

SEROLOGIC EXAMINATION OF SOME DOMESTIC ANIMALS FROM SOUTH MORAVIA ON THE PRESENCE OF ANTIBODIES TO SELECTED ARBOVIRUSES OF THE A, B, CALIFORNIA AND BUNYAMWERA GROUPS

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Abstract. Serologic examinations of some species of domestic animals from South Moravia (61 horses, 305 cows, 93 pigs, 104 hens and 47 ducks) by means of hemagglutination-inhibition test revealed HI antibodies to the viruses *Ťahyňa* and *Čalovo* in horses (34.4 and 27.9 % respectively), in cows (5.6 and 25.2 % resp.) and pigs (55.0 and 17.4 % resp.), as well as antibodies to the tick-borne encephalitis virus in horses (3.3 %) and cows (1.6 %). No antibodies against the West Nile virus were found in the animals examined. Antibodies to arboviruses of the A group (Sindbis and WEE) were detected in hens (0.9 and 1.9 %) and in ducks (8.5 and 6.4 %).

Serologic investigations among human population and populations of selected species of wild and domestic animals in the region of endemic *Ťahyňa* virus occurrence in South Moravia were carried out as far back as in 1963—1964 by Danielová and Marhoul (1968). In order to detect antibodies to the *Ťahyňa*, *Čalovo* and tick-borne encephalitis viruses, they used sero-neutralizing tests (SNT) in mice after Smithburn (1952) and in tissue cultures of rabbit lung cells (stable line).

The author was interested, whether in these sera, especially those of domestic animals (kept at -30°C since 1964), antibodies to other viruses, particularly to group A arboviruses (the occurrence of which was anticipated on Czechoslovak territory — Čupková 1964) could be detected by means of haemagglutination-inhibition test (HIT). The present communication contains the results of serologic examination of selected species of domestic animals on the presence of HI antibodies to six antigens tested — *Ťahyňa* (*Ťa*), *Čalovo* (*Ča*), tick-borne viral encephalitis (TBE), Sindbis (*Si*), West Nile (WN) and Western equine encephalomyelitis (WEE).

MATERIAL AND METHODS

Sera dating from 1964 collections and until tested had been preserved in native state at the temperature of -30°C . Blood samples from horses and cows were collected by puncture of jugular vein, and in case of pigs of marginal auricular vein. In hens and ducks the blood was collected either from ulnar vein, jugular vein or arteria carotis. Sera for HIT were purified by acetone (Clarke, Casals 1958).

Antigens for HIT were prepared by saccharose-acetone extraction (Clarke, Casals 1958) from laboratory virus strains: *Ťa*-strain "5079", *Ča*-strain "184", TB-strain "Hypr", West Nile, Sindbis and WEE. The last mentioned three strains were made available by the Virological Institute of the Slovak Academy of Sciences in Bratislava.

The HIT performance was modified for the Takátsy's "Microtimator" (8—16 antigen units in 0.025 ml, 0.025 ml of serum, 0.05 ml of goose erythrocytes).

In order to give the results the most objective evaluation, we have left out from the final findings the titres lower than 1 : 20, which represented about 1 % of all positive findings, even at a risk that in some sera a certain drop of titres might have occurred during storage.

RESULTS

In seven villages of the Břeclav district (Drnholec, Hlohovec, Lednice, Novosedly, Nový Dvůr, Nový Přerov, Sedlec), in the endemic region of the Ťahyňa and Čalovo viruses, samples of sera from five species of domestic animals were collected in 1964 and examined on the presence of HI antibodies to six virus antigens from the A, B, California and Bunyamwera arbovirus groups.

In total 61 horses, 305 cows, 93 pigs, 104 hens and 47 ducks were examined.

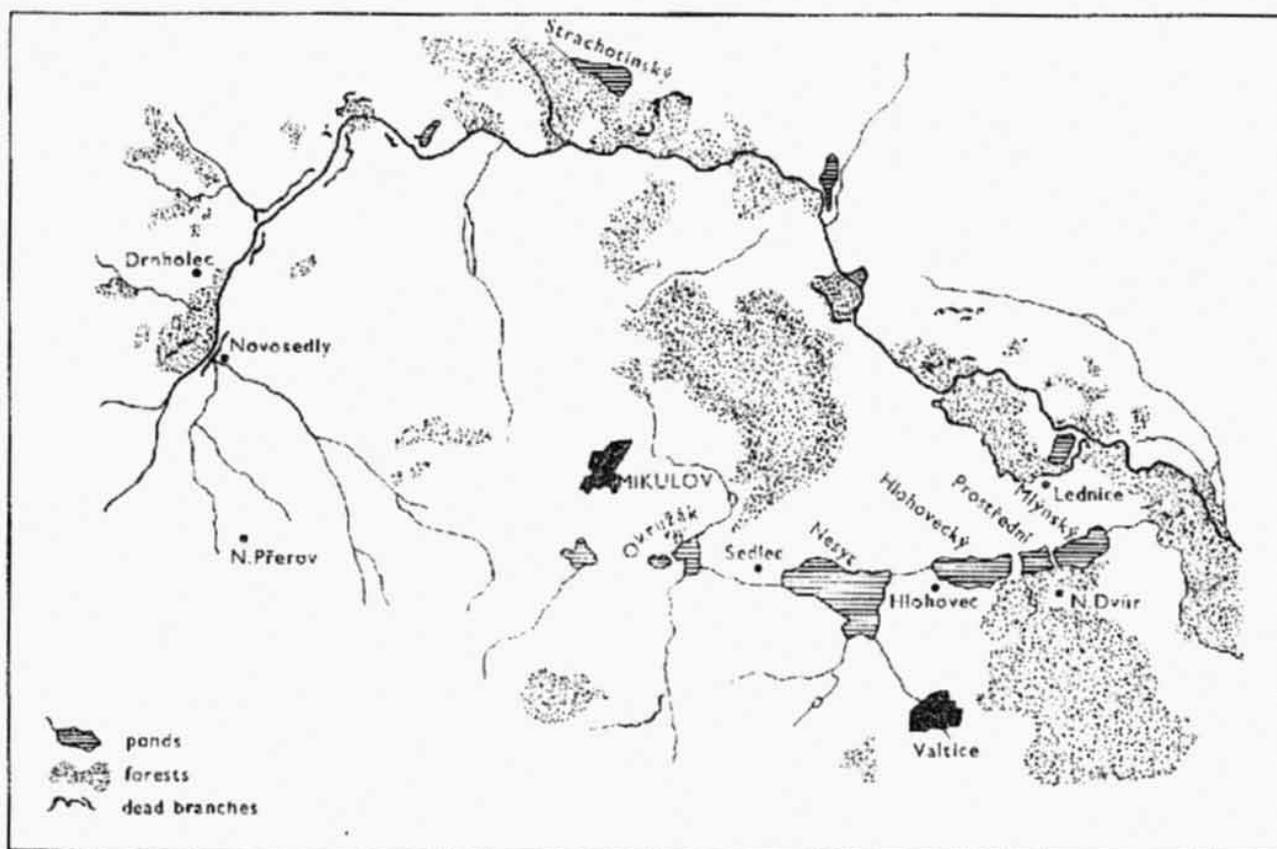


Fig. 1. Chart of the region studied.

The results given in Tables 1—4 show that 34 % and 27.9 % of HI antibodies to Ťa and Ča viruses respectively occur in horses, 5.6 and 25.2 % in cattle and 55 and 17.4 % in pigs. In the sera of some animals (pigs, horses, cows) from the localities Lednice, Sedlec, Hlohovec and Nový Dvůr antibodies to the Ťahyňa and Čalovo viruses were simultaneously detected. A survey of the frequency of these findings and titre values of both types of antibodies are presented in Table 5. Single findings of HI antibodies to the Ťa, Ča and TBE viruses in hens correspond with accidental contact with these viruses and consequently are insignificant from the epidemiological aspect. 3.3 % of HI antibodies to the TBE virus were found in horses and 1.6 % in cows. No antibodies to WN virus were detected in domestic animals examined. Repeated findings of HI

Table 1. Distribution of seropositive findings of HI antibodies in five species of domestic animals according to localities and antigens used

Species	Antigen	Horses			Cows			Pigs			Hens			Ducks		
		Examined	Positive	%	Examined	Positive	%	Examined	Positive	%	Examined	Positive	%	Examined	Positive	%
Sedlec	Āa	18	10	55.5	39	3	7.7	49	25	51.0	48	0	—	47	0	—
	ĀA	18	5	27.8	39	18	46.1	49	12	24.5	48	0	—	47	0	—
	KE	18	0	—	39	0	—	49	0	—	48	0	—	47	0	—
	WN	18	0	—	39	0	—	49	0	—	48	0	—	47	0	—
	SI	18	0	—	39	0	—	49	0	—	48	0	—	47	4	8.5
	WEE	18	0	—	39	0	—	49	0	—	48	0	—	47	3	6.4
Hlohovec	ĀA	17	3	17.6	39	1	2.6	25	13	52.0	33	0	—	—	—	—
	ĀA	17	5	29.6	39	27	69.2	25	4	16.0	33	1	3.0	—	—	—
	KE	17	0	—	39	5	12.8	25	0	—	33	0	—	—	—	—
	WN	17	0	—	39	0	—	25	0	—	33	0	—	—	—	—
	SI	17	0	—	39	0	—	25	0	—	33	0	—	—	—	—
	WEE	17	0	—	39	0	—	25	0	—	33	1	3.0	—	—	—
Lednice	ĀA	26	8	30.8	45	2	4.4	19	13	68.4	23	1	4.3	—	—	—
	ĀA	26	7	26.9	45	17	37.8	19	3	15.8	23	0	—	—	—	—
	KE	26	2	7.7	45	0	—	19	0	—	23	1	4.3	—	—	—
	WN	26	0	—	45	0	—	19	0	—	23	0	—	—	—	—
	SI	26	0	—	45	0	—	19	0	—	23	1	4.3	—	—	—
	WEE	26	0	—	45	0	—	19	0	—	23	1	4.3	—	—	—
Nový Dvůr	ĀA	—	—	—	39	2	5.1	—	—	—	—	—	—	—	—	—
	ĀA	—	—	—	39	15	38.5	—	—	—	—	—	—	—	—	—
	KE	—	—	—	39	0	—	—	—	—	—	—	—	—	—	—
	WN	—	—	—	39	0	—	—	—	—	—	—	—	—	—	—
	SI	—	—	—	39	0	—	—	—	—	—	—	—	—	—	—
	WEE	—	—	—	39	0	—	—	—	—	—	—	—	—	—	—
Drnholec	ĀA	—	—	—	—	—	—	16	9	56.2	—	—	—	—	—	—
	ĀA	—	—	—	—	—	—	16	0	—	—	—	—	—	—	—
	KE	—	—	—	—	—	—	16	0	—	—	—	—	—	—	—
	WN	—	—	—	—	—	—	16	0	—	—	—	—	—	—	—
	SI	—	—	—	—	—	—	16	0	—	—	—	—	—	—	—
	WEE	—	—	—	—	—	—	16	0	—	—	—	—	—	—	—
Novosedly	ĀA	—	—	—	44	1	2.3	—	—	—	—	—	—	—	—	—
	ĀA	—	—	—	44	0	—	—	—	—	—	—	—	—	—	—
	KE	—	—	—	44	0	—	—	—	—	—	—	—	—	—	—
	WN	—	—	—	44	0	—	—	—	—	—	—	—	—	—	—
	SI	—	—	—	44	0	—	—	—	—	—	—	—	—	—	—
	WEE	—	—	—	44	0	—	—	—	—	—	—	—	—	—	—
Nový Přerov	ĀA	—	—	—	99	8	8.1	—	—	—	—	—	—	—	—	—
	ĀA	—	—	—	99	0	—	—	—	—	—	—	—	—	—	—
	KE	—	—	—	99	0	—	—	—	—	—	—	—	—	—	—
	WN	—	—	—	99	0	—	—	—	—	—	—	—	—	—	—
	SI	—	—	—	89	0	—	—	—	—	—	—	—	—	—	—
	WEE	—	—	—	99	0	—	—	—	—	—	—	—	—	—	—

Note: — not done; % percentage of positive sera

antibodies to arboviruses of the A group (Sindbis and WEE) in hens (at a titre of 1 : 20 up to 1 : 40) and especially in ducks (at a titre of 1 : 20 up to 1 : 320) have been the first of its kind under our geographical conditions and indicate the direction of further research in the region studied.

DISCUSSION

The role of some species of domestic animals (horses, cows, pigs, dogs and cats) in the circulation of the Ťahyňa and Čalovo viruses, was discussed in some previous papers both of Czechoslovak and foreign authors. On one hand, the presence and antibody level were studied (Aspöck, Kunz 1971, Bárdoš 1960, Bárdoš, Adamecová 1960, Danielová et al. 1969, Danielová, Marhoul 1968, Sekeyová, Grešíková 1967) in the do-

Table 2. Percentage of seropositive findings of HI antibodies according to species of domestic animals

Antigen	Horses			Cows			Pigs			Hens			Ducks		
	Examined	Positive	i.e. %												
ŤA	61	21	34.4	305	17	5.6	109	60	55.0	104	1	0.96	47	0	
ČA	61	17	27.9	305	77	25.2	109	19	17.4	104	1	0.96	47	0	
KE	61	2	3.3	305	5	1.6	109	0		104	1	0.96	47	0	
WN	61	0		305	0		109	0		104	0		47	0	
SI	61	0		305	0		109	0		104	1	0.96	47	4	8.5
WEE	61	0		305	0		109	0		104	2	1.90	47	3	6.4

mestic animals mentioned, on the other, the course of disease after experimental infection of animals, with special regard to the intensity of viremic phase and dynamics of antibody production (Bárdoš 1963, 1965, Bárdoš et al. 1965, 1966, Bárdoš, Jakubík 1961).

Most of these papers were written by Slovak authors. The results published by them and relating to local epidemiological situation in Slovakia, however, could be successfully applied to other geographical conditions (Aspöck, Kunz 1971, Danielová et al. 1969, Danielová, Marhoul 1968), especially as far as the selection of animal species as indicators is concerned, and in our case to the South-Moravian region as well.

Certain differences in the prevalence of seropositive findings in particular animal species are due to local and actual epidemiological situation, during which the investigations were carried out. Thus, according to findings of Slovak authors (Bárdoš 1960, Bárdoš, Adamecová 1960) the most frequent contact with the Ťahyňa virus, according to the frequency of serologically positive findings, was detected in horses (up to 62.7 %), while according to our findings in South Moravia it varied at an average of about 34 % (Table 2), although in one of the localities studied (Sedlec) up to 55 % of antibodies were found (Table 1). Danielová et al. (1969) and Danielová and Marhoul (1968) (using the SNT test in mice and tissue cultures) reported up to 73 % of incidence of infection in pigs from the same region, while in our findings the incidence of infection

was only 55 %. Bárdoš and Adamcová (1960) recorded only 18 % of incidence of infection in Slovakia, but on the basis of data obtained by experimental infection of piglets with the Ťahyňa virus (Bárdoš 1963, Bárdoš et al. 1966, Bárdoš, Jakubík 1961) they assumed that pigs might play a much more significant role in the ecology of this virus than horses. In accordance with the findings of Bárdoš (1960), Bárdoš, Adamcová (1960), Danielová et al. (1969) as well as Sekeyová and Grešíková (1967) in the area studied cows appear to be animals which reveal a much lower seropositivity than horses or pigs. The single finding of antibodies to the Ťahyňa virus in hens may be assessed as a confirmation of the role of birds as potential blood donors for mosquitoes, the vectors of the Ťahyňa virus (Bárdoš et al. 1960), but it is known that birds under our natural conditions are of no importance to its circulation (Šimková 1967).

The presence of HI antibodies to the TBE virus was revealed in horses and cows at a low percentage only, which fact corresponds with the limited occurrence of ticks in the

Table 3. Sequence of epidemiological importance of localities studied according to the degree of infection rate in domestic animals and relation to antigens used

Anti- gen	Horses	%	Cows	%	Pigs	%	Hens	%	Ducks	%
ŤA	Sedlec	55.5	N. Přerov	8.1	Lednice	68.4	Lednice	4.3	Sedlec	0
	Lednice	30.8	Sedlec	7.7	Drnholec	56.2	Hlohovec	0		
	Hlohovec	17.6	N. Dvůr	5.1	Hlohovec	52.0	Sedlec	0		
			Lednice	4.4	Sedlec	51.0				
			Hlohovec	2.6						
		Novosedly	2.3							
ČA	Hlohovec	29.4	Hlohovec	69.2	Sedlec	24.5	Hlohovec	3.0	Sedlec	0
	Sedlec	27.8	Sedlec	46.1	Hlohovec	16.0	Lednice	0		
	Lednice	26.9	N. Dvůr	38.5	Lednice	15.8	Sedlec	0		
			Lednice	37.7	Drnholec	0				
			Novosedly	0						
		N. Přerov	0							
KE	Lednice	7.7	Hlohovec	12.8	Drnholec	0	Lednice	4.3	Sedlec	0
	Hlohovec	0	Lednice	0	Hlohovec	0	Hlohovec	0		
	Sedlec	0	Novosedly	0	Lednice	0	Sedlec	0		
			N. Dvůr	0	Sedlec	0				
			N. Přerov	0						
		Sedlec	0							
SI	Hlohovec	0	Hlohovec	0	Drnholec	0	Lednice	4.3	Sedlec	8.5
	Lednice	0	Lednice	0	Hlohovec	0	Hlohovec	0		
			Novosedly	0	Lednice	0	Sedlec	0		
			N. Dvůr	0	Sedlec	0				
			N. Přerov	0						
		Sedlec	0							
WEE	Hlohovec	0	Hlohovec	0	Drnholec	0	Lednice	4.3	Sedlec	6.4
	Lednice	0	Lednice	0	Hlohovec	0	Hlohovec	3.0		
	Sedlec	0	Novosedly	0	Lednice	0	Sedlec	0		
			N. Dvůr	0	Sedlec	0				
			N. Přerov	0						
		Sedlec	0							

Note: Digits given represent the percentage of seropositive findings in relation to antigens used

area studied. A somewhat higher incidence of antibodies in horses may be explained by the fact that horses are used for work in the local forests. The seropositive finding in one of the hens examined is insignificant. No antibodies were detected in pigs because the animals were kept in closed pigsties. No antibodies to the WN virus were found in the animals examined.

Table 4. Titre distribution of HI antibodies and their geometric mean according to species of domestic animals and antigens used

Species	Antigen	Titres of HI antibodies					GMT
		1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	
Horses	ĎA	15 ×	3 ×	3 ×	0	0	1 : 26.7
	ĎA	2 ×	13 ×	2 ×	0	0	1 : 40.0
	KE	1 ×	1 ×	0	0	0	1 : 28.3
	SI	0	0	0	0	0	
	WEE	0	0	0	0	0	
Cows	ĎA	9 ×	3 ×	5 ×	0	0	1 : 33.9
	ĎA	25 ×	41 ×	8 ×	3 ×	0	1 : 36.0
	KE	5 ×	0	0	0	0	1 : 20.0
	SI	0	0	0	0	0	
	WEE	0	0	0	0	0	
Pigs	ĎA	6 ×	15 ×	30 ×	8 ×	1 ×	1 : 66.3
	ĎA	4 ×	11 ×	4 ×	0	0	1 : 40.0
	KE	0	0	0	0	0	
	SI	0	0	0	0	0	
	WEE	0	0	0	0	0	
Hens	ĎA	0	1 ×	0	0	0)(1 : 40.0)(
	ĎA	1 ×	0	0	0	0)(1 : 20.0)(
	KE	0	1 ×	0	0	0)(1 : 40.0)(
	SI	0	1 ×	0	0	0)(1 : 40.0)(
	WEE	1 ×	1 ×	0	0	0	1 : 28.3
Ducks	ĎA	0	0	0	0	0	
	ĎA	0	0	0	0	0	
	KE	0	0	0	0	0	
	SI	0	1 ×	2 ×	0	1 ×	1 : 95.0
	WEE	2 ×	1 ×	0	0	0	

Note: GMT = geometric mean of titre

)() = value of titre given for illustration only; it is a single case in given dilution in relation to antigen used

Large domestic animals, such as horses, cows, pigs, revealed no HI antibodies to the A group arboviruses. Sekeyová and Grešíková (1967) obtained similar results during mass examination of cattle in Slovakia. However, HI antibodies to arboviruses of this group were repeatedly detected in hens (0.96—1.9 %) and especially in ducks (6.4—8.5 %). Our findings are in a certain harmony with the data of Nestorová and Likar (1961) who also detected antibodies to the A group arboviruses (EEE and WEE antigens) in poultry in Yugoslavia, but in a much higher percentage (14.8—25.9 %). Our findings also correspond with the results obtained quite recently by Ernek et al.

Table 5. Frequency of findings of HI antibodies to the Ťahyňa and Čalovo virus in sera of some domestic animals

Locality	Species	No of serum	Antibody titre		Number of sera anti ŤA/ČA	Total examined
			anti-Ťa	anti-Ča		
LEDNICE	cow	2115	20+ 40±	20-	1	45
		2135	40+	20+		
	pig	2133	80+	80+	3	19
		2146	20+	40+		
SEDLIC	pig	2420	80+	20+	8	49
		2423	320+	20+		
		2425	80+	20+ 40±		
		2443	40+	20+		
		2444	80+	40+		
		2449	20+ 40±	80+		
		2455	80+	40+		
		2447	20+	40+ 80±		
	horse	2397	40+	80+	2	18
		2406	20+	20+ 40±		
HLOHOVEC	pig	2220	80+	40+	1	25
N. DVŮR	cow	2295	20+	40+	1	39

(1971) who ascertained the presence of HI and VN antibodies to the Sindbis virus in some species of wild birds living in this country.

For the time being it is impossible to solve to utmost satisfaction the problem whether the frequent findings of antibodies to the A group arboviruses under our geographical conditions are specifically homologous in relation to antigens used, or, whether they are actually antibodies to some other, either introduced or autochthonous viral agent, listed in the A group due to its antigenous structure.

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СЕРОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ НЕКОТОРЫХ ВИДОВ
ДОМАШНИХ ЖИВОТНЫХ ИЗ ЮЖНОЙ МОРАВИИ НА НАЛИЧИЕ
АНТИТЕЛ ПРОТИВ ОТДЕЛЬНЫХ АРБОВИРУСОВ, ОТНОСЯЩИХСЯ
К ГРУППАМ А, В, CALIFORNIA И BUNYAMWE RA

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Резюме. Путем серологического исследования некоторых видов домашних животных из южной Моравии (61 лошадь, 305 коров, 93 свиньи, 104 куры и 47 уток), при помощи реакции торможения гемагглютинации, были обнаружены кроме предполагаемых находок гемагглютинацию тормозящих антител против вируса Тягиши или Чалово у лошадей (34,4 и 27,9 %), у коров (5,6 и 25,2 %) и у свиней (55,0 и 17,4 %), также антитела против вируса клещевого энцефалита у лошадей (3,3 %) и коров (1,6 %). Антитела против вируса West Nile не были обнаружены ни у одного вида исследуемых животных. Антитела против арбовирусов группы А (Sindbis и WEE) были обнаружены у кур (0,9 и 1,9 %) и у уток (8,5 и 6,4 %).

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