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PHYSICAL SIMULATION IN EPIZOOTOLOGY OF NON-TRANSMISSIVE ZOONOSES*)

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Abstract. Various methods of physical simulation of elements of an epizootic in non-transmissive infectious diseases of wild animals are discussed.

The first and most simple method of studying epizootics in wild animals is their collection by different methods and the examination of dead animals (and arthropods) on the presence of the pathogen in them. This method helps to establish the occurrence of an epizootic in a given population and to get an idea about the range of carriers and vectors of infection in nature.

The elaboration of the methods of repeated catching of marked animals has made it possible to examine several times the same representatives of a population on the incidence of infection in them. These techniques were used to investigate seasonal dynamics of epizootics of leptospiroses in populations of voles, the spatial structure of the infection foci, etc. (Karaseva 1956, Havlík et al. 1960).

The epozootic process, however, as an object of study has several peculiarities which restrict its investigation by conventional methods. Firstly, it is impossible to observe directly the progress of an epizootic in nature. Any information about the epizootic can be obtained only by disturbing, to some extent, the biocoenosis; the greater the volume of the obtained information, the greater is the extent of this interference. This fact is clearly revealed when the number of infected animals is small. Secondly, the phenomenon itself is of diverse character and the small number of frequencies due to the labour-consuming observations, is inevitable. In addition the facts obtained when conventional techniques are applied, characterize only the result of the epizootic, i.e. only the number of the animals infected in the study area.

Therefore, a necessary pre-requisite for further progress of epizootology is the search for some "roundabout" methods and techniques which would at least partially eliminate the difficulties encountered in direct observations of an epizootic process.

PHYSICAL SIMULATION AS A METHOD OF STUDYING WILD ANIMAL EPIZOOTICS

The simulation of biological systems is based on an analogy between input and output data of an artificial and a natural system while their internal structures are absolutely

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different (the "black box" method in cybernetics). Knowing the structure of a system (process) and the properties of the elements comprising it, we distinguish "essential" characteristics (properties) of elements and then build up the model preserving the „essential" characteristics. In whatever is unessential the model should, as fas as possible, differ from the original. The flimsier and narrower the model, the deeper the reasonable idealization of the original in the model, the better are the results obtained and the less effort they require, the wider is the range of the tasks tackled (Nuberg 1968, Poletaev 1968). The main point of any information model is not in copying the system's structure but in describing the principles of its functioning, which reflects the functional approach to studying biological systems. Two types of models are distinguished (Stoff 1966, Frolov 1969): material (physical) and ideal (logical-mathematical).

Physical simulation as a method of studying epizootics of zoonoses has a number of basic advantages as compared to conventional methods of investigation. The "simulated" process is much easier to observe and to repeat in an experiment. For instance, a detailed study of the most important part of an epizootic cycle, the mechanism of infection transmission (in Gromashevsky's interpretation, 1962) has been impossible to accomplish as yet. This is due to the fact that the transition of a pathogen from one habitat to another cannot be immediately registered and its distribution in the environment realized. If the pathogen is substituted by an agent which is easily recorded in the vector or environment fine prospects open up for a detailed quantitative study of these problems at population and biocenotic levels.

The physical simulation of a complete epizootic is unfeasible at present owing to the absence of a suitable "model of the pathogen". We should deal only with the simulation of individual elements of the epizootic process whose direct study is either impossible or extremely difficult.

METHODS OF PHYSICAL SIMULATION OF ELEMENTS OF AN EPIZOOTIC IN NON-TRANSMISSIVE INFECTIOUS DISEASES

The range of non-transmissive diseases suitable for a quantitative study of an epizootic by application of material simulation, is sufficiently wide. It includes at least the following diseases: leptospiroses, erysipeloid, listeriosis, pseudotuberculosis. The possibility of applying the simulation techniques to the study of epizootology of these diseases depends on a number of common features. In all these diseases, the pathogen is eliminated into the environment with the excreta of the animals, the carriers of infection, and is maintained in the environment for quite a long period. The main routes of transmission of the pathogens of the mentioned diseases are water-borne and alimentary. In addition, the application of physical simulation is also expedient in the study of non-transmissive routes of pathogen dissemination in some facultative-transmissive diseases, particularly, in tularemia. Let as discuss the structure of an epizootic in non-transmissive diseases and the ways of expressing individual elements in a quantitative manner. An epizootic in non-transmissive diseases with natural foci represents a branching chain of infectious processes in a population of the carrier which might alternate with the existence of the pathogen's micropopulation in the environment. As a rule, it consists of two stages, each of them being typical of a definite environment of the pathogen and composed of several elements (Litvin 1971).

All the elements of the first stage of the epizootic process (the existence of the micropopulation of the pathogen in the carrier's organism) are available for investigation under conditions of a direct experiment. The second stage of the epizootic process, i.e. the existence of the pathogen micropopulation in the environment, comprises four elements: 1. the elimination of the micropopulation of the pathogen in the environment,

2. the distribution of the micropopulation of the pathogen in the environment, 3. the maintenance (multiplication) of the pathogen in the environment, and 4. the entry of the pathogen in the organism of a healthy susceptible animal.

The natural laws governing the elimination of the pathogen from the organism of the carrier into the environment may be investigated, as a rule, by direct observation under experimental conditions. The quantitative characteristics of the distribution of the pathogen's micropopulation in the environment is practically impossible when using any technique of direct study. Apparently, the only method of investigating this element of the epizootic is the substitution of some agent for the pathogen, which, unlike the pathogen is easily recorded in the environment. The agent which simulates the pathogen should be eliminated together with the excreta from the animal's organism just as the pathogen proper, and be sufficiently physiological. In this case radioactive isotopes have proved to be adequate "models of the pathogen". The applied dose of the isotope should ensure the registration of excreta in the locality with a definite period of time without causing any changes in the animal's life. For the *Microtus* voles these requirements are met by a 0.25 mCu dose of Na_2HPO_4 administered subcutaneously. Radioactive phosphorus P^{32} eliminated with the urine of voles is registered in the soil within 5—6 days (Litvin 1967). The registration of traces of radioactive excreta is carried out in periods necessary for the investigator (daily registration is possible) by field radiometers with an extension probe. Every spot with radioactive urine (feces) of the animal is marked in the locality, numbered and mapped using a convenient scale. The diameter of the spot may be calculated rather accurately by using the radiometer probe: in the experiments with *M. oeconomus* Pall. it was 5—7 cm. In case of individual labelling the study of every animal is carried out in a separate site. The presented technique makes it possible to obtain approximate estimates of a natural focus territory contaminated with the infected urine of carriers (Karaseva and Litvin 1968).;

Much more valuable data may be obtained by applying mass labelling of the infected animals with radioactive phosphorus. In such a case 0.25 mCu of radiactive phosphorus is administered to each animal, in whose urine (feces) the pathogen has been detected. Thereafter the animals are released in the capture area. After mass labelling of the voles *M. oeconomus* infected with leptospirosis in 1970—1971 a territory of one hectare was inspected with radiometers one day after the introduction of the isotopes and the spots with radioactive urine of the labelled animals were marked both in the locality and in the map. Repeated survey of this territory after eight days showed that the new spots were primarily situated within the bounds of the former "contaminated" territory. In 10 spots the probes were taken from the upper soil layer and washed with a physiological solution. The microscopy of these washings against a dark field helped detect mobile leptospirae in 9 out of 10 probes. Thus, the technique of concurrent mass labelling of animals, the carriers of infection, with radioactive phosphorus, makes it possible not only to detect the real distribution of the pathogen's micropopulation in the environment, but to study several problems relating to its ecology (time of survival, the possibility of reproduction, stability in changing a number of properties in the environment, etc.) as well.

Radioactive phosphorus is eliminated to the environment not only with urine but with the animal's feces as well. Hence it is applicable as a simulating agent in some diseases whose pathogens are eliminated with the feces of the carrier. However, the preparation used for these purposes and containing P^{32} should be administered per os.

Particularly interesting among other isotopes tested is Co^{58} with a sufficiently powerful radiation and a comparatiely small half-life, easily eliminated with urine within 1—2 days. The spots with P^{32} (beta-radiation) and Co^{58} (gamma-radiation) are easily recognized in the locality and this fact makes it possible to use a "pair of isotopes" and con-

currently label any two groups of animals (infected and uninfected, males and females, adult and young, etc.).

Several dyes have also been tested as "pathogen models". Quinacrine and trypan blue proved to be most suitable dyes which are eliminated with the urine of voles (Kulik et al. 1967). Among those eliminated with the feces of animals the best results have been obtained with fluorescein and eosine (Litvin and Kulik 1969). The dyes as simulating agents have a number of considerable drawbacks which render their application less expedient in comparison to radioactive isotopes.

The quantitative study of periods of existence (reproduction) of the pathogen in different environment conditions is undertaken by direct investigations. These can be carried out with no great difficulty now since the application of radioactive isotopes makes it easy to register the exact trace of the pathogen in the environment. When simulating the elements of the second stage of the epizootic process, the duration of the simulating agent registered in the environment should accurately correspond to the periods during which the pathogen is maintained in nature.

The possible infection of healthy animals in a population under natural conditions is primarily determined by the intensity of their contacts with areas of the territory contaminated with the excreta of animals, the carriers of the infection. As has been noted above, such contaminated spots of the territory can be easily registered in the given area by administering radioactive phosphorus to infected animals. The next task is the elaboration of methods facilitating to assess the intensity of contacts between voles and the given contaminated spots in the territory and, consequently, the risk at which healthy animals in the population may be infected. In this case the "model of the pathogen" should be transmitted from the environment to the animals which have visited the contaminated spot. One of the possible variants is reduced to the following. All contaminated spots are dusted with the methylene blue powder (within the confines of the spot) while live traps are set in the area of labelling and around the spots. Within several days the labeled voles are caught in this area. Naturally not all animals which have come into contact with the contaminated spots can be caught, but their number can be estimated by a simple proportion (Litvin and Proshina 1971). The value of this technique consists in the possibility of establishing the particular animals which have visited the contaminated spots within the given period of time. However, when dyes are used it remains unknown what particular spots and with what frequency have been visited by the labelled animals. To solve these problems it is necessary to register the animal visits to a contaminated spot not on the animal but in the spot itself.

In such a case it is expedient to make use of soot-covered slips of paper (Justice 1961, Sheppe and Carnes 1965), which are placed on the contaminated spots. When these soot-covered papers are checked the numbers of the spots visited by the animals is recorded and a new coat of soot is put on the slips of papers, or they are replaced. The traces on the papers are sufficiently clear and, as a rule, do not leave any doubt when being identified. With some caution we can make a valid judgement about the approximate number of voles visiting the spots on definite days of observation (Litvin and Proshina 1971). The experiments with soot-covered slips of paper have shown that with the increase in the population density there is an increase in the average level of contacts made by the voles with contaminated spots situated in the territory of natural foci of leptospiroses. At the same time there is an increase in the number of spots visited by voles at a definite unit of time as well as the frequency of visits to contaminated spots (Litvin and Proshina 1971). Most likely other ways of a quantitative estimation of the level of contacts of healthy animals with contaminated spots in a natural focus are possible. They may include the autoradiography in contaminated spots of healthy animals labelled by a radioactive isotope.

Up to now we have worked out and tested techniques of physical simulation of the elements of an epizootic during acute infections of rodents in hay ricks and straw stacks in winter with transmission of the pathogen through cannibalism (Karulin et al. in litt.). The operation of the model commences with the zero point of epzootic (the arrival of an infected animal into a straw stack, its death and the ingestion of the infected carcass by healthy animals). A dose of 2—3 mCu of P^{32} is administered to a vole in vivo then its carcass is placed in a straw stack. A complete, layer by layer, examination of the stack with radiometry using the radiometer probe of every removed layer of straw reveals the complete picture of the distribution of contaminated spots (radioactive excreta). Concurrently, the entire population of the straw stack is caught, the animals are sacrificed, dissected and their internal organs are subjected to radiometry in field laboratory. This method makes it possible to reveal all "infected" animals which have received P^{32} while devouring the carcass. The model of the epizootic can be continued by introducing the isotope to all animals which were "infected" in the first stage by sacrificing them at periods of their natural deaths due to the studied infection and by placing the radioactive carcasses at sites of captures in the straw stack, etc. The physical simulation is most likely the sole way of obtaining accurate parameters of definite elements of winter epizootics of tularemia and other diseases in hay ricks and straw stacks, i.e. a quantitative estimation of the development (not the result!) of an epizootic in time and space.

It follows that the methods of physical simulation may already reveal strict parameters of those elements of an epizootic which are inaccessible to direct examination or to check the operation of the model by the results of a real epizootic under the same conditions. It should be pointed out that a complete registration and preservation in the models of all the characteristics of a real epizootic is absolutely necessary (susceptibility and infection sensitivity of different species and groups of mammals, periods of infection, development of the infectious process, death or bacteria-carriage, the level of the number of immune animals in the population, periods of survival, the possibility of reproduction of the pathogen in the external environment, the risk of infection of healthy animals contacting the pathogen and the infective dose, etc.). These parameters should determine the sequence and time of implementing individual operations in physical simulation; they also serve as adjustment factors when elaborating the programme and "runs" on the computer in mathematical simulation of the entire epizootic.

At present, the first mathematical model of a plague epizootic has been worked out in a population of *Rhombomys opimus* Licht. (Soldatkin et al. 1973), the programme of which incorporates the parameters obtained in physical simulation of individual epizootic elements (Soldatkin 1968, Soldatkin et al. 1966, Rudenchik 1963, et al.).

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МАТЕРИАЛЬНОЕ МОДЕЛИРОВАНИЕ В ЭПИЗООТОЛОГИИ НЕТРАНСМИССИВНЫХ ЗООНОЗОВ

В. Ю. Литвин

Резюме. Сделана оценка разных методов материального моделирования элементов эпизоотии нетрансмиссивных инфекций диких животных.

REFERENCES

FROLOV I. T., Genetika i dialektika. Izd. Nauka Moscow, 1968.

GROMASHEVSKY L. V. (Editor), Mekhanizm peredachi infektsii. Kiev, 1962.

HAVLÍK O., POKORNÝ J., ZÁSTĚRA M., Experimental method of studying a focus of leptospirae. J. Hyg. Epidemiol., Microbiol. Immunol. 4: 488—496, 1960.

KARASEVA E. V., Some peculiarities of leptospirosis epizootic in root voles investigated by the labelling method. Zool. Zh. 35: 1384—1389, 1956. (In Russian.)

—, LITVIN V. Y., A new method of studying natural foci of leptospiroses by labelling of animals with radioactive phosphorus). Zool. Zh. 47: 444—450, 1968. (In Russian.)

KARULIN B. E., LITVIN V. Y., NIKITINA N. A., DUNAEVA T. N., OKHOTSKY Y. V., KHLYAP L. A., TESLENKO E. P., ALBOV S. A., Methods of radioisotope labelling of rodents in hayricks and straw in ecological and epizootological investigations. Zool. Zh. 6, 931—938, 1973. (In Russian.)

KULIK I. L., KARASEVA E. V., LITVIN V. Y., New methods in the study on individual home ranges of small mammals. Zool. Zh. 46: 264—271, 1967. (In Russian.)

LITVIN V. Y., Optimal doses of P^{32} for isotope labelling of gray voles in nature. Zool. Zh. 46: 1088—1093, 1967. (In Russian.)

—, Epizootic process in leptospiroses and ways of quantitative expression of some of its elements. In: Leptospirozy. Tr. 5. Vsesoyuz. konfer. po leptospirozam, Kazan: 293—299, 1971. (In Russian.)

—, KULIK I. L., Search for dyes eliminated from an organism with feces for the purpose of labelling mice and voles. Zool. Zh. 48: 920—924, 1969. (In Russian.)

—, PROSHINA T. F., Elaboration of methods and experience in studying the contacts of *Microtus oeconomus* with contaminated spots in a territory in a natural focus of leptospiroses. Zool. Zh. 50: 572—581, 1971. (In Russian.)

NUBERG N. D., On cognitive opportunities of simulation. In: Matematicheskoe modelirovanie zhiznennykh processov. Moscow, 1968. (In Russian.)

POLETAEV I. A., Some mathematical models of biogeocoenoses and comments on simulation. In: Matematicheskoe modelirovanie zhiznennykh processov. Moscow, 1968. (In Russian.)

RUDENCHIK Y. V., Application of radioactive indicators for studying intrapopulation relations as an epizootological factor in communities of great gerbils. Zool. Zh. 42: 1849—1856, 1963. (In Russian.)

SOLDATKIN I. S., Epizootic of plague as self-regulating process. Doctoral Dissertation. Saratov, 1968. (In Russian.)

—, RODNIKOVSKY V. B., RUDENCHIK Y. V., Experience in statistical simulation of an epizootic process in plague. Zool. Zh. 52: 751—756, 1973. (In Russian.)

—, RUDENCHIK Y. V., OSTROVSKY I. B., LEVOSHINA A. I., Quantitative characteristics of conditions for the development of a plague epizootic in communities of great gerbils. Zool. Zh. 45: 481—486, 1966. (In Russian.)

STOFF V. A., Modelirovanie i philosophiya. Izd. Nauka, Moscow-Leningrad, 1966.

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