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ENTEROTOXIGENIC PROPERTIES OF THE STRAINS OF *Y. ENTEROCOLITICA*, ISOLATED FROM ANIMALS AND ANIMAL PRODUCTS

The results of determination of enterotoxigenic properties of Y. enterocolitica strains, isolated from animals and products of animal slaughter are presented. 11 strains were toxigenic among the 20 researched. They led to catarrhal inflammation and in one case to hemorrhagic inflammation and accumulation of fluid in the isolated loops of small intestine guinea pigs that were researched.

Yersiniosis (intestinal yersiniosis) – a disease of humans and animals, referred to sapronotic infections, pathogens are characterized by ecological plasticity, the ability to flexibly switch from saprophytic to parasitic existen ce, and vice versa. Last determines epizootological (epidemiological) features of Yersiniosis in humans and animals, argues the importance of study of its strains isolated from different sources [2].

Y. enterocolitica is oligotrophic and termotolerant. Microorganism can grow in the poorest nutritionally environmental media, proliferate even in a domestic refrigerator. These properties provide a very wide circulation in nature. Now *Y. enterocolitica* is isolated from almost all species of mammals, fish, amphibians, mollusks and insects [2].

In Ukraine *Y. enterocolitica* remains little studied pathogen, in particular, such as pathogenicity factor toxigenicity of microorganisms isolated from animals.

Yersinia infection in humans is characterized by polymorphism of clinical symptoms. Quite often, it is typical for food poisoning. On the prevalence of the latter is second only to Salmonella toxicoinfection [1, 2].

In animals *Y. enterocolitica* causes mainly gastroenteritis, but also occasionally lesions and other organs and tissues are observed, in particular, arthritis, rhinitis, tracheitis (mostly pigs), mastitis (cows and sows), abortion, and birth of nonviable piglets and recorded conjunctivitis arise and erythema of the skin, signs of cardiovascular disease as well. Manifest during Yersinia infection is diagnosed mainly in infants and young animals, in adults it usually occurs in latent form [2].

Virulent strains of Yersinia have factors sufficient to ensure their adhesion to the cells, colonization on the tissue surface colonization and penetration, overcoming mechanisms of specific and nonspecific immunity, the ability to proliferate in vivo, inducing pathological phenomena. Part of pathogenicity factors is determined by the microbial cell chromosome, the other by the plasmid pYV – plasmid associated with Yersinia virulence [1, 5, 6].

Chromosomes are determined, in particular, inter alia, such pathogenicity factors: urease (neutralize acidity in the stomach), catalase (owners antifagocitos activity) and proteins HtrA GsrA (inhibit phagocytosis), outer membrane protein of 103 kDa – invasin (provides pathogen adhesion and penetration into the cell), outer membrane protein 17 kDa (causes adhesion and penetration of the pathogen into the cell detects anticomplementary action), a protein Ymo A (promotes transcription of genes Yop-virulona), enterotoxin Yst (stimulates guanylate cyclase enterocytes, causes accumulation of cGMP), and others. The plasmid are determined: proteinase mucin (provides mucin coating enterocytes overcoming) adhesin YadA (causes adhesion and colonization of the pathogen, and antifagocitos anticomplementary activity), protein Vir (protein YadA transcriptional activator),

outer membrane proteins of *Y. enterocolitica* – Yops (YoE, YopH, YopB, YopD, YopN) – (cause immunosuppression in the body, ensure dissemination of the pathogen in the tissues), and others. [3].

Aggressive characteristics *Yersinia enterocolitica* is largely due to the ability of the strain to produce enterotoxin – molecular weight of its toxic component of 12.4 kDa. It is thermostable protein – not destroyed by boiling for 5 minutes. In the body-like enterotoxin enterotoxins other enterobacteria activates adenylate enterocytes synthesis, increases the permeability of membranes to water and electrolytes, which leads to the development of diarrhea syndrome, intoxication dehydration and [4].

Enterotoxin synthesis can be controlled by two chromosomal genes – ystA and ystB. Regulation of expression of enterotoxins occurs at the transcriptional level and depends essentially on various factors, such as temperature, pH, and others. In the in vitro gener are transcribed ystB ystA and preferably at temperatures below 30 °C, however, and in the body at higher temperature values the synthesis caused by their products is observed, which is obviously due to the influence of some factors, such as pH in the intestine [3].

The objective of this study – to investigate the properties of enterotoxigenic strains of *Yersinia enterocolitica*, isolated from patients with symptoms of diarrhea, products of slaughter cattle, pigs and livestock feed.

MATERIALS AND METHODS

The studies were carried out in the laboratory department microbiology, virology and biotechnology NUBIP Ukraine. 20 strains of *Y. enterocolitica*, isolated from various sources (from the feces of sick calves, dogs, products of slaughter cattle, pigs, dairy products) were studied.

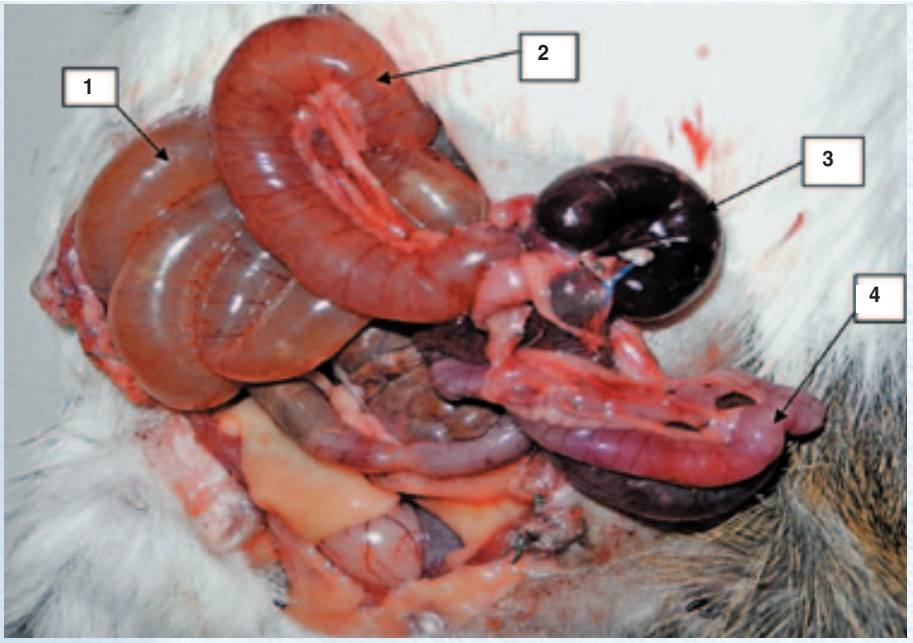
Enterotoxigenic properties of strains were examined on isolated pattern portions of the small intestine of guinea pigs. For this purpose outbred animals were used at the age of 10–12 months old, weighing 280–300 g. Prior to surgery the animals were kept for 48 hours with free access to water. They were operated using the ether anesthesia. Incision of 3–4 cm was carried out by the white line. Small intestine was put it on a sterile gauze, periodically, wetting it with sterile 0.9% sodium chloride solution through 5–7 min. Before ligation intestines were washed with saline. Silk ligature was applied so as to form segments of length 1–1,5 cm. The test material – 48-hour broth culture of *Y. enterocolitica* strain grown on Hotingera broth (pH 7,1) was injected into the lumen of the isolated of fragment small intestine guinea pigs (at a dose of 0,1 cm^3, a concentration of 5×10^6 CFU). For the control – a sterile nutrient medium (0,1 cm^3 per dose) was injected. After the injection of materials a gut was placed into the abdominal cavity, the peritoneum and the skin were sutured with a continuous suture. In 18 hours after the infection the euthanasia was carried out, animal carcass was dissected and analyzed for changes in isolated sections of the intestine, the number and nature of fluid therein was determined. The liquid was aspirated from the isolated segment and its volume was determined using a syringe. Segment length (in cm) was measured by calipers. Dilatation index was calculated by determining the ratio of the amount (cm^3) of the liquid to the length (cm) segment. Enterotoxigenic strain was considered assuming that the index dilatation was 1 or higher.

THE RESULTS OF THE RESEARCH AND DISCUSSION

All investigated strains *Y. enterocolitica*, irrespective of the source of selection, caused an accumulation of fluid in the isolated areas of the intestine of guinea pigs. However, the diagnostic significance of the volume of fluid (dilation index 1 and above) was caused only 11 strains.

Table – **The results of determination of enterotoxigenic strains of *Y. enterocolitica* on guinea pigs**

Strain name	Material from which was selected strains of *Y. enterocolitica*	Positive reaction	Negative reaction
		Dilation index ≥1,0	Dilation index ≥1,0
E 35	Calf faeces	–	+
E 41	Calf faeces	–	+
E 47	Calf faeces	+	–
E 61	Calf faeces	+	–
E 122	Veal liver	+	–
E 139	Calf retropharyngeal lymph nodes	–	+
E 142	Veal liver	–	+
E 345/1	Swine tongue	+	–
E 511	Raw milk	+	–
E 560	Raw milk	–	+
E 614	Swine retropharyngeal lymph nodes	+	–
E 616	Swine tongue	–	+
E 652	Pork liver	+	–
E 656	Swine tongue	+	–
E 670	Swine retropharyngeal lymph nodes	–	+
E 744	Swine tongue	–	+
E 986	Pork liver	+	–
E 816	Swine tongue	+	–
E 1037	Dogs faeces	+	–
E 1086	Meat pork mince	–	+



Enterotoxigenic signs of *Y. enterocolitica* strains in isolated loops of small intestine of guinea pigs: 1, 2 – segments filled with transparent liquid – strains № 5, 9; 3 – segment with signs of hemorrhagic inflammation – strain №11; 4 – control segment

They are allocated from various sources, including the three strains (E47, E61, E1037) isolated from the feces of animals with symptoms of diarrhea , seven strains (E122, E345 / 1, E614, E652, E656, E816, E986) –- from the slaughter products and one strain (E511) from raw milk (*see* Table).

When studying the properties of enterotoxigenic strains *Y. enterocolitica* isolated from different sources, varying degrees of occurrence of these properties have been found.

When an autopsy guinea pigs which were administered the test material was found signs of intestinal inflammation individual sites (*see* Figure).

Clear signs of inflammation are clearly correlated with the degree of accumulation of fluid in the isolated areas of the intestine and were observed only in cases where the dilation index was 1,0 and above.

Leading mechanism of accumulation of fluid in the intestine is the activation of adenylatecyclase by the action of the intestinal epithelium enterotoxin *Y. enterocolitica*. Regarding hemorrhagic inflammation in one of the segments may strain *Y. enterocolitica*, unlike the others, has expressive invasive properties.

The presented data indicate the complexity of the processes of interaction of Yersiniosis with the body of animals, in which a variety of specialized structures of the microorganism are involved.

CONCLUSION

In the study of enterotoxigenic strains *Y. enterocolitica*, isolated from sick animals and products of animal slaughter it was found that 55 % of the strains resulted in an accumulation of fluid in the isolated segment of the small intestine of guinea pigs. During the guinea pigs autopsy, it signs of catarrh were found and in some cases, hemorrhagic inflammation of intestinal in guinea pigs was found. For more information on this phenomenon will be given after the histological research of selected materials.

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Ентеротоксигенні властивості штамів *Y. enterocolitica*, ізольованих від тварин та тваринницької продукції. *Г.В. Козловська, П.К. Солонін, В.Г. Скибіцький, В.І. Білоконь*

Наведено результати дослідження токсигенності штамів *Y. enterocolitica*, виділених від тварин, продуктів забою та тваринницької продукції. Токсигенними виявились 11 штамів серед 20 досліджених. Вони обумовили катаральне, а в одному випадку геморагічне запалення та накопичення ексудату в ізольованих петлях тонкого кишечнику дослідних мурчаків.

Энтеротоксигенные свойства штаммов *Y. enterocolitica*, изолированных от животных и животноводческой продукции. *А.В. Козловская, П.К. Солонин, В.Г. Скибицкий, В.И. Белоконь*

Изложены результаты исследования токсигенности штаммов *Y. enterocolitica*, выделенных от животных, продуктов убоя и животноводческой продукции. Токсигенными оказались 11 штаммов среди 20 исследованных. Они обусловили катаральное, а в одном случае геморрагическое воспаление и накопление экссудата в изолированных петлях тонкого кишечника исследованных морских свинок. ○

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