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RESULTS OF THE INVESTIGATION OF SOIL FOR CONTAMINATION WITH PATHOGENIC LEPTOSPIRES

E. V. KARASEVA, Yu. G. CHERNUKHA and T. F. SAKHARTSEVA

WHO Collaborating Centre on Epidemiology of Leptospiroses and Laboratory of Medical Zoology, the Gamaleya Institute of Epidemiology and Microbiology, the USSR Academy of Medical Sciences, Moscow

Abstract. In the natural focus of leptospirosis at the lake Nero (Yaroslav region, USSR) 630 samples of soil were investigated for the presence of leptospires. Seven cultures of leptospires were isolated from the soil; five of them were pathogenic (four belonged to the serogroup *Grippotyphosa* and one to the serogroup *Hebdomadis*) and two were saprophytic. Among the cultures of pathogenic leptospires isolated from soil there was observed the same quantitative ratio of serougroups as among cultures obtained from rodents, which suggests that the leptospires circulate from mammals to soil and vice versa.

A knowledge concerning details of existence of pathogenic leptospires in the environment outside the animal organism is still very scarce. Cultures of these leptospires were first isolated from water of natural basins and moist soil in Malaya and the Northern Kalimantan. About 1,500 strains belonging to 29 various serovariants (Alexander et al. 1966) were obtained. In this country no similar works have been carried out.

The objective of our study was to isolate cultures of pathogenic leptospires from moist soil and to establish the frequency with which these microbes can be detected in the area. The work was carried out in the natural foci of leptospirosis in the hollow of the lake Nero (Yaroslav region, USSR),where the main carriers of pathogenic leptospires were small mammals (*Microtus oeconomus*). Among these animals most circulate leptospires of the serogroup *Grippotyphosa* and *Hebdomadis* (Ananyin and Karaseva 1961).

MATERIAL AND METHODS

The observations were carried out in 1974, from June to September inclusive, on two plots in places typical of the focus. One of them, measuring 1 hectare, was situated in a very marshy hassock thickly overgrown with sedge, canary-grass, horse-tail and other boggy plants. The other plot of 0.8 hectare extended along the lake Nero and was covered with a thicket of tall reed. The average moisture of the soil and pH in both plots were the same. At different places of the plots the moisture ranged from 69.5 to 70.3 %, and pH from 7.4 to 7.8. The soil was of the humus boggy-peaty type.

On hassocks the work continued during the entire season whereas on the reed thicket only in August and September. Numbered stakes were placed with an interval of 10 cm each other on both plots. A total of 100 stakes were inserted on hassacks and 75 on the reed thicket. The plan of the plots and disposition of stakes were mapped. Every day 1 g samples of soil were taken near 10 of the stakes and investigated for the presence of leptospires. When samples of soil near all stakes were taken, the process was repeated from the beginning. On hassocks, investigations of the entire plot (i.e. soil sampling at every stake) were conducted in June, July and August. In September samples were taken only at 64 stakes. In the reed thicket full survey was carried out twice.

Every sample of soil was suspended in saline (5 ml) and after 1.5—3 hours examined by microscope. If mobile leptospires were detected, the material was injected intraperitoneally to golden hamsters. Thirty days later the hamsters were sacrificed, the suspension of the kidney was examined

and inoculation of Vervoort-Wolf's medium was carried out by conventional method. All positive cases were plotted on the plan of the areas. If hamsters failed to get infected, in many cases soil sampling was repeated in the same place. Bacteriological technique using 0.22 μm millipore filter was then applied in order to get the strai. In all, 630 samples of soil were taken and investigated.

Non-killing traps were placed on the plots near every stake and examined daily. The trapped rodents were marked, investigated for leptospirosis and set free at the place of catching (Karaseva 1956).

RESULTS

At the hassock plot during the whole period of the study leptospires were revealed in 24 various points, i.e. approximately 1/4 of the entire area proved to be contaminated. Of 364 samples of soil, leptospires were identified in 43 cases, i.e. in 11.8 %. It should be noted that the distribution of leptospires in the locality was increasing every month. In June the leptospires were identified only in two points, in July in 11 points, in August in 14 points, and in September in 18 points.

It is noteworthy that in many cases leptospires were found repeatedly in the same place. Thus, they were found once in 12 places, twice in eight places, three times in one place, and four times in three places.

Two cultures were isolated at the entire hassock plot. One strain was obtained by infecting a hamster and identified as belonging to the serogroup *Grippotyphosa*, the other one was isolated by bacteriological method. Injection of the latter to hamster produced no effect, no renal leptospirosis appeared during 30 days after inoculation.

In the year in which the study was conducted there was a very low number of rodents, and during the period of the study the experimental plot was practically uninhabited. Not a single animal was entrapped on the plot.

Quite a different picture was noted on the other plot which was covered with thickets of reed. Although they were not numerous, *Microtus oeconomus* were trapped systematically in this area. The density of their population amounted to 18 individuals per 1 hectare on the average. An intensive epizootic of leptospirosis was developing among these animals (11—12 % of investigated specimens carried the leptospires). At this experimental field leptospires were found in 28 of the 150 investigated soil samples, i.e. in 11.6 % of cases, in the vicinity of 20 stakes.

On the whole, 5 cultures of leptospires were obtained on this plot. Four of them were isolated through hamsters and one by direct culturing of the filtrate. Out of four strains of pathogenic leptospires three were identified as *Leptospira* of the serougroup *Grippotyphosa* and one as *Hebdomadis*.

Thus, 7 cultures of leptospires have been isolated from moist soil: five pathogenic (four of the serogroup *Grippotyphosa* and one *Hebdomadis*) and two saprophytic.

DISCUSSION

Pathogenic leptospires circulate in populations of mammals of many species (Alston and Broom 1958). There have been different opinions on the significance of the environment in the existence of these microbes. One group of investigators considered that the main pathway of *Leptospira* transmission from animal to animal was a sexual one and thus excluded entirely the significance of the environment in the circulation of pathogenic leptospires (Kiktenko 1954, 1962). The others, on the contrary, assumed that the leptospires could exist for a long time outside the organism of warm-blooded animals and be source of infection of healthy individuals (Terskikh 1945). However, no evidence confirming any concept was available.

The results of our studies indicate that in natural foci, besides the circulation of

leptospires in animal populations, their existence in the environment is of considerable importance. There on the boggy experimental plot with hassock at the period of the studies were no rodents, but besides saprophytic leptospires there were also pathogenic ones. In earlier experiment it was shown that in artificial application of the urine of spontaneously infected *Microtus oeconomus* to the soil alive leptospires retaining their pathogenic properties survived as long as 279 days (Karaseva et al. 1973).

Tagging of animal carriers of leptospires with radioactive phosphorus and subsequent radiometer recording of portions of the urine with leptospires disseminated on the locality revealed that up to 600 infective points were concentrated on 1 hectare (Karaseva and Litvin 1968). Consequently, pathogenic leptospires can exist in the environment for a long time; they are able to survive freezing, thawing and exposure to other abiotic factors, the extent of their dissemination in the territory being very great.

It was of interest to compare our findings with the results of investigations conducted in Malaya. A comparison of quantitative ratios of strains of various serogroups isolated from water and soil (Alexander et al. 1966) and those obtained from wild animals (Smith et al. 1961) in Malaya revealed that there is no correlation between them. Cultures of the serogroup *Javanica* were isolated predominantly from animals, whereas the strains belonging to the serogroups *Icterohaemorrhagiae*, *Canicola*, *Pyrogenes* and *Hebdomadis* were isolated from the environment. Only leptospires of the serogroup *Bataviae* were isolated both from animals and the environment.

A different picture was observed at the lake Nero. From soil cultures were isolated mainly leptospires of the serogroup *Grippotyphosa*, and less frequently leptospires of the group *Hebdomadis*, i.e. there was the same ratio as that observed among cultures obtained from rodents (Ananyin and Karaseva 1961). In other words, a continuous circulation of leptospires from mammals to soil and vice versa, seemed to take place there.

The results obtained are of epidemiological significance, and should be taken into account in planning measures aimed at sanitation of natural foci of leptospirosis.

РЕЗУЛЬТАТЫ ИССЛЕДОВАНИЯ ПОЧВЫ НА ЗАРАЖЕННОСТЬ ПАТОГЕННЫМИ ЛЕПТОСПИРАМИ

Е. В. Карасева, Ю. Г. Черпуха и Т. Ф. Сахарцева

Резюме. В природном очаге лептоспироза на оз. Неро (Ярославская обл., СССР) было исследовано на наличие лептоспир 630 проб почвы. Из почвы изолировано 7 культур лептоспир — 5 патогенных (4 отнесены к серогруппе *Grippotyphosa* и одна *Hebdomadis*) и 2 сапрофитических. Среди изолированных из почвы культур патогенных лептоспир имело место то же количественное соотношение серогрупп, которое было среди культур, полученных от грызунов, что говорит о циркуляции лептоспир от млекопитающих в почву и обратно.

REFERENCES

ALEXANDER A. D., EVANS L. B., LAMBERT W. H., et al., Preliminary leptospiral culture typing findings on 1476 cultures from Malaya and 60 cultures from North Borneo. In: First Inform. exchange in leptospirosis, Wld. Hlth. Org.: 45—49, 1966.

ALSTON J. M., BROOM J. C., Leptospirosis in man and animals. Edinburgh—London, 1958.

ANANYIN V. V., KARASEVA E. V., Prirodnaya ochagovost leptospirozov (Natural focality of leptospirosis). Izd. Meditsina, Moskva, 1961. (In Russian.

KARASEVA E. V., Some peculiar features of the development of epizootic of leptospirosis in *Microtus oeconomus* studied by the method

of radioactive labelling in animals. Zool. Zh. 35: 1384–1389, 1956. (In Russian).
–, LITVIN V. YU., A new method of investigation of natural foci of leptospirosis. Labelling of animals with radioactive phosphorus. Zool. Zh. 47: 344–450, 1968. (In Russian).
–, ZAITSEV S. V., CHERNUKHA YU. G., PISKUNOVA L. A., Some characteristics of ecology of leptospires under natural conditions of natural focus of leptospirosis. Zh. Mikrobiol. Epidemiol. Immunobiol. (Moskva) No 5, 36–40, 1974. (In Russian).
KIKTENKO V. S., Leptospirozy cheloveka (Leptospiroses of man). Izd. Meditsina, Moskva 1954. (In Russian).
–, On natural focality of leptospiroses. Zh. Mikrobiol. Epidemiol. Immunobiol. (Moskva) No 12: 49. 1962. (In Russian).
SMITH G., TURNER C. E., HARRISON L. H., BROOM I. L., Animal leptospirosis in Malaya. I. Methods, zoogeographical background and broad analysis of results. Bull. Wld. Hlth. Org. 24: 5–21, 1961.
TERSKIKH V. I., Leptospirozy lyudey i zhivotnykh (Leptospiroses in man and animals). Izd. Meditsina, Moskva, 1945. (In Russian).

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E. V. K., Laboratory of Medical Zoology, the Gamaleya Institute of Epidemiology and Microbiology of the USSR Academy of Medical Sciences, Moscow, USSR

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J. M. Franz, A. Krieg: Biologische Schädlingsbekämpfung.

(2nd ed.). P. Parey, Berlin 1976, 222 pp., 25 Figs., 14 Tables. Price 29 DM.

The books which appear in a new edition are interesting because they signalize not only their usefulness but also the development in the field during the time from their first edition. The reviewed book definitely has proven its usefulness. It brings a well balanced review of all aspects of biological control and many answers to actual questions of environmentalists concerning techniques and means in pest control with the use of entomophagous insects and pathogens. This main topic is complemented with short outlines of biotechnical methods including the use of atractants, repellents, pheromones, sterile male technics and hormone analogues. In all chapters older data of the first edition are complemented, in many cases whole sections are re-written according to the most recent reports of efficient field trials or large-scale applications. The main changes are in the section on pathogens of invertebrates where four new subdivisions are introduced, treating the use of pathogens in the control of insects and other arthropods, of helminths and snails. General aspects of production, mode of application, side reactions, resistance, impact on useful non-target organisms including man and vertebrates are discussed on the basis of published reports. With the increased selection of references cited at the end of the book it is a useful reference book for insect pathologists and entomologists as well as for workers in the general field of plant protection and environmental management.

Dr. J. Weiser, D.Sc.