



## DETECTION OF ARBOVIRUSES IN IXODIC TICKS OF MIGRATING BIRDS VIA ZMIINYI ISLAND

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The Zmeinyy island is key point of «Aristotle's» way of birds' passage. Its geographical position enables to conduct ornithological observations of birds which migrate from African continent to Europe. In 1928 notable German ornithologist R. Drost used to mark, that in the migratory understanding there is no such similar place in Europe, while in the spring during a day on island 74 species and subspecies of birds are being registered.

Birds serve as reservoirs for arboviruses- causative agents of infectious diseases; they also serve transporters and feeders for ticks – ectoparasites, which could contain pathogenic viruses. In conditions of Ukraine and Europe ticks have been detected in more than 60 species of birds, mainly in sparrows. The organism of tick has favorable conditions for virus replication. Once a causative agent has been received from a sick animal, ticks can store it for long period of time in their organism, and pass from generation to generation. Causative agent remains virulent even during long starvation periods of ticks or at during feeding on a healthy animal. Due to these observations there is an obvious need to conduct virological and parasitological researches on island.

The purpose of our work was to reveal an antigen of a tick borne encephalitis virus from ticks, which have been removed from birds that migrated via Zmeinyy island.

Birds were caught on Zmeinyy island during period from 9<sup>th</sup> of May to 18<sup>th</sup> of May in year 2009. Birds' head (at crow, eyes, eardrums) as well as area of cloacal opening were examined for detection of ticks in birds. All found ticks were placed in standard moist chambers. Mixture from ticks was prepared by standard methodic. The immuno-fermental analysis (IFA) (test-system Vector-Best, Russia) was used for detection of tick borne encephalitis. The record of an IFA reaction was carried out by means of vertical spectrophotometer (reader) “Sanofi Dianostics Pasteur PR2100”.

As a result of researches it was established, that the ticks removed from birds have been present in 7 cases by species of *Ixodes ricinus*, in 11 cases - *Hyalomma plumbeum plumbeum*. Larva of *Ixodes ricinus* have been removed from Red-breasted Flycatcher, and nymphs of larva ticks of *Ixodes ricinus* were removed as well from Blackcap Warbler, Barred Warbler and Redstart nymphs of *Hyalomma plumbeum plumbeum* have been detected. It is interesting to point out, that all previously listed ticks were not contaminated with tick borne encephalitis virus, while in a brain of Blackcap Warbler the antigen of this virus has been detected.

The most with tick inhabited were synanthropic birds nightingales – 13 nymphs have been removed from two individuals, including 4 - *Ixodes ricinus* and 9 - *Hyalomma plumbeum plumbeum*. Laboratory researches have showed that 2 nymphs of *Ixodes ricinus* were contaminated with tick borne encephalitis, and 5 nymphs of *Hyalomma plumbeum plumbeum* were contaminated with same virus. However, in a brain of nightingales the antigen of tick borne encephalitis has not been detected.

These data have important epidemiological meaning, as it provides with evidence of possible transcontinental transport of arboviral infections from Africa and Near East to Europe.

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## COMPLEX RESEARCHES OF THE PROFILES OF *ERWINIA CAROTOVORA* “S” DEFECTIVE PHAGE PARTICLES

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**Introduction.** Bacteriophages of *Erwinia carotovora* are rarely discovered comparing to other enterobacteria. Lysogenic induction of cultures of this bacterium, are resulted in formation of defective particles of phage nature, such as: capsids, basal plates, tail fibers. It is assumed that these particles are synthesized by different prophages and characterize defective lysogeny of *E. carotovora* subsp *carotovora* [1]. Complex approach was used for the analyses of obtained virus like particles (VLP). It included: chromatological and biophysical researches, and also electron microscopy. Stage-by-stage method of the particles“ elution through ion-exchange chromatography on fibrous DEAE –cellulose was developed for more detailed study of defective particles. Four types of particles have been identified according to received data. The first two were identified as phage tail-like particles, the third type of particles – were capsids (of different sizes), and precise nature of the last one was not possible to establish.

**Aim.** The purpose of the work was to develop the defective lysogenicity identification method of *E. carotovora* subsp *carotovora*. It would include clearing, separation, concentration and obtainment of the defective phage particles preparative quantity.

**Materials and Methods.** Objects of research were the laboratory strains of *E. carotovora* subsp *carotovora* ZM1, Ec153 and 48A. Bacteria were cultivated on high-grade LB medium and the liquid minimum medium A?1, with the addition of MgSO<sub>4</sub> ?7H<sub>2</sub>O and glucose. Mitomicin C in 1 ìg/ml concentration was used as an inducer [2].

For simultaneous concentration and clearing of particles fibrous DEAE cellulose 23SS «Serva» was used. After lysates application, 1,6?32,5 cm column was eluted with 0,15Ì NaCl, and then fractionated with 0,25 and 0,4Ì NaCl. VLP preparations received by ultracentrifugation or ion-exchange chromatography were used for electron microscopy researches. Preparations were stained by 2 % uranyl acetate or 2 % solution of phosphotungstic acid. Electron-microscope ÅÌ-100BR (Sumi) was used for electron-microscopy researches. VLP were observed at tool amplification 30000-40000 ? [3].

The HPLC method with liquid chromatograph Agilent 1200 (Agilent Technologies, Waldbronn, USA), equipped with the diode-matrix detector, and analytical column Zorbax 300SB-C18 2,1 mm ? 150 mm ? 5 ìm (Agilent Technologies) was used for analysis of proteins preparations. Detection was carried out on wavelength of 254 nanometers and 280 nanometers. Received chromatograms were analyzed with the help of program package Chemstation (Agilent Technologies). Standard program Excel 2000 was used for statistic data processing.

**Results.** The elution profiles of virus like particles of *E. carotovora* subsp *carotovora* ZM1, Ec153, 48A were completely identical according to the researches. Strain ZM1 is characterized by a wide spectrum of defective virus like particles according to earlier received data. It is capable to synthesize such types of VLP as phage tail particles, basal plates and capsids while the lysogenic induction [4]. We used the ion-exchange chromatography on fibrous DEAE-cellulose for their nature better understanding. Four VLP types have been received after carrying out stage-by-stage elution. The first type was received after the 0,15Ì NaCl elution, then it was precipitated by ultracentrifugation. It was named BS (sedimentation bacteriocin) because it only partially

bounded with the column. The second one was eluted by 0,25 M NaCl and it was named  $\hat{A}15$  (according to the number of peak fraction). It was established that these bacteriocins belonged to the group of macromolecular carotovoricins — the phage tail-like bacteriocins. VLP  $\hat{A}19$  was also received in fractions eluted by 0,25 M NaCl. It differed from two types received earlier, and it was capable to cause the effect of phage-phage induction. The fourth type of particles named  $\hat{A}22$  had the greatest affinity with DEAE-cellulose and it was eluted by 0,4 M NaCl. All four biologicaly active fractions differed according to the electron-microscopic data and to the lyses specificity and inducing activity on different indication cultures. So bacteriocins BS and  $\hat{A}15$  had an identical host range and they were identified as macromolecular carotovoricins, phage tail-like bacteriocins, upon the lyses activity, in difference to VLP  $\hat{A}19$  and  $\hat{A}22$  which have different killer specificity. Electron-microscopy researches revealed that fractions B15 and VLP-BS contained only tail-like structures. Fraction B19 was characterized by capsids and procapsids structures of five kinds and structures of false tail particles type (LT). VLP- $\hat{A}22$  had no distinct structure. This type of particles was classified as small colicin-like bacteriocins according to their biological activity. The analysis chromatographic data completely confirmed and widespread earlier received data. VLP  $\hat{A}15$  and BS are found in the form of two-component peaks with different time of retention that testifies that these VLP represented tail particles of two different types. The data of VLP  $\hat{A}19$  HPLC-analysis completely confirmed the presence of five types capsid structures. VLP  $\hat{A}22$  HPLC-profiles also confirmed earlier received data.

**Conclusion.** On the basis of the received results it is possible to claim with confidence that the approach offered in this work has justified itself as a method of identification of a various kind of defective phage particles. The method essence consists of profiles elution research of the total VLP preparations put on a column with fibrous DEAE-cellulose 23SS. The complex of methods allowed to separate 4 peak fractions with carotovoricins  $\hat{A}S$ ,  $\hat{A}15$ ,  $\hat{A}19$  and  $\hat{A}22$  which according to received data can be considered the macromolecular complexes with virus nature.

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