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EXPERIMENTAL INFECTION WITH THE VIRULENT, CENTRAL-EUROPEAN, MURINE LEPTOSPIRA POMONA STRAIN IN THE PIG

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Abstract. The virulent, murine *Leptospira pomona* strain isolated from *Apodemus agrarius* was used in an experimental infection of six pigs aged 4—5 months. The clinical course of the infection was inapparant, both the blood picture and the uptake of food were normal. All infected pigs produced antibodies against *L. pomona* at titres from 1 : 3 200 to 1 : 50 000. The reisolation of leptospires from the blood of the infected pigs was successful in one case only, and that on day two p.i. Throughout the course of our experiment, no microscopic evidence was obtained of the presence of leptospires in the blood of the infected animals. Of the six guinea pigs injected repeatedly with the urine of the infected pigs, antibodies against *L. pomona* were detected in two of these at titres 1 : 3 200 and 1 : 6 400. However, no direct proof was obtained of leptospires in their kidneys. Leptospires were isolated from the kidneys of two of the infected pigs, at days 10 and 21 p.i. respectively. As suggested by our results, the Central European, murine *Leptospira pomona* strain should be regarded as an independent biovar uncapable of causing a long-term leptospiruria and, hence, apparently unable to result in an epizooty in intensive pig husbandry. According to experimental evidence, *Mus musculus* can be a potential reservoir of the murine *L. pomona* biovar in Central Europe.

In Europe, where the serovar *pomona* is widely distributed (Kathe and Mochmann 1967), it produces two types of leptospirosis foci (Mittermayer et al. 1961, Kmety 1967), a natural focus with *Apodemus agrarius* as its main reservoir, and an anthropourgic focus with the domestic pig as its main reservoir. It is the most important serovar in the etiology of leptospirosis of pig and cattle (Zwierzchowski 1967). In some countries, e.g., in the USSR (Lubashenko 1962) and in the USA (U.S. Department of Agriculture 1954), it causes considerable losses to the breeds of domestic animals particularly cattle, pig and horse.

In the USSR, strains of leptospires of the serogroup *Pomona*, isolated from the common vole (*Microtus arvalis*), have been described as a new, independent subtype under the name *Leptospira pomona mozdok* (Semenova 1965). Later, this subtype has been placed in identity to the serovar *pomona* (Kmety 1971, Borg—Petersen 1974), but Nicolescu and Moldoveanu (1974) continued to regard it as an independent subtype. Chernukha et al. (1974) obtained results of great interest and epizootological importance in that they disclosed biological differences between strains isolated from small mammals and those isolated from domestic animals (*L. pomona, L. "monjakov"*). The disclosure of the differences emerged from the fact that they were unable to infect small mammals with strains isolated from domestic animals. This finding has been the reason for investigating in the pig the course of an experimental infection with the virulent, Central European, murine *L. pomona* strain.

MATERIAL AND METHODS

We examined serologically six clinically healthy pigs aged 4—5 months with the microscopic agglutination-lysis test. We used these serovars and strains in the basic solution 1 : 100: 1. *icterohaemorrhagiae* Fryšava, 2. *sorex-jalna* Sorex Jalná, 3. *canicola* Canis 7, 4. *arboreae* M 7, 5. *pyrogenes*

Table 1. The virulence of the strain *L. pomona* 7130/78 retested on white laboratory mice

Killed and examined on:	On January 31, 1979, 0.25 ml of the culture was administered to each mouse by an i.p. injection							
	1		2		3		4	
	Bacterio-logically	Serologically	Bacterio-logically	Serologically	Bacterio-logically	Serologically	Bacterio-logically	Serologically
7. II. 1979	0	a 1 : 160 b 1 : 320 c 1 : 640	—	—	—	—	—	—
14. II. 1979	—	—	+	a negative b 1 : 320 c 1 : 640	—	—	—	—
21. II. 1979	—	—	—	—	+	a negative b 1 : 160 c 1 : 1 280 d 1 : 1 280	—	—
28. II. 1979	—	—	—	—	—	—	+	a 1 : 40 b 1 : 640 c 1 : 2 560

+ = reisolation of the strain, a = *L. pomona* Šimon, b = *L. pomona* Borg—Petersen, c = *L. pomona* 7130/78, d = *L. pomona* Jug. 32/II./77

Salinem, 6. *bulgarica* Nikolaevo, 7. *bratislava* Jež Bratislava, 8. *pomona* Šimon, 9. *grippotyphosa* P 125, 10. *sejroe* M 84, 11. *bataviae* Moldava, 12. *tarassovi* S 42. Whenever *L. pomona* reacted in the basic solution (1 : 100), we also used two other strains of the serovar, i.e., the strain Borg—Petersen and the strain 7130/78. In one instance, we also used the strain *L. pomona* 32/II./77 isolated from *A. agrarius* in Yugoslavia. The strain 7130/78 was isolated from *A. agrarius* on October 12, 1978 at Karvinná, NE-Moravia. We inoculated the primoculture of the strain into a biphasic Korthof medium (1 % agar in the Korthof medium with 7.5 % rabbit serum slant solid in the tube and filled to one half with the liquid Korthof medium with 7.5 % of rabbit serum), and stored it without reinoculation until we used it in the present experiment. We knew from our earlier experience that leptospira strains remained virulent in this medium for a very long period. Each pig was kept in a separate box, examined clinically each day, and also its temperature was taken daily. Prior to our trials of reisolating leptospires from both the blood and the urine of our experimental animals, the Korthof medium with 7.5 % rabbit serum to be used in the test was examined for a good growth of leptospires. Urine samples were examined for the presence of leptospires by means of a dark-field examination, at a magnification of 10 × 15. Simultaneously, from the second week p. i. onwards, always one guinea pig was injected with the urine of each pig (4 repetitions in intervals of 3 days). The guinea pigs were killed three weeks after the first injection, and examined both serologically and bacteriologically. The blood picture of the pigs was examined with a standard method.

RESULTS

One week before the experimental infection (February 14, 1979), all pigs were examined serologically with the 12 serovars of leptospires at the dilution 1 : 100 and, apart from these, with the three strains of *L. pomona* referred to earlier in the text, also at the titre 1 : 20. Pigs no, 1, 4, 5 and 6 reacted negatively, pigs no. 2 and 3 positively with *L. icterohaemorrhagiae* at the titre 1 : 800 and 1 : 100 respectively, pig no 3 also with *L. bratislava* (1 : 200). Before using the strain *L. pomona* 7130/78 in an experimental infection of the pigs, we tested its virulence on 4 white laboratory mice. Three days before the experiment, we examined the mice serologically, and their urine microscopically, three times, for the presence of leptospires. Bacteriologically negative was mouse no. 1 only, killed after one week p.i. Leptospires were reisolated from the kidneys of the remaining three mice at weeks 2, 3 and 4 p.i. respectively. All four mice were serologically positive with *L. pomona* 7130/78 at titres from 1 : 640 to 1 : 2 560. It was noteworthy that the titres of mice no. 2, 3 and 4 to the strain Šimon differed greatly from those to strains Borg—Petersen, 7130/78 and Yug. 7130/77. The results remained unchanged in all three repetitions of the examination. By contrast, strains Yug. 32/II./77 and 7130/78 isolated from *A. agrarius*, reacted in the same titres. The results of the experiment are surveyed in Table 1.

In addition to confirming the virulence of the strain, the experiment made with mice showed that the infection with the murine biovar of the Central European *L. pomona* strains caused a prolonged leptospiruria in the house mouse (*Mus musculus*) that persisted for one month at the minimum. This finding provided reliable evidence that the species might be capable of acting as an important, potential reservoir.

We infected pigs no. 3, 4, 5 and 6 with the strain 7130/78 on February 19, 1979: pigs no. 3 and 5 perorally (5 ml of the culture in one liter phosphate buffer pH 7.2 administered as a drink), pigs no. 4 and 6 by injecting intramuscularly 2.5 ml of the culture. Pigs no. 1 and 2 served as controls. Owing to negative results, we infected the controls, pigs no. 1 and 2, on April 23, 1979 by injecting intramuscularly 2.5 ml of the mentioned culture of leptospires. Table 2 surveys the results of repeated, serological examinations of the pigs. All six pigs reacted to the infection with the production of antibodies against *L. pomona*. It was of interest that pig no. 1 reacted as early as on day 4 p.i. to *L. pomona* Borg—Petersen at a titre of 1 : 1 600. The titres attained their peak at weeks 2 and 3 p.i. respectively, i.e., 1 : 50 000 (pigs no. 2, 3, 4), 1 : 6 400 (pig no. 6) and 1 : 3 200 (pig no. 5). In pig no. 5 killed at week

Table 2. Results of the serological examination of six experimentally infected pigs

Blood samples drawn on	Positive with *L. pomona* in the titre:																							
	1			2			3			4			5			6								
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Day 4 p.i.	400	1 600	200	100	100	neg	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Week 1 p.i.	1 600	3 200	1 600	1 600	3 200	1 600	400	1 600	1 600	400	1 600	1 600	3 200	200	neg	—	—	—	—	—	—	—	—	—
Day 10 p.i.	3 200	3 200	3 200	6 400	12 800	25 000	—	—	—	—	—	—	3 200	—	—	—	—	—	—	—	—	—	—	—
Week 2 p.i.	—	—	—	—	—	—	12 800	25 000	50 000	12 800	25 000	50 000	3 200	1 600	1 600	1 600	1 600	1 600	1 600	800	3 200	800	—	—
3 p.i.	—	—	—	12 800	25 000	50 000	12 800	12 800	50 000	6 400	12 800	25 000	3 200	1 600	3 200	3 200	1 600	3 200	1 600	1 600	1 600	1 600	800	3 200
4 p.i.	—	—	—	—	—	—	12 800	12 800	25 000	6 400	6 400	25 000	3 200	800	neg	3 200	800	3 200	3 200	1 600	1 600	6 400	1 600	3 200
5 p.i.	—	—	—	—	—	—	6 400	6 400	6 400	6 400	3 200	6 400	3 200	1 600	neg	3 200	1 600	3 200	6 400	3 200	neg	6 400	3 200	6 400
6 p.i.	—	—	—	—	—	—	3 200	1 600	6 400	1 600	1 600	6 400	1 600	800	neg	1 600	800	3 200	3 200	1 600	neg	6 400	1 600	6 400
8 p.i.	—	—	—	—	—	—	1 600	1 600	6 400	—	—	—	800	800	neg	800	800	3 200	3 200	1 600	neg	3 200	—	—
10 p.i.	—	—	—	—	—	—	1 600	3 200	3 200	—	—	—	400	800	neg	400	800	1 600	800	400	neg	—	—	—
12 p.i.	—	—	—	—	—	—	1 600	1 600	3 200	—	—	—	200	400	neg	200	400	800	400	200	neg	—	—	—
14 p.i.	—	—	—	—	—	—	—	—	—	—	—	—	100	200	neg	100	200	800	200	100	neg	—	—	—
16 p.i.	—	—	—	—	—	—	—	—	—	—	—	—	100	200	neg	100	200	400	100	100	neg	—	—	—
21 p.i.	—	—	—	—	—	—	—	—	—	—	—	—	200	100	neg	200	100	800	200	100	neg	—	—	—

a = *L. pomona* Šimon, b = *L. pomona* Borg—Petersen, c = *L. pomona* 7 130/78

21 p.i., it was still possible to detect titres against *L. pomona*, of relatively low values (1 : 100, 1 : 200 and 1 : 800).

Throughout our experiment, there were no apparent clinical signs of the infection, the temperature, the blood picture and food intakes remained normal. We attempted to reisolate leptospires from the blood of the experimental pigs on days 2 and 4 p.i., but succeeded in one instance only (pig no. 1) on day 2 p.i. From the second week p.i., we tried without success to detect leptospires in the urine by means of a dark-field examination. Similarly, we failed to recover leptospires from the kidneys of guinea pigs injected repeatedly with the urine of infected pigs; three of the animals were negative even serologically. One guinea pig injected with the urine of pig no. 1 responded positively to *L. pomona* (strain Šimon) at the titre 1 : 1 600, strain Borg—Petersen 1 : 3 200 and strain 7130/78 at 1 : 3 200; another guinea pig injected with the urine of pig no. 2 was positive at titres 1 : 1 600, 1 : 3 200 and 1 : 6 400, one guinea pig infected with the urine of pig no. 5 was positive at titres 1 : 200, 1 : 400 and 1 : 3 200.

Using the Korthof medium with 7.5 % rabbit serum, we recovered leptospiresr. from the kidneys of pig no. 1 and pig no. 2 killed at days 10 and 21 p.i. respectively.

DISCUSSION

As suggested by a comprehensive survey of literary data on an infection of the pig with *L. pomona* (Zwierzchowski 1967), clinical symptoms are indistinct particularly in the not quite young piglets. A temporary loss of appetite and an increase in the temperature are fairly frequent. On the other hand, leptospiruria becomes considerably prolonged (e.g., 122) and agglutination titres have been found to be present even after a longer period. Similar results were obtained by Shmatkova (1965) from an experimental infection of pigs. The author found neither clinical symptoms nor pathological changes in the organs; both leptospiruria and the serological positivity persisted from many months. Malakhov and Alekhin (1976) stated that the course of leptospirosis in the pigs was mostly asymptomatic and the rate of mortality low. According to these authors, leptospiruria persisted in pigs with a spontaneous infection for as long as 13 months. The temperature was increased for a short period, from several hours to 1—3 days. The course of the infection was completely asymptomatic in our six experimental pigs, their temperature, the blood picture and the food intakes, were normal. Apart from a prolonged leptopsiruria, the course of our experimental infection with the murine *L. pomona* strain was similar to an infection with the pig biovar. While Morse et al. (cit. Zwierzchowski 1967) reisolated leptospires from pigs infected with the *L. pomona* pig biovar at as late a time as day 10 p.i., we succeeded to recover them from one of our six experimental pigs infected with the murine *L. pomona* biovar, i.e., from the blood of pig no. 1, on day 2 p.i. However, there could not have been many leptospires in the blood of the pig, because we inoculated with it 4 test tubes but obtained a culture from one of these only. A fundamental difference was observed in the duration of leptospiruria in our experimental pigs as evident from the fact that we succeeded in reisolating leptospires from the kidneys of pigs that were killed on days 10 and 21 p.i. respectively. Owing to the small number of leptospires present in the kidneys of the two animals (pig. no 1 and pig no. 2), we failed to detect them in a dark-field examination of kidney preparations, and obtained a culture of leptospires from one of the four test tubes only inoculated with material from pig no. 1. The test tubes inoculated with material from pig no. 2, also contained at first very few leptospires. On the other hand, the kidneys of pigs infected with the *L. pomona* pig biovar, contain mostly large numbers of leptospires and, generally, these are released in masses. We failed to detect microscopically

leptospires in the urine of our experimental pigs. In support of our results were those obtained from our experiment with the guinea pigs. In spite of a repeated infection, by way of injection, with the urine of the individual, experimental pigs, we failed to detect leptospires in their kidneys, and two guinea pigs only were serologically positive to *L. pomona*.

The postmortem examination of the six experimental pigs showed no pathological changes in their organs.

The results of our experiment confirmed the statement of the soviet authors (Chernukha et al. 1974, 1975) that strains of *L. pomona* isolated from small mammals are host-specific. Also the Central European strains isolated from *A. agrarius* are an independent biovar adapted to *A. agrarius* as their main reservoir, and to other species of small rodents (*A. sylvaticus, A. flavicollis, M. musculus*) as their potential reservoir. Owing to the fact that the murine biovar is uncapable of producing a long-term leptospiruria in the pig, its survival in pig breeds is most unlikely.

ЭКСПЕРИМЕНТАЛЬНОЕ ЗАРАЖЕНИЕ СВИНЕЙ ВИРУЛЕНТНЫМ СРЕДНЕЕВРОПЕЙСКИМ ШТАММОМ *LEPTOSPIRA POMONA*

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Резюме. Вирулентным мышиным штаммом *Leptospira pomona*, выделенным из *Apodemus agrarius*, экспериментально заражали 6 свиней в возрасте 4—5 месяцев. Заболевание было клинически незаметно, температура, картина кровы и прием корма были нормальными. После заражения у всех свиней образовывались антитела против *L. pomona* в титрах от 1 : 3 200 до 1 : 50 000. Только в одном случае удалось реизолировать из крови лептоспиры через два дня после заражения. В течение эксперимента нельзя было обнаружить лептоспиры в моче свиней. Мочу повторно впыскивали для заражения 6 морских свинкам. Антитела против *L. pomona* были обнаружены только у 2 из них в титрах 1 : 3 200 и 1 : 6 400, но прямое доказание лептоспир в их почках не удалось. Лептоспиры выделяли из почек только у двух свиней через 10 и 21 день после заражения. Результаты эксперимента показывают, что среднеевропейские мышиные штаммы *L. pomona* является самостоятельным биоваром, не вызывающим у свиней долгосрочной лентоспирурии и поэтому не способным вызывать эпизоотию в промышленном свиноводстве. Экспериментально было доказано, что *Mus musculus* в средней Европе может служить потенциальным резервуаром мышиного биовара *L. pomona*.

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