Ann Agric Environ Med 2007, 14, 51-56

AN ATTEMPT TO CONTROL A NATURAL FOCUS OF LEPTOSPIROSIS GRIPPOTYPHOSA BY RODENTICIDE – A LONG-TERM STUDY (1977-2004)

Pavol Bakoss[1], Jana Jareková[1], Milan Labuda[2]

[1]Department of Epidemiology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic
[2]Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovak Republic

Bakoss P, Jareková J, Labuda M: An attempt to control a natural focus of leptospirosis grippotyphosa by rodenticide – a long-term study (1977-2004). *Ann Agric Environ Med* 2007, **14**, 51-56.

Abstract: The purpose of the study was to suppress a known natural focus of field fever exerting an influence on animal hosts of leptospires – small terrestrial mammals (s.t.m.) by rodenticide. After repeated application of the zinc phosphide rodenticide, the s.t.m. were regularly live-trapped and checked for leptospirosis by dark-field microscopy and culture of their renal tissue and serological examination. Isolated leptospira strains were typed by help of rabbit factor sera. The deratization influenced the s.t.m. structure considerably: the proportion of the dominant *Microtus arvalis* subjects – the main reservoirs of *Leptospira kirschneri* serovar Grippotyphosa – were gradually and substantionally reduced and, contrarily, the percentage of the potetial hosts subjects – *Clethrionomys glareolus* and *Apodemus flavicollis* – rose decisively over time. Changes in culture and serological positivity for leptospirosis of s.t.m. have also been enregistered. The highest original infestation of *M. arvalis* have slowly but strongly decreased while that of *C. glareolus* and *A. flavicollis* has increased decisively and reached its maximum within the last years of investigation. It is probable that these two animal species have undertaken the leading role in the maintenance of the natural focus of the field fever. In other animal species analogous trends were not registered. Based on these long-term findings, there exists the possibility to suppress only partially and temporarily the epizootic process of leptospirosis in a natural focus that can be desirable in some circumstances (building or free time activities, etc.).

Address for correspondence: Prof. Dr. med. Pavol Bakoss, Dr Sc., Department of Epidemiology, Faculty of Medicine, Comenius University, Špitálska Street 24, 813 72 Bratislava, Slovak Republic. E-mail: pavol.bakoss@fmed.uniba.sk

Key words: leptospirosis grippotyphosa, natural focus, control, rodenticide.

INTRODUCTION

Leptospiroses are worldwide occuring diseases of animals and man occurring worldwide and present a public health and economic problem. Many of them are bound to natural foci. In many respects, leptospiroses may be wieved as emerging or re-emerging infections [13].

Long-term surveys of natural foci of leptospirosis extending beyond 9 years were rarely realized untill now [2]. Also, some attempts to relieve/clear such foci were carried out for only a few months [1].

From November 1978 – November 1980, on an area of 4 hectares a five times repeated application of rodenticide containing zinc phosphide was carried out [10] in a known natural focus of field fever [8] and tularemia in the lowland of Zahorie (Western Slovakia, district of Senica) in order to suppress both infections. The focus was an abandoned orchard covered with shrubs, localized among fields where the climate is warm and relatively dry (average yearly temperature 9ºC with precipitations of 600-650 mm). Eleven years after finishing the deratization, the focus appeared to undergo gradual extinction [5]. This fact led to the

Received: 7 October 2005
Accepted: 28 August 2006

observation of its further existence. The obtained results are presented in this paper.

For a more complex view on the changes registered in this natural focus within 28 years, some findings related to the beginning of its monitoring are briefly demonstrated.

MATERIAL AND METHODS

In the natural focus, small terrestrial mammals (s.t.m.) were regularly live-trapped using Swedish grid metal traps, once quarterly in the years 1977-1984, once monthly in the period from 1991-1994, and only once yearly, in November, in 1998-2004, except for 1998 when the animals were trapped also in March and July, and for 2001 and 2004 when the s.t.m. were examined also in September and June respectively. The November date was based on the highest values obtained of the number of trapped and for leptospiroses positive anaimals in these months during our investigations in 1981-1994.

The renal tissue of the animals was checked for leptospires by dark-field microscopy; since 1991 serological examination of the s.t.m. was also undertaken by micro-agglutination test (MAT) with a battery of leptospiral antigens currently used in our laboratory [9]. If at least one of these examinations gave a positive result, small pieces of kidney tissues were cultured in Korthof's medium in order to isolate leptospires; in the opposite case, the culture was not realized.

Identification of isolated leptospira strains was accomplished with the aid of serogroup specific hyperimmune rabbit sera, and by factor sera [3].

RESULTS

Changes of the structure of s.t.m. in particular years of investigation are presented in Table l and summarized for five different periods of time in Figure 1: years prior the deratization (1977 and 1978), years of deratization (1979 and 1980), the early post-deratization period (1981-1984), the later post-deratization period, i.e. 11-14 yars after deratization (1991-1994) and the last period of examination, i.e. 17-24 years after deratization (1998-2004).

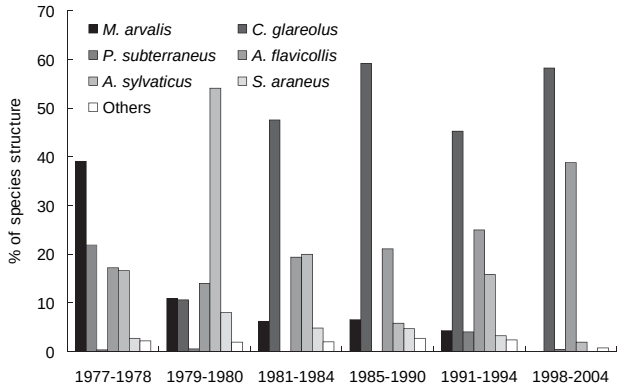

Figure 1. Species structure (%) of small terrestrial mammals in dependence on different time periods in the natural focus of field fever Šaštín.

The proportion of originally dominant species of s.t.m. – *Microtus arvalis* – the main host of *Leptospira kirschneri* serovar Grippotyphosa (out of all trapped animals 39.1%) decreased in the years of deratization by almost ¾ (to 10.10%) and in subsequent years continued to decrease; in the last period of investigation when only a small number of animals was checked, this species was no longer found.

On the contrary, the percentage of the occurence of *Clethrionomys glareolus* – a potential reservoir of the field fever agent – fell from the original 21.9% to a half of this value (10.6%), but in the next two periods of time it strongly increased to 47.6% and 45.3% respectively, and in the last years of investigation even to 58.3% on the yearly average.

Table 1. Species structure (%) of small terrestrial mammals in the natural focus of field fever Šaštín.

Animal species	1977	1978	1979	1980	1981	1982	1983	1984	1991	1992	1993	1994	1998	1999	2000	2001	2002	2003	2004
	Numbers of investigated small terrestrial mammals																		
	351	347	167	219	174	80	152	140	185	211	166	255	35	46	40	46	36	14	54
	Percentage of small terrestrial mammals																		
Microtus arvalis	58.4	19.6	7.2	13.6	13.8	0.0	6.6	0.0	2.7	4.3	5.4	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Clethrionomys glareolus	21.9	22.0	13.2	8.7	23.6	61.2	41.4	76.4	57.3	44.0	35.0	44.4	68.6	82.6	62.5	47.8	50.0	71.4	38.9
Pitymys subterraneus	0.3	0.3	0.0	0.9	0.0	0.0	0.0	0.0	5.4	5.2	0.6	4.3	0.0	0.0	0.0	2.2	0.0	0.0	0.0
Apodemus flavicollis	14.0	20.4	25.1	5.5	15.0	13.8	30.9	15.7	20.5	28.0	24.1	26.3	31.4	17.4	30.0	45.7	50.0	28.6	57.4
Apodemus sylvaticus	1.7	31.7	49.7	57.5	35.6	18.8	14.5	7.2	9.7	15.2	27.1	13.3	0.0	0.0	7.5	0.0	0.0	0.0	3.7
Sorex araneus	3.4	2.0	2.4	12.4	8.0	3.7	5.3	0.7	2.2	2.4	4.2	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Others	0.3	4.0	2.4	1.4	4.0	2.5	1.3	0.0	2.2	0.9	3.6	3.1	0.0	0.0	0.0	4.3	0.0	0.0	0.0

Table 2. Percentage of positive small terrestrial mammals on leptospirosis established by culture and serological examination in the natural focus of field fever Šaštín.

Animal species		1978-1980	1981	1982	1983	1984	1991	1992	1993	1994	1998	1999	2000	2001	2002	2003	2004
Microtus	C	Not examined	8.7	x	40.0	x	20.0	0.0	0.0	0.0	x	x	x	x	x	x	x
arvalis	S		•	x	•	x	20.0	11.1	0.0	16.7	x	x	x	x	x	x	x
	No		24	0	10	0	5	9	9	12	0	0	0	0	0	0	0
Clethrionomys	C		2.3	4.3	6.3	1.9	0.0	0.0	0.0	1.8	4.2	21.1	12.0	9.1	0.0	0.0	0.0
glareolus	S		•	•	•	•	1.9	1.1	0.0	4.4	8.3	15.8	0.0	18.2	0.0	0.0	0.0
	No		41	49	63	107	106	93	58	113	24	38	25	22	18	10	21
Pitymys	C		x	x	x	x	10.0	0.0	0.0	9.1	x	x	x	0.0	x	x	x
subterraneus	S		x	x	x	x	20.0	27.3	0.0	9.1	x	x	x	0.0	x	x	x
	No		0	0	0	0	10	11	1	11	0	0	0	1	0	0	0
Apodemus	C		0.0	0.0	2.1	0.0	0.0	1.7	0.0	3.0	0.0	0.0	8.3	9.5	0.0	0.0	19.4
flavicollis	S		•	•	•	•	0.0	1.7	0.0	8.9	0.0	37.5	0.0	14.3	0.0	0.0	3.2
	No		26	11	47	22	38	59	40	67	11	8	12	21	18	4	31
Others[a]	C		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	x	x	0.0	0.0	x	x	0.0
	S		•	•	•	•	0.0	5.1	0.0	1.9	x	x	0.0	50.0	x	x	0.0
	No		83	20	32	11	26	39	58	52	0	0	3	2	0	0	2
All species of	C		1.7	2.7	5.9	1.4	1.1	0.5	0.0	2.0	2.9	17.4	10.0	8.7	0.0	0.0	11.1
animals	S		•	•	•	•	2.2	3.8	0.0	5.5	5.7	19.6	0.0	17.4	0.0	0.0	13.0
	No		174	75[b]	152	140	185	211	166	255	35	46	40	46	36	14	54

C – % of positive animals by culture; S – % of serologically positive animals only; No – numbers of examined small terrestrial mammals; • – not examined; x – data logically not possible; [a] – all animals as on Table 1 including *A. sylvaticus* and *S. araneus;* [b] – 80 animals captured, 5 out of them not examined.

The percentual occurence of the Pitymys subterraneus subjects was low in all periods of time (0.3-0.5%), in some years even zero, except for the period 1991-1994 when the members of this species were captured annually and their proportion increased significantly (by as much as 4.0%).

The proportin of the subjects of *Apodemus flavicollis* which similarly to the members of *A. sylvaticus* were represented by approximatively l/6 of s.t.m. in the years prior to deratization (17.2% and 16.6% respectively), after its slight decrease during the application of rodenticides it gradually increased in the next periods of time, and in the last period of monitoring reached more than twofold values (38.8%) compared to those obtained prior deratization.

On the other hand, the relative occurence of the members of *A. sylvaticus* after its temporary increase in the years of deratization (as much as 54.1%) decreased gradually in the later periods of time, and finally become only insignificantly representative (1.9%).

Although the percentage of the subjects of Sorex araneus increased threefold in the time of rodenticide application (from 2.7% to 8.0%), it decreased to the initial values in the later years.

During the whole period of investigations, there were in additionally trapped 17 subjects of *Crocidura leucodon* (predominantly, i.e. 14 × in years 1991-1994), 13 × *Talpa europea* (only within the years 1977-1980), 6 × *Mustala nivalis*, 6 × *A. microps*, 5 × *Sorex minutus*, 2 × *Mustela minuta*, 2 × *Micromys minutus*, 2 × *Apodemus* spp. (species not determined), 1 × *Neomys fodiens* and l × *Mus musculus*.

In the course of focus monitoring, there were also registered changes in serological and culture positivity of s.t.m. (Tab. 2, Fig. 2).

In the early post-deratization period (1981-1984), the highest culture positivity was found in the already strongly decimated *M. arvalis* subjects (17.6%), while it was five times lower among *C. glareolus* members (3.5%). In 1983, an epizootic of leptospirosis was recorded: the culture positivity in all animal species together reached as much as 5.9% and at this time also one leptospira strain was isolated from a further animal species – *A. flavicollis*.

In the later post-deratization period (1991-1994), only 2.9% culture positivity was found in *M. arvalis* subjects; this means that their positivity was six times lower than in the previous period. Moreover, a further 8.6% of subjects belonging to this species and giving negative results of the culturing, were serologically positive for leptospirosis grippotyphosa. The percentage of culture positive members of. *C. glareolus* decreased to 0.5%; nevertheless, the number of isolated leptospira strains was l and 2 in favour of *C. glareolus*. On the other hand, the culture positivity in *A. flavicollis* subject increased to 1.5%, above all in *P. subterraneus* members to 6.1%; moreover, an other 15.2% of animals of the latter species had positive serology. In this way, *P. subterraneus* became the most infected species among the s.t.r. at that time. There were also three serologically positive animals belonging to other species, namely 2 × *M. nivalis* a 1 × *C. leucodon*. In 1994, a new activation of the natural focus was recorded – the overall culture positivity of s.t.m. achieved 2.0% and 5.5% of other subjects had positive serology.

In the last period of observation (1998-2004), the culture positivity was found in 8.9% of animals *C. glareolus* and a further 7.6% members of this species were serologically positive. Analogous findings were obtained with subjects of *A. flavicollis* – 8.6% and 6.7% respectively. In addition,

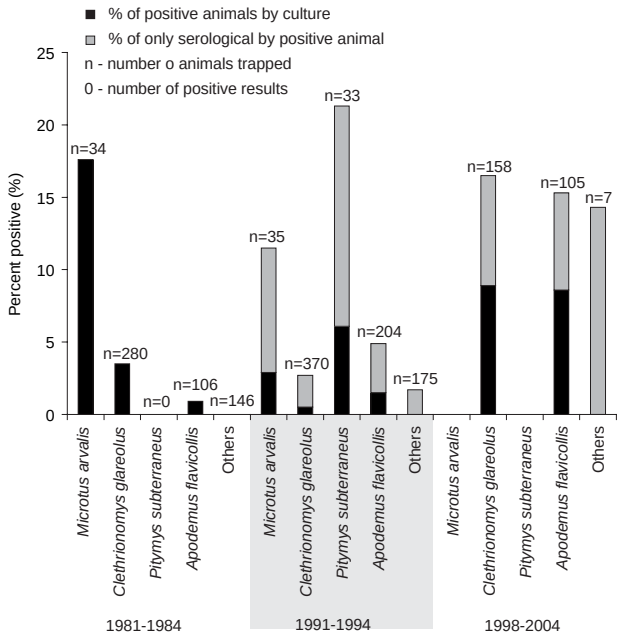

Figure 2. Percentage of positive small terrestrial mammals on leptospirosis established by culture and serological examination in dependence on three time periods in the natural focus on the field fever Šaštín.

one animal belonging to *Apodemus* spp. was only serologically positive. The positivity found only in two animal species is evidently due to the low number of trapped animals. In this period of time, two epizootic waves of leptospirosis among s.t.m. were also detected. The first one occured in 1999 when the overall culture positivity of checked animals was very high for this focus of leptospirosis – 17.4%, and a further 19.6% presented only positive serology. The second epizootic wave occured in 2004 when these values reached 11.1% and 1.9% respectively.

Out of 47 leptospira strains isolated within the whole period of investigation, 42 of them belonged to the serovar Grippotyphosa, 3 belonged to the serovar Jalna (serogroup Australis), one to the serogroup Sejroe, and one strain was lost prior to its identification.

DISCUSSION

The repeated application of a rodenticide on the territory of a natural focus of field fever and tularemia in l978-1980 had a significant and long-term effect (28 year period of investigation) on the structure of s.t.m. and their infestation with leptospires. The originally numerous main reservoir of the strains belonging to the serovar Grippotyphosa – *Microtus arvalis* – has gradually lost its dominant positioun (Tab. 1). In some years after deratization [1982, 1984], the subjects of this species were not trapped at all. Nevertheless, the culture positivity of *M. arvalis* was still high in the post-deratization period (1981-1984) (Fig. 2). However, *Clethrionomys glareolus* successively became the dominant species. Even though the culture positivity of this species was low at that time, 9 leptospira strains were isolated from the latter species while only 6 strains from the former. In 1983, during an epizootic, one strain was isolated also from *A. flavicollis*. In this way, the participation of *C. glareolus* in the maintenance of the natural focus must have been non negligible already at that time. The role of other animal species in the circulation of leptospires was not detected.

In the later post-deratization period (1991-1994), the picture of animal structure did not change substantionally, except for the species *P. subterraneus*, the frequency of which rose considerably to 4%. At that time, the culture positivity of *M. arvalis* was only 2.9% even though 8.6% of other members of this species gave positive serologic results; therefore approximatively 1/10 of these animals must have been afflicted with leptospirosis.

Serological positivity of s.t.m. without culture positivity can be explained by the fact that some animals are able to get rid of leptospires [the majority of them remain lifelong infected], and by the possibility that not in every case a proper/suitable piece of renal tissue containing leptospires is taken for culture [2].

Transient low culture and serological positivity were also found in *C. glareolus* subjects at that time. The relatively most infected species was *P. subterraneus*. *A. flavicollis* also participated in the circulation of leptospires to a certain extent. All 4 mentioned species exhibited positivity for leptospirosis, especially during the epizootic wave in 1994 when the overall culture and serological positivity alone reached 2% and 5.5% respectively. The smaller number of animals captured in 1993 (Tab. 1) is evidently due to the epidemic of tularemia the previous year which partially eliminated the population of s.t.m.

In absolute terms, the number of culture positive subjects relative to different species of s.t.m. in the period 1991-1994 was as follows: *M. arvalis* – 1×, *C. glareolus* – 2×, *P. subterraneus* – 2× and *A. flavicollis* – 3×; the total number of infected animals (including serologically positive ones) in these species was 4, 10, 7 and 10. Thus, the number of the reservoirs of infection was the lowest in the main host of the serovar Grippotyphosa i.e. *M. arvalis*.

In the last period of observation (1998-2004) the number of animals caught was very small. Therefore the trapping of *M. arvalis* and *S. araneus* was not expected. A different situation was with the species *P. subterraneus* – on the basis of previous results about 11 trapped animals were expected instead of one actually obtained. This gives evidence of the decreased frequency of members of this species. To a certain extent, an analogous situation was observed with *A. sylvaticus*, too. In this way, *C. glareolus* and *A. flavicollis* remained the dominant species (average yearly percentage 97.1). Only from these subjects were leptospira strains isolated (positive culture in 8.9% and 8.6% respectively), and only among the members of these species were further serologically positive animals found (7.6% and 6.7% respectively). In absolute terms, 14 leptospira strains were isolated from *C. glareolus* and 9 from *A. flavicollis*, the

total number of culture positive and serologically positive animals was 26 and 16 respectively. Even though it is not possible to exclude the participation of the main host, i.e. *M. arvalis*, in the circulation of *Grippotyphosa* strains, it seems probable that the leading role in the maintenance and casual activation of the natural focus of leptospirosis have been undertaken by the potential reservoirs *C. glareolus* and *A. flavicollis*.

In 1999 and 2004, the activation of the investigated natural focus was registered. In the first case, the culture positivity reached for this focus an unprecendented value of 17.4% and an overall infectivity rate of 37%; the previous year these values were 6-times and 4.3-times lower. In 2004, 11.1% of culture positivity and 24.1% of overall positivity (including serologically positive animals) was found (two years before these values were equal to zero). It is curios that the positive animals belonged only to *A. flavicollis* (except for one serologically positive subject of *A. sylvaticus*) and none to *C. glareolus*, in spite of the fact that 38.9% of the trapped animals were members of the latter species. Another surprising fact was the isolation of not only Grippotyphosa strains but for the first time, within 23 years of observation, also the isolation of strains belonging to the serovar Jalna (serogroup Australis) whose more important reservoirs in our region are *Apodemus* spp., *C. glareolus*, *M. arvalis*, and to the serogroup Sejroe which has different reservoirs, namely *Mus musculus*, *Apodemus* spp., hedgehogs, dogs and pigs. It is worth mentioning that the s.t.m. trapped from 1981-1985 in a nearby wooded area and checked for leptorspiroses were negative [6]. On the other hand, in the district where the natural focus is situated, serological positivity of bovine [4] and exceptionally of small terrestrial mammals [8] with the Sejroe group strains was observed. However, this can hardly explain the isolation of the leptospira strain from the group Sejroe in the monitored natural focus of field fever in 2004. Thus, this finding remains unexplained and therefore further investigations in this focus are needed.

The activation of the field fever focus occured in the years 1983, 1994, 1999 and 2004 (the presumptive activation of the focus in 1988 or 1989 could not be verified as at that time the investigation had not been realized). In spite of this, the activation of the focus seems to be a cyclic natural process. When such a phenomenon occurs, the infection can temporarilly spread from the main host(s) to other animal species, which is happened in 1983 (the participation of *A. flavicollis* in the circulation of leptospires) and in 1994 (the link of *P. subterraneus* in the chain of infection spread).

The role of *M. arvalis* in the epizootics in years 1994 and 2004 could not be judged because no subject of this species was trapped. With regard to the long-term excretion of leptospires via urine of these animals and the persistance of the etiological agent in the soil for months also in the winter season (if there are convenient humidity and pH) [14], *M. arvalis* can participate in the spread of infection among s.t.m., even though the number of its subjects is small. If the population density of s.t.m. increases simultaneously, the number of reservoirs of leptospires will also grow [1]. Indeed, in our cases too a greater number of s.t.m. was captured in the years of epizootics compared to the years before activation of the focus using the same method of capture (equal number of traps posted on the same lines). The climate conditions at the time of activating of the natural focus were also convenient: the average temperature above normal or at least some warm summer months (2004), and at least some wet months (June, October, November in 2004), or normal intensity of precipitations (1983).

It is difficult to evaluate the influence of the deratization in the natural focus of field fever on the occurance of this disease in humans. The reason is the small number of diagnosed and notified human field fever cases. In the district where the natural focus is localized, occurence of this disease is registered exceptionally. However, if we take into account a region larger than this district, e.g. Western Slovakia (to which the mentioned district also belongs), a certain, approximately a 10-year cycling of the increase of the incidence rate can be found. This was also proved by chronobiometric analysis [11]. However, the years of the increased incidence rate of field fever in humans do not necessarilly correspond with those of the activation of the natural focus. For example, in years 1983 and 2004, at the time of epizootics among s.t.m., no rise in the number of human cases occured. However, such a situation was registered during another epizootic wave in 1999 (as well as also two years before) and at the time of presumptive activation of the focus in 1988. Evidently, the occurence of human cases of field fever is influenced also by factors other than the actually occuring epizootic among s.t.m. themselves.

CONCLUSION

Application of a rodenticide in a natural focus of field fever influenced significantly the species structure of s.t.m for a long time. Instead of the dominant species, *M. arvalis* – the main host of the leptospira strain Grippotyphosa [the percentage of which gradually decreased 10-fold], the occurence of potential reservoirs of this infection – *C. glareolus* and *A. flavicollis* – markedly increased (approximately 3-times and 2 to 3-times, respectively). The degree of infestation of these three animal species with leptospires also changed: the circulation of field fever agents progressively began to be materialized, largely among the subjects of the potential hosts, instead of among those of the main reservoir.

This long-term study, like some older sporadic short-time investigations [1, 2, 7], points out that it is possible to suppress, at least partially and for a short period of time, the activity of the epizootic process in a natural focus of field fever. In our case, this was due to the significant decrease of the percentual proportion of *M. arvalis* subjects

(but not of the degree of their infestation by leptospires!). In spite of a marked percentage rise of *C. glareolus* and *A. flavicollis* species members among s.t.m., their infestation remained low for many years, and became significantly higher only within last years of the investigation. At that time, the subjects of these two animal species have undertaken the principal role in the maintenance of the natural focus in which, even 24 years after deratization, epizootic waves of leptospirosis could be observed. Thus, our original opinion that due to the chemical treatment the natural focus progressively becomes extinct [5], was not confirmed.

On the basis of our long-term findings, there exists the possibility to suppress temporarily and partially the epizootic process of leptospirosis in a natural focus. This can be desirable in the case of necessity to adapt the ground for building or for varied free time activities [e.g. camping], etc. However, an entire extinction or at least a long-term suppression of a natural focus of field fever by chemical intervention cannot be expected.

Acknowledgements

This study was supported in part by the Slovak Grant Agency VEGA, Projects No. 1/54/92, No.1/5160/98, No.1/8293/01, No. 1/1166/04 and APVT Project No.51-004702.

The authors wish to thank Drs J. Lysý and O. Kožuch for trapping and species identification of small terrestrial mammals and Mrs I. Takáčová, T. Smidová, J. Pribullová, M. Siebenstichová, J. Benčíková for their excellent technical assistance.

REFERENCES

1. Ananyin VV (Ed.): *Leptospiroses in Men and Animals.* Medicina, Moscow 1971 (in Russian).

2. Ananyin VV, Karaseva JV: *Natural Foci of Leptospiroses*. Medgiz, Moscow 1961 (in Russian).

3. Dikken H, Kmety E: Serological typing methods of leptospiroses. In: Bergan T, Norris JR (Eds): *Methods in Microbiology*, 259-307. Academic Press, London, New York, San Francisco 1978.

4. Hudecová H: *Epizootologic Study of Domestic animals' Leptospiroses Pertaining to the Region of the State Veterinary Institute of Bratislava*. Dissertation. Institute of the Teaching and Education of Veterinarians, Košice 1997 (in Slovak).

5. Jareková J, Bakoss P, Lysý J: Influence of rodenticide on natural focus of field fever. Preliminary report. *Biológia* (*Bratislava*) 1993, **48**, 279-281.

6. Jareková J, Kmety E: Investigation of the evolution in natural foci of leptospiroses in selected localities of the lawlands Záhorie. Entomol. *Problems* (*Bratislava*) 1989, **19**, 347-355 (in Slovak).

7. Karaseva JV, Ananyin VV, Aguzarova MCh: Experience in reducing the natural leptopsirosis focus. *Zh Mikrobiol Epidemiol Immunol* 1965, **4**, 65-69 (in Russian).

8. Kmety E: Field voles as reservoirs of field fever and other leptospiroses. *Bratisl Lekár Listy* 1951, **31**, 118-125 (in Slovak).

9. Kmety E, Bakoss P: To the question of the selection of antigens for micro-agglutination test in leptospiroses. *Czechoslov Epidemiol* 1978, **27**, 247-252 (in Slovak).

10. Lysý J, Řeháček J, Kocianová E, Kožuch O, Nosek J, Grulich I: Results of zoological and parasitological survey in some localities of the lawlands Záhorie in years 1977-1985. *Entomol Problems* (*Bratislava*) 1989, **19**, 297-317 (in Slovak).

11. Mikulecký M Sen., Bakoss P, Kmety E: Leptospiral infections in Slovakia 1949-1995: Trends and periodicities. **In:** Mikulecký M Sen. (Ed.): *Proceedings of the 3rd Internat. Workshop Chronobiology and its roots, High Tatras,* Slovakia 2-6 September 1997. Slovak Med. Soc., Bratislava 1997.

12. *Surveillance of Zoonoses. Slovak Republic. Leptospiroses 1970-2004*. State Veterinary and Food Administration, Bratislava 1970-2004.

13. WHO: *Human Leptospirosis: Guidance for Diagnosis, Surveillance and Control*. Internat. Leptosp. Society, WHO 2003.

14. Zaytsew SV, Chernukha JG: Possibility of survival of pathogenic leptospirae in the soil of a natural focus. **In:** Litvin VJ, Chernukha JG, Nikitina NA, Tartakovskij IS, Volk AV, Kotovich MN (Ed.): *Ecology of Agents of Sapronoses*, 85-94. Gamaleya Institute, Moscow 1988 (in Russian).