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PŮVODNÍ PRÁCE

Human Mozdok leptospirosis first diagnosed by serum agglutinin-absorption tests in the Slovak Republic

Bakoss P.[1], Awad-Masalmeh M.[2], Resch G.[2, 3], Jareková J.[1], Stanko M.[4], Perželová J.[1]

[1]Comenius University, Faculty of Medicine, Department of Epidemiology, Slovak Republic
[2]University of Veterinary Medicine, Clinic for Ruminants, Department of Food Animals and Herd Medicine, Vienna, Austria
[3]Ministry of Agriculture, Environment and Watermanagement, Vienna, Austria,
[4]Slovak Academy of Sciences, Institute of Zoology and Institute of Parasitology, Košice, Slovak Republic

ABSTRACT

Introduction: The serovar Mozdok related leptospirosis in humans were not yet feasibly diagnosed using merely the standard micro-agglutination test (MAT) what was perhaps due to the impossibility to distinguish them from illnesses that are caused by *Leptospira* strains belonging to other serovars of the serogroup Pomona. On the contrary, leptospires of the Mozdok serovar were cultured from rodents and domestic animals world-wide including Central Europe where only *Leptospira* strains of the serovars Pomona and Mozdok are known to be present till now.
Study objective: The aim of the study was to discover if leptospires of Mozdok serovar may cause human leptospirosis that remained hidden till now among infections diagnosed merely by MAT as Pomona illnesses.
Material and Methods: The reference *Leptospira* strains of Pomona and Mozdok serovars (Pomona and 5621), as well as three endemic, and in some tests only two strains of human and pig origin (Šimon, S-23, Pöstényi), and two strains of rodent provenance – *Apodemus agrarius* (M-210/98 and M-71/01) were used for this purpose. First, the endemic strains were assigned to one of the afore-mentioned two serovars by agglutinin cross-absorption tests performed using rabbit immune sera, monoclonal antibodies and random amplified polymorphic DNA methods. Afterwards, twenty-one sera of patients with a Pomona leptospirosis confirmed by MAT were examined by agglutinin absorption test (AAT).
Results: Based on the results of the mentioned laboratory method used, the endemic *Leptospira* strains of human and pig origin could be affiliated to the serovar Pomona, while those of rodent origin were classified as serovar Mozdok strains.
Out of the 21 patients sera, an illness caused by the serovar Mozdok strains was found out in 13 cases and a disease caused by serovar Pomona strains in 8 cases. Their differentiation was made on the strength of the following results of AATs: All strains from the serovar Mozdok have completely absorbed antibodies (anti-Pomona and anti-Mozdok) from the tested sera, however following the absorption of these sera with the Pomona strains, high levels of residual antibodies reacting in MAT with the Mozdok strains have still persisted. In this way, it was possible to prove the Mozdok infection in thirteen patients.
On the contrary, following the absorption of the sera with the strains of the serovar Pomona, a complete absorption of all antibodies (anti-Pomona and anti-Mozdok) was achieved in seven cases using the strain Šimon, and in one case with the strain S-23, whereas after absorption using the Pomona strain, the residual antibodies were still present in all sera, and also in the majority of them when they were absorbed using the strains S-23 and Pöstényi. In this context, the Pomona infection was determined in the case of eight patients. Hence it follows that not all strains of the Pomona serovar were suitable for the AATs.
Conclusion: The presence of the human Mozdok leptospirosis was confirmed for the first time by the use of the agglutinin absorption test. A clear correlation between the habitat areas of the *A. agrarius* and the patients who were infected with the strains of the Mozdok serovar was determined.

KEYWORDS

***Leptospira* – serovar Mozdok – human diseases – natural foci**

SÚHRN

Bakoss P., Awad-Masalmeh M., Resch G., Jareková J., Stanko, M., Perželová J.: Leptospiróza Mozdok u ľudí diagnostikovaná po prvý raz v Slovenskej republike absorpčnými testami sérových aglutinínov
Úvod: Leptospiróza ľudí spôsobená sérovarom Mozdok nebola doposiaľ jednoznačne diagnostikovaná, čo bolo možno spôsobené tým, že mikroaglutinačným testom (MAT) ju nemožno odlíšiť od ochorení spôsobených kmeňmi iných sérovarov leptospír patriacich do sérologickej skupiny Pomona. Naopak, leptospíry sérovaru Mozdok sa izolovali z hlodavcov a domácich zvierat na celom svete, vrátane strednej Európy, kde sa vyskytujú iba kmene sérovarov Pomona a Mozdok.
Cieľ práce: Cieľom bolo zistiť, či leptospíry sérovaru Mozdok môžu spôsobiť ochorenia ľudí, ktoré mohli byť doposiaľ skryté medzi leptospirózami zapríčinenými leptospírami sérovaru Pomona a diagnostikovanými iba použitím MAT.
Materiál a metódy: Použili sa referenčné leptospírové kmene sérovarov Pomona a Mozdok (Pomona a 5621) a tri, v niektorých testoch iba dva endemické kmene izolované od ľudí a ošípaných (Šimon, S-23 a Pöstényi), a dva domáce kmene od hlodavcov – *Apodemus agrarius* (M-210/98 a M-71/01). Prvým nevyhnutným krokom bolo zatriedenie endemických kmeňov do jedného z dvoch uvedených sérovarov pomocou krížových absorpčných testov aglutinínov králičích imúnnych sér (ACAT), monoklónových protilátok a metódou náhodnej amplifikácie polymorfnej DNA. Následne sa vyšetrilo absorpčnými testami aglutinínov (AAT) dvadsaťjeden sér pacientov diagnostikovaných iba pomocou MAT ako leptospirosis Pomona.
Výsledky: Na základe výsledkov použitých metód sa dali zatriediť endemické leptospírové kmene izolované z ľudí a ošípaných do sérovaru Pomona a izoláty z hlodavcov do sérovaru Mozdok.
Z 21 pacientskych sér zistila infekcia leptospírami sérovaru Mozdok 13 krát a ochorenie vyvolané kmeňmi sérovaru

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Pomona 8 krát, a to podľa týchto výsledkov AAT: Všetky kmene sérovaru Mozdok absorbovali z testovaných sér všetky protilátky (anti-Pomona aj anti-Mozdok), kým po absorpcii týchto sér kmeňmi Pomona perzistovali ešte protilátky reagujúce v MAT s kmeňmi Mozdok. Tak sa dokázalo ochorenie Mozdok u trinástich pacientov.
Na druhej strane po vysýtení týchto sér kmeňmi sérovaru Pomona sa absorpcia všetkých protilátok (anti-Pomona a anti-Mozdok) dosiahla iba kmeňmi Šimon – 7 krát a S-23 – 1 krát, zatiaľ čo po ich vysýtení kmeňom Pomona ostávali reziduálne protilátky vo všetkých sérach a vo väčšine z nich aj po absorpcii kmeňmi S-23 a Pöštényi. Takto sa dokázalo ochorenia Pomona u ôsmich pacientov. Ukázalo sa, že nie všetky kmene sérovaru Pomona boli vhodné na AAT.
Záver: Po prvý raz sa potvrdila leptospiróza Mozdok u ľudí absorpčnými testami sérových aglutinínov. Zistila sa tiež jasná korelácia medzi areálmi prírodných ohnísk *A. agrarius* a pacientmi infikovanými leptospírovými kmeňmi sérovaru Mozdok.

KĽÚČOVÉ SLOVÁ
***leptospira* – sérovar Mozdok – ochorenia ľudí – prírodné ohniská**

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INTRODUCTION

In Central Europe, within the serogroup Pomona besides leptospires of Pomona serovar (strain Pomona, genospecies *interrogans*) the occurrence of leptospires of Mozdok serovar (strain 5621, genospecies *kirschneri*) was recorded till now. Both strains have been approved as reference strains by the Subcommittee on the Taxonomy of Leptospira [1]. The latter strain was isolated in 1961 [2]. The strains of the two serovars have been reliably distinguished by the use of monoclonal antibodies [3, 4, 5] while the classical polyclonal antibody cross-absorption tests performed using rabbit antisera yielded equivocal results obtained by different authors [quoted 6].
Regarding the strains of serovar Pomona, their maintenance reservoirs are mainly cattle and swine, for those of the serovar Mozdok, they are particularly wild mammals i.e. in mainland Europe above all the black-striped field mouse – *Apodemus agrarius* while in England the short-tailed vole – *Microtus agrestis* presents the maintenance hosts [7, 8]. Domestic animals can be also afflicted with this infection [7, 9].
The presence of serovar Mozdok related infections in feral and domestic animals, mostly pigs, have been proved worldwide. In Central Europe, it was recorded in Poland [10], probably in the Czech Republic [11] and among the distant European countries in Germany [9], Portugal [12], and England [13]. In spite of this, Mozdok infections in humans were not yet feasibly diagnosed, which is probably due to the impossibility to distinguish them using merely micro-agglutination test (MAT) from illnesses related to Pomona strains. Therefore the patients whose sera reacted in MAT with the generally used strain Pomona had simply been qualified as having Pomona disease (swineherd's disease). Nevertheless, in a recent study the presence of possible Mozdok infection in humans was assumed but not proved; this was based only on the assumption that the infected person, as well as the reservoirs such as wild small rodents and pigs (from which the Mozdok strains were isolated), were all present in the same region of the country [14].
In the Slovak Republic, cases of human Pomona related illnesses have been recognized by serological examination (MAT) since 1949. In the following years, endemic strains of animal and human origin from the Pomona serogroup were isolated.
On the basis of our preliminary experience [15] leptospires belonging to Pomona and Mozdok serovars both occurring in Slovakia (the latter originate mainly in black-striped field mice) the present study was aimed toward the determination of whether the serovar Mozdok strains may also play a role in human pathology in the country.

MATERIAL AND METHODS

Serum samples of 21 patients were identified by MAT to be positive with serogroup Pomona strains. They all were examined using a battery of *Leptospira* strains that occur in Slovakia and present the serogroups Icterohaemorrhagiae, Grippotyphosa, Sejroe, Javanica, Pomona, Canicola, Australis, Tarassovi, Bataviae, Ballum, Autumnalis and Pyrogenes. Sera having a titre not less than 1:3200 to the strains of both Pomona and

Table 1. Number of patients relative to their regional distribution and causal serovar of leptospires

Profession or social group	Geographic regions of Slovakia					
	East		West		Middle	
	Mozdok	Pomona	Mozdok	Pomona	Mozdok	Pomona
Butchers, pig breeders	1	0	2	5	1	1
Pensioners	4	0	1	1	0	1
Others *	4	0	0	0	0	0

*milk made, locomotive driver, unemployed, unknown profession

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Figure 1. Chart of the Slovak republic illustrating the regional distribution of patients with Pomona and Mozdok diseases, sites of isolation of Mozdok strains from Apodemus agrarius and areas of this feral rodent

Mozdok serovars were chosen as suitable candidates for further examination.

Nine of the patients were originally from the East Slovakia, 9 from the West Slovakia and 3 from the Middle Slovakia (Table 1). Sixteen were males and five were females. Ten were butchers, seven pensioners (one of them came into contact with pigs), and a milk maid on a farm, a locomotive driver, an unemployed while the profession of the last one was not recorded.

***Leptospira* strains** used in this study were the reference strains Pomona (serovar Pomona) and 5621 (serovar Mozdok), the endemic strains Šimon (human origin, isolated in East Slovakia, 1954), S-23 (pig, West Slovakia, 1955) and Pöštényi (human, from West Slovakia, 1961) as well as M-210/98 and M-71/01 (both isolated from *A. agrarius* (East Slovakia, 1998 and 2001 respectively) – Table 2.

Microscopic agglutination tests were performed in the regular way using double dilution technique (geometrical progression), starting with a serum dilution of 1 : 100 [16].

Agglutinin cross-absorption tests (ACATs) realized with **rabbit immune sera** were used in order to class the endemic strains of leptospires into one of the two afore-mentioned serovars Pomona and Mozdok. The technique was repeatedly described in the professional literature [e.g. 16, 6].

Rabbit immune sera against *Leptospira* strains were prepared conventionally [16]. The titres of non-absorbed sera were 1:6400 or 1:12800. Endemic *Leptospira* strains Pöštényi and M-71/01 were only used in MATs performed with absorbed rabbit sera, as "twin" strains of those belonging to serovars Pomona and Mozdok respectively. The majority of ACATs was repeated more than twice.

Monoclonal antibodies (MAbs) F48C3, F58C1-2 and F61C7-1 [3] kindly provided by the Royal Tropical Institute, Amsterdam were used for MAT (starting dilution 1:20) with the *Leptospira* strains under study.

The random amplified polymorphic DNA (RAPD) was realized with each examined *Leptospira* strain and a phylogenetic tree was constructed according as described recently [15].

The **agglutinin-absorption test** (AAT) with **patient's sera** was performed in a reversed arrangement, i.e. reversely to the classic agglutinin cross-absorption test. Concerning the sera of our 21 patients, with the aim of distinguishing etiology of the diseases on serovar level, the 10% criterion of the Subcommittee on the Taxonomy of *Leptospira* [1] was applied reversely, as was described earlier [17, 18] as well as in this study: for the causal Leptospira strain of the illness was considered that strain after absorption by which less than 10% of antibody titre of the unabsorbed patient's serum remained in repeated tests. Later to the *Leptospira* strains used for absorption of rabbit immune sera, absorbing strains Pöštényi (serovar Pomona) and M-71/01 (serovar Mozdok) in case of human sera were also added.

RESULTS AND DISCUSSION

Serovar affiliation of the endemic *Leptospira* strains to the serovars Pomona or Mozdok by different laboratory methods was the first inevitable step of the study.

The results of ACAT are on Table 2. They demonstrate that the endemic strains Šimon and S-23 belong to serovar Pomona (lines 2, 3, 7, 8, 12, 13) even though the antigenic composition of the latter strain revealed to be a little more complex compared with the strains Pomona and Šimon (lines 12, 13). The strain Pöštényi reacted analogously to the strain S-23. On the other hand, the endemic strain M-210/98 could be affiliated to the serovar Mozdok but seems to be slightly richer in antigenic components in comparison with the reference strain 5621 (lines 20, 25).

The immune sera prepared against those three strains of the serovar Pomona after absorption with the serovar Mozdok strains still agglutinated agglutinated in MATs the homologous Pomona strains over the 10% antibody level (lines 4, 5, 9, 10, 14, 15). Vice versa, in both immune sera of serovar Mozdok after their absorption with serovar Pomona strains, there were still residual antibodies sufficient to agglutinate mainly the endemic Mozdok strains but to a lesser extent the reference 5621 strain over the 10% level (lines 17–19, 22–24). The outcome obtained with the last-named strain corresponds with the discrepancy related to its separate serovar status observed by different authors performed with ACATs method [6]. Nevertheless, the results of MATs with MAbs and those of RAPD used in this study confirmed that all three Mozdok strains form a homogenous group distinct from the Pomona strains on serovar level.

The results of MAT with MAbs confirmed the results obtained by ACATs and showed that all strains of the Pomona and Mozdok serovars could be distinguished unambiguously. The strains of both groups reacted with MAbs F48C3 proving their affiliation to serogroup Pomona. In contrast, the strains of Mozdok serovar reacted with the MAbs F58C1-2 to the titres 1 : 640 (strain 5621) and to 1 : 1240 (strains M-210/98 and M-71/01) and with the MAb F61C7-1 to the titre 1 : 2560, while all the three strains of the serovar Pomona gave negative reactions at serum dilution 1 : 20. The outcome corresponds to that obtained by other authors with the MAbs used in our tests [3, 4, 5].

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Table 2. Results of agglutinin cross-absorption tests with reference and endemic *Leptospira* strains

Line	Immune serum	Absorbing strain	Microagglutination test with antigens						
			Pomona	Šimon	S-23	Pöstényi	5621	M-210/98	M-71/01
1	Pomona	unabsorbed	100.0	100.0	50.0	50.0	100.0	100.0	50.0
2		Šimon	– [a]	–	–	–	–	–	–
3		S-23	–	–	–	–	–	–	–
4		5621	12.5-25.0[b]	12.5	12.5	12.5	–	–	–
5		M-210/98	1.6-12.5	1.6-12.5	1.6-12.5	< 1.6-12.5	–	–	–
6	Šimon	unabsorbed	100.0	100.0	50.0	100.0	50.0	100.0	50.0
7		Pomona	–	–	–	–	–	–	–
8		S-23	–	–	–	–	–	–	–
9		5621	50.0	100.0	50.0	.	–	–	–
10		M-210/98	12.5-50.0	12.5-50.0	6.3-50.0	6.3-50.0	–	–	–
11	S-23	unabsorbed	50.0	50.0	100.0	100.0	25.0	25.0	50.0
12		Pomona	–	6.3-12.5	6.3-12.5	6.3-25.0	–	–	–
13		Šimon	–	–	6.3-12.5	6.3-12.5	–	–	–
14		5621	12.5	50.0	25.0	.	–	–	–
15		M-210/98	12.5	50.0	25.0	.	–	–	–
16	5621	unabsorbed	100.0	100.0	50.0	100.0	100.0	100.0	100.0
17		Pomona	–	–	–	–	–	25.0	12.5-50.0
18		Šimon	–	–	–	.	12.5	25.0	25.0
19		S-23	–	–	–	.	12.5	25.0	25.0
20		M-210/98	–	–	–	–	–	–	–
21	M-210/98	unabsorbed	50.0	50.0	25.0	50.0	50.0	100.0	100.0
22		Pomona	–	–	–	–	–	25.0	50.0
23		Šimon	–	–	–	–	–	12.5-50.0	12.5-50.0
24		S-23	–	–	–	–	3.1-12.5	25.0	12.5-25.0
25		5621	–	–	–	–	–	6.3-12.5	6.3-12.5

Note: Antibody titres expressed by the reciprocal of serum dilution
Titres of unabsorbed sera were 1:6400 – 1:12800
[a] < 10 % of reciprocal antibody titre of unabsorbed immune serum
[b]result of repeated test
.not realized

By the used RAPD, an apparent differentiation between the strains belonging to Pomona serovar and those assigned to Mozdok serovar was made possible, their affiliation to one of the two serovars was therefore feasible. Even though some differences among the serovar Pomona strains were found they all form a cluster of closely related strains yet with a slightly different DNA patterns (Figure 2). This method with the used primer(s) allowed not only to differentiate between the two genospecies of the genus *Leptospira* (*interrogans* from *kirschneri*) but also showed a good correlation between these genospecies and the phenotypes of serovars Pomona and Mozdok. Such a correlation was also proved with *Leptospira* strains belonging to different serovars from other serogroups [15]. Using each of the three methods, the isolates M-210/98 and M-71/01 of *Apodemus agrarius* origin could be affiliated to serovar Mozdok while the strain S-23 of pig origin, and Šimon and Pöstényi of human origin could be assigned to serovar Pomona. In this way the suitability of endemic besides reference strains for examination of patient's sera by absorption tests was revealed.

Patients' sera. Based on our earlier experiences regarding the proof of the usefulness of the serum AATs in the serovar diagnosis of human Australis and Sejroe leptospirosis [17, 18], the sera of patients diagnosed merely by MAT and classified simply as Pomona disease were examined using AATs. It was ascertained that both the Pomona and Mozdok human infections may be also serologically well discriminated using this method. Thirteen of 21 examined patients were affected by Mozdok illness and eight by Pomona illness.

Without going through the details, it is necessary to stress that the serum antibodies of the patients who were affected by Mozdok after absorption by using all the strains of the Mozdok serovar (e. g. 5621, M-210/98 and M71/01) gave virtually the same, unequivocal results using MAT: All three strains of the Mozdok serovar did not only absorb the corresponding Mozdok antibodies but

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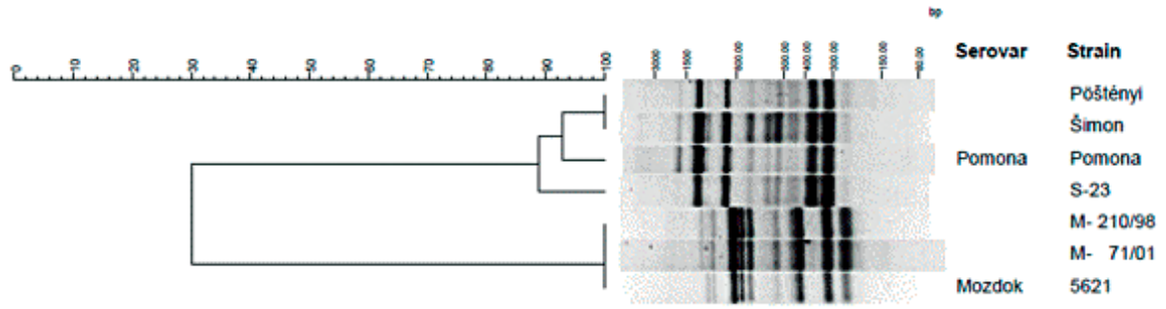

Figure 2. DNA fingerprints and relatedness between reference and endemic *Leptospira* strains of serovars Mozdok and Pomona (serogroup Pomona)

also those reacting in MAT with all strains of the serovar Pomona (e.g. Pomona, Šimon, S-23, Pöštényi). On the other hand after absorption of these sera with the four strains of the Pomona serovar, very high levels of residual Mozdok antibodies still persisted. In this way, it was possible to confirm Mozdok infection in these patients, as well as the identity or at least a very close antigenic relation of these absorbing strains (Table 3).

It is also inevitable to point out that in Mozdok sera absorbed by the serovar Pomona strains, differences in residual antibodies among the Pomona strains were registered in MAT, considering only this group of leptospires and not the Mozdok group. While in all Mozdok sera except for one of them, absorbed with the strain Šimon, no residual antibodies were found to be present, a considerable number of these sera still reacted after absorption with the strains S-23 and Pöštényi but rarely after absorption with the strain Pomona (see Table 3).

On the contrary, MAT results of the absorbed eight human Pomona sera with the strains of the serovar Pomona indicated some differences of these strains: All antibodies (anti-Pomona and anti-Mozdok) of all sera except one were regularly absorbed with the strain Šimon, nevertheless they were irregularly absorbed by the strains Pomona, S-23 and Pöštényi (Table 4, patient A). However, according to the results of the reference method ACAT realized using the rabbit's sera which is the reference method for serovar typing of leptospires, all the Pomona strains may be affiliated to the serovar Pomona (see Table 2).

It should be noticed that the strains S-23 and Pöštényi bore virtually the same results with the absorbed sera in MAT. On the other hand, the strains Šimon and Pomona proved to be a little distinct from one another, and that they also differ from the strains S-23 and Pöštényi. The differences among the various Pomona group strains can also be seen in the results of AATs realized with these sera with the Mozdok group strains.

Antibodies from the last (eighth) patient's serum were entirely absorbed only with the strain S-23 (see Table 4, patient B). Based on this observation it can be derived that not each Leptospira strain belonging to the serovar Pomona group may be convenient for discrimination between the two serovars.

The fact that the strains of the serovar Mozdok are able to also absorb antibodies in some sera produced against the Pomona strains and vice versa gives the evidence for the presence of common antigenic determinants between both groups of these *Leptospira* strains. It is noteworthy that the revealed results that were obtained using the endemic strains of leptospires were more convincing than those that were achieved using the reference strains.

The results of AATs realized with human sera do not entirely correspond with those results that were obtained by the ACATs using the rabbit immune sera. The major determined discrepancy was that in ACATs the *Leptospira*

Table 3. Results of agglutinin absorption tests of patient's serum with Mozdok leptospirosis

Patient's serum	absorbed with strain	Microagglutination tests with antigens						
		Pomona	Šimon	S-23	Pöštényi	5621	M-210/98	M-71/01
Patient pensioner East Slovakia	–	100.0	100.0	50.0	50.0	50.0	100.0	100.0
	Pomona	< 3.1	6.3	< 3.1	< 3.1	50.0	50.0	100.0
	Šimon	< 3.1	3.1	< 3.1	< 3.1	25.0	50.0	100.0
	S-23	25.0	100.0	< 3.1	< 3.1	25.0	25.0	50.0
	Pöštényi	50.0	100.0	< 3.1	< 3.1	25.0	100.0	100.0
	5621	< 3.1	3.1	< 3.1	< 3.1	3.1	3.1	3.1
	M-210/98	< 3.1	3.1	< 3.1	< 3.1	< 3.1	< 3.1	< 3.1
	M-71/01	3.1	3.1	< 3.1	< 3.1	6.3	6.3	3.1

Note: Antibody levels expressed in percent of reciprocal values of the serum titre prior to absorption

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Table 4. Results of agglutinin absorption tests of patients' sera with Pomona leptospirosis

Patient's serum	Absorbed with strain	Microagglutination tests with antigens						
		Pomona	Šimon	S-23	Pöštényi	5621	M-210/98	M-71/01
Patient A butcher West Slovakia	–	50.0	100.0	100.0	100.0	50.0	50.0	50.0
	Pomona	< 1.6	25.0	25.0	50.0	1.6	3.1	6.3
	Šimon	< 1.6	< 1.6	3.1	< 1.6	< 1.6	< 1.6	< 1.6
	S-23	< 1.6	6.3	< 1.6	3.1	12.5	25.0	12.5
	Pöštényi	1.6	1.6	< 1.6	< 1.6	6.3	12.5	25.0
	5621	6.3	50.0	100.0	50.0	3.1	< 1.6	1.6
	M-210/98	3.1	50.0	12.5	12.5	<1.6	<1.6	1.6
	M-71/01	12.5	100.0	25.0	25.0	6.3	<1.6	3.1
Patient B butcher East Slovakia	–	6.3	25.0	100.0	.	6.3	12.5	.
	Pomona	0.8	25.0	100.0	.	< 0.8	1.6	.
	Šimon	0.8	1.6	100.0	.	< 0.8	< 0.8	.
	S-23	0.8	1.6	0.8	.	< 0.8	1.6	.
	5621	6.3	12.5	100.0	.	< 0.8	0.8	.
	M-210/98	3.1	12.5	50.0	.	< 0.8	< 0.8	.

Note: Antibody levels expressed in percent of reciprocal values of the serum titre prior to absorption
.not realized

strains Pomona and Šimon were found to be identical, which was not the case in the human sera (see Tables 2, 3, 4). This might be explained by inter-species (human – rabbit) differences in the antibody formation that was also found in our former study [quoted 16] or by intra-species differences in the serological response to the antigenic stimulus known in men and animals.

It was found that nine patients from the East Slovakia (out of all 13 patients from the whole Slovak Republic) were afflicted with Mozdok leptospirosis what corresponds well with the existence of multiple known natural foci of *A. agrarius* as the maintenance host of the serovar Mozdok leptospires in this part of the country. Indeed, in the east part of Slovakia nearly 90 *Leptospira* strains belonging to serovar Mozdok were isolated from this animal species within the period between 1997 and 2010 (not published).

Four of the nine Mozdok patients from the East Slovakia were pensioners, the professions of the remaining five were a pig-breeder, a milk made, a locomotive driver, an unemployed and one with an unknown profession. All patients were villagers and therefore they could have been indirectly or directly exposed to rodents' excrements. It is noteworthy that the majority (> ⅔) of all 13 Mozdok cases from the whole Slovak Republic occurred in the warm months of the year, while on the other hand, Pomona infections were recorded through all seasons. This epidemiological feature was noted in East Slovakia in 1961 [19] even prior to the publication of the first isolation of the serovar Mozdok strain in 1965 [2].

In the West Slovakia, habitats of *A. agrarius* are geographically not extended till now, yet the species is spreading continuously from the south part of the region toward the north [20]. Even though members of *A. agrarius* species were not yet examined for the presence of leptospires in this region, the fact that two cases of human Mozdok illness detected in the southern part of the West Slovakia within the area of its habitat, as well as one case in the western part of the same region of the country and not far away from a known habitat of *A. agrarius*, indicate the possible existence of natural foci of this leptospirosis and could explain the related recorded human Mozdok diseases.

The origin of one case of the Mozdok illness in the northern part of the Middle Slovakia might be explained by the fact that this patient resided in the close vicinity to an assumed natural focus of this leptospirosis. Indeed, in this area, eight *Leptospira* strains belonging to the Pomona serogroup were isolated from *A. agrarius* in 1958 [21]. It can be presumed that these isolates might have belonged to the serovar Mozdok. However, the antigenic structure of these strains was not thoroughly examined at that time. Occurrence of Pomona (slaughter-house) disease above all in butchers in the West Slovakia and the Middle Slovakia was not a surprising finding.

Revising the results of the study, it is possible to conclude that leptospires belonging to the Mozdok serovar may represent a possible causative agent of human illness. However, a definitive proof needs to be issued and that is based on isolation of a Mozdok strain from patients afflicted with this leptospirosis. Nevertheless, the presented results prove that AAT is a useful tool to differentiate between human Mozdok and Pomona illnesses.

It should be noted that even though the occurrence of human Mozdok infections seems to well correspond with the presence of habitats of the black-striped field mouse, the number of examined patients do not allow us to draw a final conclusion about the geographical distribution of the illness. Nevertheless, it is noteworthy to mention

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that such a correlation was recently noted in a region in Portugal (14).

CONCLUSION

On the basis of the obtained results it can be stated that leptospires of the Mozdok serovar can be safely considered as a causative agent of human illness and can be diagnosed by serologic methods. The possibility of acquiring this infection should be taken into account by physicians examining patients who stayed at places around the natural foci i.e. habitats of infected small mammals, due to either professional or leisure activities. The obtained results can be useful for extended surveillance of leptospirosis and for the determination of appropriate epidemiological measures.

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Conflict of interest
The authors declare that there is no conflict of interest.

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Adresa pro korespondenci:

Prof. MUDr. Pavol Bakoss, DrSc.
Lekárska fakulta Univerzity Komenského
Špitálska 24
813 72 Bratislava
Slovenská republika
e-mail: pavol.bakoss@fmed.uniba.sk

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