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STUDY OF STRAINS YERSINIA ENTEROCOLITICA ASSOCIATED WITH EPIDEMIC OUTBREAKS BY DIFFERENT TYPING METHODS

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L'étude de l'information complète sur les caractéristiques biologiques de Yersinia intestinales, qui possèdent des systèmes d'adaptation liés au changement des conditions d'existence (« organisme-environnement »), est particulièrement actuelle pour définir la source d'infection et les voies de transmission de Y. enterocolitica pendant la recherche étiologique de yersiniose épidémique. Cette étude a été entreprise pour trouver les méthodes permettant d'identifier plus correctement les souches de même clone et de déterminer leur origine commune, en particulier sous forme d'étude comparative des caractéristiques écologiques et épidémiologiques de circulation de populations naturelles ou parasites de Y. enterocolitica.
L'étude du phénotype de 64 isolats sapophytes et de 25 souches de cas cliniques de yersinia intestinales, provenant de 5 foyers du nord-ouest de la Russie, a révélé la variabilité de toutes les caractéristiques biologiques à la fois au plan des différents isolats et de l'ensemble des souches. Les caractéristiques se comportent comme marqueurs absolus de clones pathogènes. Pour ce faire, nous avons utilisé les résultats d'un typage génique comparé avec AP-PCR, à partir des cultures de yersinia intestinales de foyers. Des locus utilisés en PCR pour toutes les souches épidémiques ont permis de distinguer non seulement les variants mais aussi le clone jouant le rôle essentiel dans la situation épidémique.

Epidemiologic importance of enteric yersiniosis, in modern stage, in general is due to diversity of serologic varieties cultured from sick people and animals *Yersinia enterocolitica* and to ability of yersinia to exist in the nature in form of "saprophitic" and "parasitic" populations. It makes the yersiniosis diagnostics more difficult. Therefore, it is also difficult to definite how mentioned populations, heterogeneous by antigen structure, influence to morbidity rate of that infection pathology in investigated anthropurgic outbreak. At the same time, having the fact of serologic resemblance among clinic isolates, *Y. enterocolitica,* and strains of enteric yersinias obtained from environment, beyond doubt, it is posssible to establish only the common character of these populations and their correlance. However, we can't do any conclusions about their potential pathogenicity in explored anthropurgic outbreaks and, moreover, about the part of natural strains in the development elaboration of epidemic with enteric yersinias.
In connection with this fact, the aim of our study was to investigate, with use of epidemiological techniques, five outbreaks of enteric yersiniosis in the North-West region of Russia, the biologic properties *Y. enterocolitica*. It makes it possible, on one hand, to identify pathogenic strains of yersinia which are connected etiologically with the morbidity in such focusis and, on the other hand, to confirm the common character of "natural" and clinic isolates, derived during these outbreaks decoding. We carried out biological properties of cultured strains *Y. enterocolitica* by using a series of methods. These methods allowed to get more clear picture of potential ability to provoke the infection process, i.e. their pathogenicity. There was defined: biochemical activity, ability to autoagglutination, growth in enyironment (Ca2+- dependence), morphology changes of colonies at different temperatures of incubation (28 ^{0}C and 37 ^{0}C) (temperature dependence of morphometry), ability for esculin fermentation, determination of Yad A outer membrane protein and ability to induce ceratoconjunctivitis in mouses and guinea-pigs (Sereny test).
Evaluation of pathogenic potential made by such traditional laboratorial methods covered 64 isolates *Y. enterocolitica* of serologic varieties 0:5; 06,30; 07,8; 0:9; 05,27. They formed saprophytic population (39 strains were cultured from waters washed of vegetables and 25 ones were from waters of pork) in explored anthropurgic focusis. We also evaluated 25 clinical yersinia that were analogue to serovars (parasitic population). Research setting of above mentioned isolates *Y. enterocolitica* showed irregularity of distribution phenotypic markers of virulence in indicated populations enteric yersinia.
So, from 64 assessed saprophitic isolates *Y. enterocolitica* signs of autoagglutination and morphometry changes of colonies of enteric yersinia against the cultivation temperature were discovered in 19 strains(29,7%) depended on Ca++-ions content in nutritious environment were 15 isolates *Y. enterocolitica*(23,4%). Only 3 strains of enteric yersinia from environment (4,7%) gave positive results in Sereny ceratoconjuctival test and 1 isolate *Y. enterocolitica* (1,6) couldn't ferment esculin. The signs of "expression" of own membrane's plasmid coded protein were not revealed in none of isolates enteric yersinia, derived from abiotic objects in explored anthropurgic seats. By biochemical activity 89,1% of strains *Y. enterocolitica* among all investigated saprophitic strains were bearing to first biologic type. And only 10,9% of isolates of explored population have shown the signs of second biovar considering biochemical activity. During the study of phenotype 25 enteric yersinia clinical strains of enteric yersinia, identical in respect of serogroup, which demonstrated certainly contrary results in each phenotypic sign ($p<0,001$). Yet 72% of all isolates *Y.enterocolitica* of this population in autoagglutinative reaction were positive. The symptom of Ca++-dependence and of temperature-dependent morphometry of colonies were present in

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60% and 68%(respectively) strains of enteric yersinia, cultured from the patients. 20% of analysated isolates had ability to cause the ceratoconjuctivitis in guinea-pigs. 32% of all *Y. enterocolica* had been esculinonegative. And "expression" of extern protein of own membrane have found in 4% of enteric yersinia (05,27 serologic type), being in parasitic phase of circulation.

Generation of saprophitic bacterias of enteric yersinia turned out to be more heterogeneous. It proved also by their biochemical activity than their identical by O-antigenetic construction clinical isolates *Y. enterocolitica.* Though, heterogenicity in biochemical respect remained also among the isolates cultured from the patients, i.e. in parasitic population *Y. enterocolitica*. However, already 56% of clinical isolates enteric yersinia were concerned to second biologic type. All studied strains derived from patients 0:9 and 05,27 of serologic species and 75% of enteric yersinia 0:5 serovar and unidentified *Y. enterocolitica* belonged to mentioned serovar. Biochemical activity of second serovar has been found by *Y. enterocolitica* of serologic type 06,30 (out of 5 strains - 1 isolate) and 07,8 (out of 5 strains -1 isolate).

Thus, the variability of almost all standard phenotypic signs is revealed both on the level of alone isolate *Y. enterocolitica*, derived either from environment or from a patient and in respect of studied natural and parasitic population of enteric yersinia on the whole. It is clear evidence of different speed of dissociate process in natural and clinical strains of enteric yersinia, measuring intensity of which depends on orientation of selective pressure. It is especially important for epidemic conception of pathogenesis nature by "host-parasit" relations into population with Y. enterocolitica infection.

So, we can come to the conclusion: as far as the studied phenotypes' stability is provided firstly by selection, it is insufficient to utilize only phenotypic methods for pathogenesis determination to evaluate what epidemiologic part of concrete enteric yersinia microb or, the more, of their population plays in mechanism of development of epidemic at explored seat now days of evolution of mentioned microorganisms. It should be also beared in mind the complication of genetics organs that is responsible for the replication of this or that phenotypic feature. We must remember that some structures of it are exposed to the mutations (for ex., deletion, insertion, acquisition of supplementary stuff from plasmids) (because of phenotypic properties of bacteria can be due, on one hand, by expression of unique gene (protein of invasia *Y. pseudotuberculosis* - chromosome gene *inv*) and on the other hand, by functioning of integral operon (the whole complex of genes) of bacterial strain (triptophan operon, lactose operon E.colli). It also brings in evidence to not consider phenotypic signs of virulence as pathogenetic clon that is responsible for morbidity exciting. This point of view is strengthened by a hypothesis of directional reorganisation. According to it, during the spreading of pathogenes some varieties of microorganisms are appearing and their features are different from primary ones despite of their common clonal origin.

To solve this problem we have utilized the results of polymeras chain reaction with universal primers (AP-PCR), which has first elaborated in Russia and then in USA. These results, in our opinion, define at most pathogenic features of mentioned microbes both on system level (phenotyping) and on molecular one (genotyping), i.e. in all bacterial genom. As a result of AP-PCR - genotyping of strains *Y. enterocolitica* 06,30, which have been isolated in different abiotic objects of studied outbreaks (the factors of yersinia transmitting - pork products and vegetables). It turned out that the whole coincidence of PCR-patterns in separate isolates correlated with their common location. On the contrary, the difference of phingerprints of mentioned strains has been in agreement with the data about different sources of getting. Besides that, judging by gotten results, we came to the conclusion that heterogeneous population *Y. enterocolitica*, inhabiting in studied objects that are epidemiologically important, is characterized by polyclonality.

Consequently, the identification of genom locuses into genom of all participated in epidemic process isolates that is discovered by PCR would make to definite simply enough and for sure the clon influencing most etiologically in epidemic situation.

To check this suggestion we carried out arbitrary PCR-genotyping with olygosystem 45 of 8 isolates *Yersinia enterocolitica*, cultivated from sick people and from environment objects in one anthropurgic focus.

The analysis of turned out genom's "imprints" of all cultivated isolates *Y. enterocolitica*, from serovars 06,30 and 0:9, was evidence of absence of etiological importance of strains, isolated in environment, for the exciting of infection, due to mentioned serovars.

At the same time, genoidentification of strains *Y. enterocolitica* 0:5 derived almost simultaneously in studied outbreak from a patient and from environment objects (in vegetable's and pork's waters) showed coincidence of all strains. It made it possible to consider all received strains *Y. enterocolitica* from the serovar 0:5 as clonal-combined isolates. And the genotype identification of saprophitic and parasitic strains, on one hand, showed very clearly etiologic part of natural strain to provoke enteric yersiniosis in studied collective and, on other hand, indicated simply enough the epidemic part of vegetables and meat products in exciting of this sickness in investigated outbreak.

Thus now, for receiving of information that describes wholly and adequately the biological features of yersiniosis during the different life's stages of their existence, it is useful to utilize molecular-biologic methods. These methods allowed more certainly to identify clonal-combined isolates and to determinate their common origin especially when studying of ecological and epidemiological properties of circulation of natural and parasitic populations *Y. enterocolitica.* In our opinion, the last fact has direct applied importance for veterinary epidemiology. It could make the decoding of separate epidemiological aspects of microbes'transmitting must more easier and could replace probabilitible character of discourses about the mechanism of development of population'relations of micro- and macroorganisms in studied anthropurgic outbreak by real proofs of such interrelations despite of revealing of enteric yersinia in definite moment of the level in laboratory conditions.

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