mollaretii (n= 3)* did not grow on CAY. Contrary to that, *Y. frederiksenii (n= 3)* and *Y. bercovieri (n= 4)* produced colonies looking the same way as the non-pathogenic (blue metallic) and pathogenic (purple) colonies of *Y. enterocolitica* respectively (table 11) (Renaud, et al., 2013).

CIN agar and modified CIN agar (Cefculodin-Irgasan-Novobiocin) (Renaud, Lecci, Courcol, Simonet, Gaillot, 2013).

Both CIN and the modified CIN contributed to the growth of all tested strains of *Yersinia*, excluding the non-pathogenic strain IP102 (Y*. enterocolitica* bioserotype 1A/O:6,30) that was inhibited on both media. In addition, all Yersinia colonies demonstrated the same explicit feature of the "bull's red eye".

Both CIN and the modified CIN acted in the same way as inhibitors for the growth of different species of *Salmonella, E. coli, Shigella, Proteus, Vibrio, Pseudomonas, Enterococcus, Listeria, and Staphylococcus.* Two media enabled the growth of *C. freundii, C. koseri, S. odorifera, S. marcescens,* and *Pantoea*, which do not produce H_2S, and created colonies with the "bull's red eye" morphology. Hence, the CIN modification has not improved the differentiation of these bacteria from *Yersinia spp.* (Tan, et al., 2014).

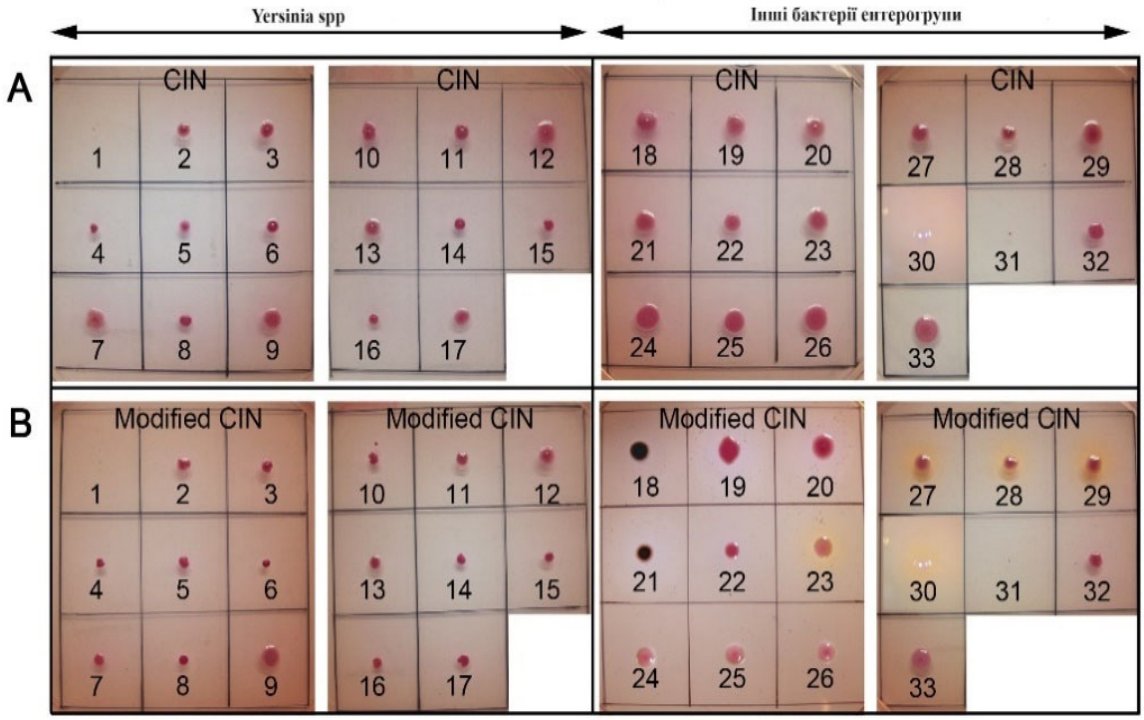
Contrary to that, the modified CIN, but not CIN, made it possible to differentiate *Yersinia* spp. from several other *Enterobacteriaceae* and *A. hydrophila*. The *C. braakii* colonies and H_2S-producing *C. freundii* on the modified CIN demonstrated the black middle, the *P. rettgeri* colonies were encircled with a brown diffusion pigment, the *E. cloacae* colonies were light pink, *A. hydrophila* looked like pink colonies encircled with a brown pigment, while *M. morganii* produced tiny colourless colonies encircled with a brown pigment. Thus, the modified CIN is

more effective that CIN for discerning *Yersinia* spp. from these species of *Yersinia* bacteria (Tan, et al., 2014).

Table 11

Growth specifications of different species of Yersinias on CHROMagar Yersinia after 48 hours of incubation at 28 ^{0}C

Species of Yersinia		*Strains*	*Growth specifications*	
			Colour of colonies	*Size of colonies , mm*
Yersinia enterocolitica	Biovar 1A	NEM7, LUMC3192, LUMC3847, RDH25383, RDH30963, RUMC646896, RUMC648126, RUMC663603	Blue metallic	1-3
	Biovar 1Б	8081, NEM6, WA, LUMC1497, LUMC 3100	Purple	1-2
	Biovar 2	NEM3, NEM5, LUMC1484, LUMC1503, LUMC1681, LUMC1682	Purple	1-2
	Biovar 3	IP383, IP864, LUMC1135, RDH29135, RDH30976	Purple	1-2
	Biovar 4	LUMC992, NEM1, NEM2, NEM4, LUMC4173, LUMC1491, LUMC1683, LUMC1996, LUMC4035, RDH25203, RDH28460, RDH29088 , RDH30205	Purple	1-2
	Biovar 5	LUMC2491, LUMC1502, LUMC1680	Purple	1-2
Y. pseudotuberculosis		IP1553, IP2775, IP2790, IP2926, YPIII, KM, ST, LUMC1136, LUMC1498, LUMC1499, LUMC1500, LUMC1501, LUMC1504, LUMC1505, LUMC1506, LUMC1507, LUMC2085, LUMC2149, RDH2 8967	No growth detected	-
Y. kristensenii		IP7577, IP7117, IP24418, LUMC1573, LUMC2147	No growth detected	-
Y. aldovae		LUMC2151, LUMC2152, LUMC2153, LUMC2219	No growth detected	-
Y. bercovieri		IP24495, IP24511, IP25595, RUMC521167	Purple	1-2
Y. frederiksenii		IP14523, IP24548, IP24551	Blue metallic	1-3
Y. intermedia		IP24596, IP24604, IP24617	No growth detected	-
Y. mollaretii		IP24077, IP24084, IP24538	No growth detected	-



Notes: 1, *Y. enterocolitica* 1A/O:6,30 (IP102);
2, *Y. enterocolitica* 1B/O:8 (IP11105);
3, *Y. enterocolitica* 2/O:9 (IP383);
4, *Y. enterocolitica* 3/O:1,2,3 (IP135);
5. *Y. enterocolitica* 4/O:3 (IP134);
6. *Y. enterocolitica* 5/O:2,3 (IP178);
7. *Y. enterocolitica* 1B/O:8 (ATCC 9610);
8. *Y. enterocolitica* 3/O:3 (PC-M1-K1);
9. *Y. enterocolitica* 1A/O:5 (PC-M16-2);
10. *Y. aldovae* (IP6005);
11. *Y. bercovieri* (IP3443);
12. *Y. frederiksenii* (IP3842);
13. *Y. intermedia* (IP955);
14. *Y. kristensenii* (IP105);
15. *Y. mollaretii* (IP33766);
16. *Y. pseudotuberculosis* (IP34476);
17. *Y. enterocolitica* 1B/O:8 (YE036c-CY);
18. *C. freundii*, H_2S-produces;
19, 20. *C. freundii*, does not produce H_2S;
21. *C. braakii*;
22. *C. koseri*;
23. *A. hydrophila*;
24, 25, 26. *E. cloacae*;
27, 28, 29. *P. rettgeri*;
30, *M. morganii*;
31. *Pantoae* spp.;
32. *S. odorifera*;
33. *S. marcescens*.

Figure 2. Bacteria on CIN (A) and modified CIN (B).

Conclusions. The research results demonstrate that infections caused by *Yersinia* spp. remain a relevant health issue for animals and humans worldwide; the disease has episootic, economic, and epidemiological relevance. The main sources of infection for humans are contaminated animal and plant products. Presently, Ukraine has no domestic diagnostics tools to identify the types of all pathogenic Yersinia sero- and biovars. The regulations on the diagnostics and control of Yersiniosis in animals in Ukraine need to be updated and improved in line with up-to-date international requirements.

Further research prospects. Future research will be focused on summarizing the data concerning the laboratory diagnostics of Yersiniosis and their inclusion into methodological guidelines.

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ЛАБОРАТОРНА ДІАГНОСТИКА ІЄРСИНІОЗІВ

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Резюме. *Стаття присвячена аналізу сучасного стану діагностики ієрсиніозів тварин і важливості вивчення цього бактеріального зоонозу. Використовуючи джерела вітчизняної та закордонної літератури, у статті представлена інформація щодо розповсюдження ієрсиніозів та лабораторної діагностики цієї хвороби. Ієрсиніозні інфекції, зумовлені патогенними для людини та тварин Yersinia pseudotuberculosis і Yersinia enterocolitica, відносяться до широко розповсюджених у світі гострих кишкових захворювань. Біологічне споріднення збудників псевдотуберкульозу й кишкового ієрсиніозу забезпечує подібність клінічних форм. До недавнього часу кишковий ієрсиніоз та псевдотуберкульоз вважалися доволі рідкісними захворюваннями і не привертали до себе особливої уваги фахівців. Останнім часом проблема ієрсиніозів привертає все більше уваги ветеринарних та медичних працівників, що обумовлено поширеністю ієрсиній у природі (ґрунт, вода, каналізація, рослини, пил), збільшенням кількості хворих на ієрсиніоз людей, та недосконалими методами лабораторної діагностики цих захворювань. Заготівля, транспортування і зберігання харчових продуктів (молока, м'яса, овочів, коренеплодів, фруктів) або кормів для тварин з недодержанням санітарно-гігієнічних норм і правил, підвищує ризик накопичення ієрсиній та інфікування людей і тварин. В даний час епізоотолого-епідеміологічною особливістю збудників зазначених захворювань є зміна популяцій циркулюючих Y. enterocolitica, зокрема, зниження питомої ваги серотипів, які домінували в попередні десятиріччя і поява нових серо- і біотипів, що раніше вважалися непатогенними. У зв'язку із різноманітними клінічними проявами, множинністю ураження органів та систем кишковий ієрсиніоз ховається під маскою різних захворювань чим ускладнює та обумовлює трудоємкість процесу діагностики даної хвороби.*

Враховуючи високу потенційну небезпеку бактерій роду Yersinia для людини через контамінацію харчових продуктів тваринного і рослинного походження, актуальність проблеми лабораторної діагности ієрсиній не знижується.

Ключові слова: бактеріальні зоонози, ієрсиніоз, лабораторна діагностика, бактеріоз, зооноз.

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