Ann. Soc. belge Méd. trop.
1977, **57**, 4-5, 409-423

COMPARISONS OF VARIABLE ANTIGENIC TYPES PRODUCED BY TRYPANOSOME STRAINS OF THE SUBGENUS *TRYPANOZOON*

N. VAN MEIRVENNE, E. MAGNUS & T. VERVOORT

Summary. — Antigenic variation was studied in 8 strains of trypanosomes belonging to different species and subspecies of the genus *Trypanozoon.* Thirty-one distinct antigenic types were recognized. Each strain produced its own fairly characteristic series of predominant variants. However, several similar variable antigens were found in different strains.

KEYWORDS : Antigenic variation, Immunofluorescence, Immunolysis, *Trypanozoon*, *Trypanosoma (T.) brucei*, *Trypanosoma evansi*, *Trypanosoma equiperdum.*

Introduction

Different strains of a given species of salivarian trypanosomes often produce different series of « predominant » variable antigenic types during the first few weeks of relapsing infections and strains can be typed serologically on this basis (Gray and Luckins, 1976). Variant-specific immunofluorescence and immunolysis tests proved particularly suitable for such purposes (Van Meirvenne *et al.*, 1975b). The present paper describes the relationships of 34 variable antigenic types produced by 8 strains of the subgenus *Trypanozoon.*

Materials and Methods

Strains of trypanosomes

Rodent-infective lines of the following strains were studied (for the purpose of this paper the strains were arbitrarily designated by a capital letter) :

Strain A : *T. b. brucei* isolated by the EATRO from a bushbuck in Uganda in 1966. Trypanosomes of this strain are neutralized by normal human serum (Van Meirvenne *et al.*, 1973).

Strain B : *T. b. rhodesiense* isolated from *Glossina pallidipes* captured in Uganda in 1960 (Lumsden and Herbert, 1975). Trypanosomes of this strain caused an accidental laboratory infection in man and may remain infective when incubated in normal human serum (Van Meirvenne *et al.*, 1976).

Strain C : *T. b. rhodesiense* isolated at the Institute of Tropical Medicine in Antwerp (ITMA) in 1971 from a tourist who got infected in the Akagera Park in Rwanda.

Strain D : *T. b. rhodesiense* isolated at the ITMA in 1971 from a second tourist who got infected in the Akagera Park.

Strain F : *T. b. gambiense* isolated from a patient in Ivory Coast in 1952. Trypanosomes of this old laboratory strain are still resistant to normal human serum (personal observations).

Strain H : *T.* (*T.*) *brucei* (subspecific identity unknown) isolated at the NITR in 1970 from *Glossina morsitans* captured in Northern Nigeria.

Strain I : *T. evansi* isolated from a South American Capybara in 1969 (Kageruka and Mortelmans, 1971).

Strain J : *T. equiperdum*, old laboratory strain.

VAT reference clones

Reference clones of variable antigenic types (VATs) were set up in mice by a few passages at 2-3 day intervals and then preserved as frozen stabilates. The VATs were designated by a laboratory code reference (Lumsden *et al.*, 1967) followed by the capital letter of the strain and a serial number. The 34 VATs isolated are listed in table 1. The stabilate pedigrees of the clones and of some other reference populations are given in figures 1 to 8. The AnTat clones were prepared by the present authors, the ETat clones were received from Professor W. H. R. Lumsden, the LiTat clones were provided by Professor A. Capron.

TABLE 1

Origin and code of the 34 VATs isolated as clone populations

Strain	VATs isolated	
	Total number	Code
A	13	AnTat A1 to A13
B	12	ETat B1 to B12
F	2	LiTat F1 and F2
H	1	AnTat H1
I	5	AnTat I1 to I5
J	1	AnTat J1

Serological methods

Serotyping of VATs was done by means of immunolysis and immunofluorescence tests using mono-specific antisera from clone-infected rabbits. The range of the variant-specific protective immunity developing in rabbits with long-term infections was studied by trypanolysis tests. Antisera collected several weeks or months after inoculation were tested at the 1 : 5 dilution for the presence of lytic antibodies to trypanosomes of different VAT clones. All test procedures were the same as those described by Van Meirvenne *et al.* (1975a) except for one technical improvement : the dried blood smears used for immunofluorescence tests were fixed with acetone for 15 minutes.

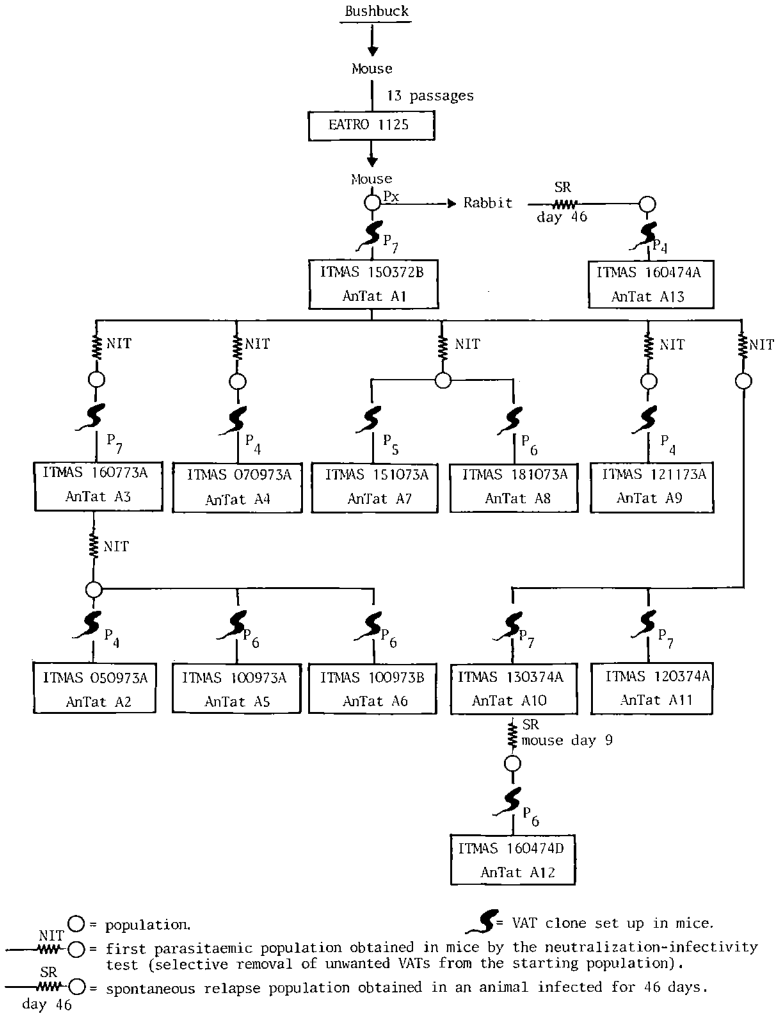

Figure 1

Stabilate pedigrees of the VAT clones AnTat A1 to A13
(for details see Van Meirvenne *et al.*, 1975a).

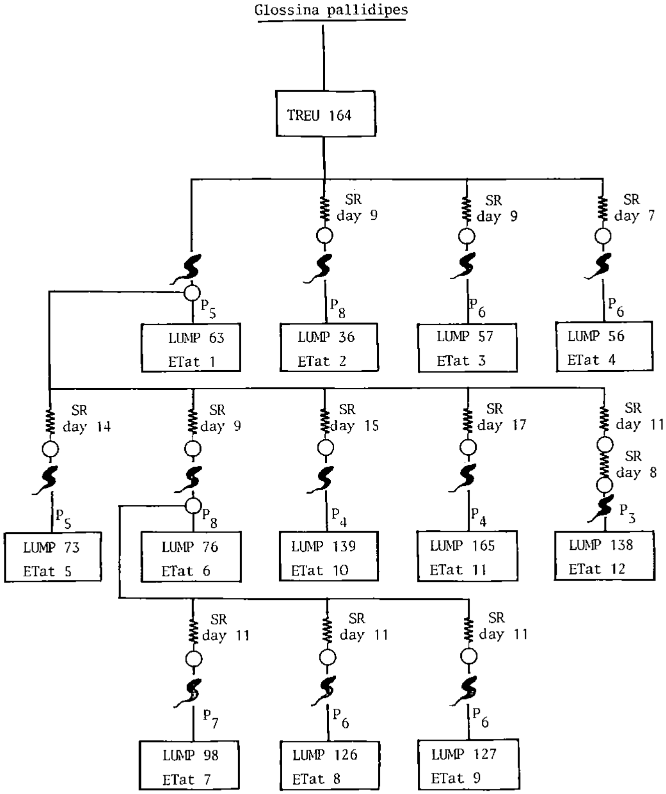

Figure 2

Stabilate pedigrees of the VAT clones ETat B1 to B12 (for details see Lumsden and Herbert 1975). All spontaneous relapse populations (SR) were obtained in mice.

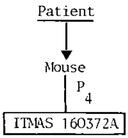

Figure 3

Pedigree of the first stabilate of strain C.

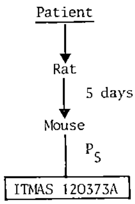

Figure 4

Pedigree of the first stabilate of strain D.

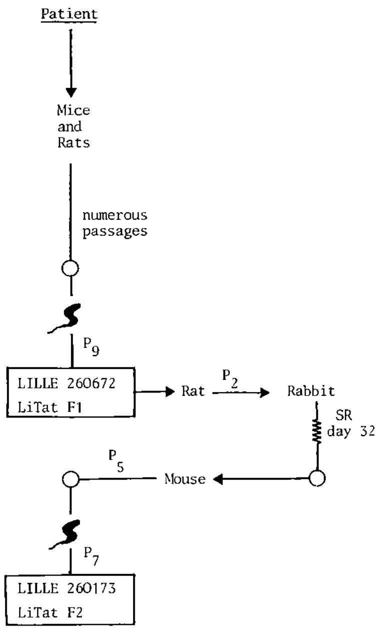

Figure 5

Stabilate pedigrees of the VAT clones LiTat F1 and F2 (for details see Afchain *et al.*, 1975).

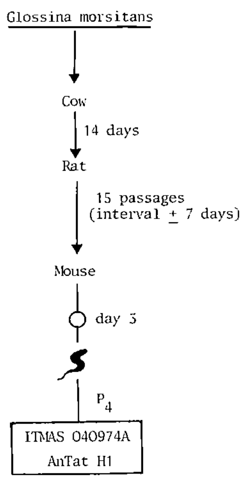

Figure 6

Stabilate pedigree of VAT clone AnTat H1.

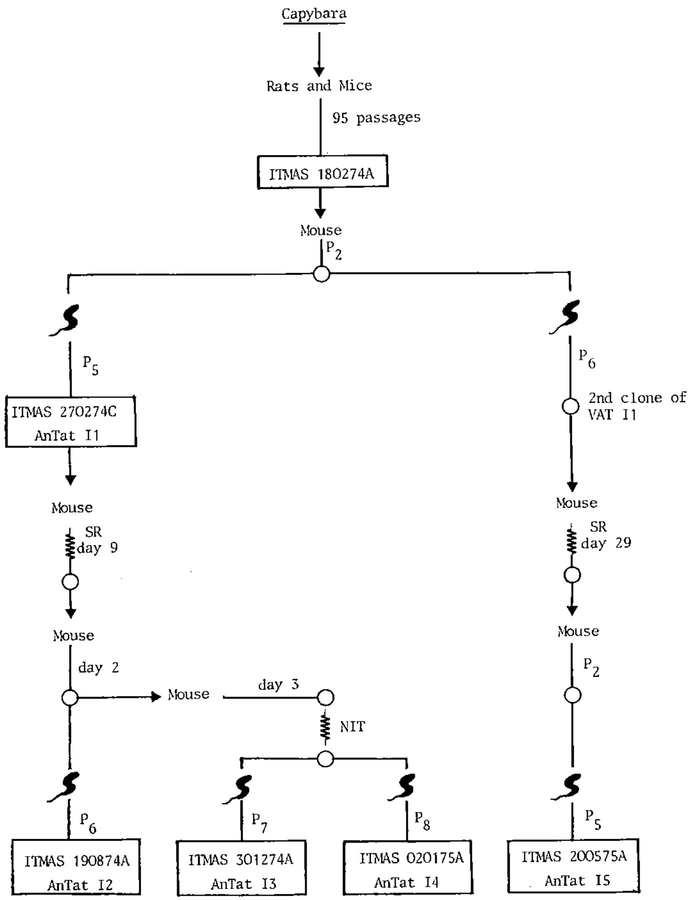

Figure 7

Stabilate pedigrees of the VAT clones AnTat I1 to I5.

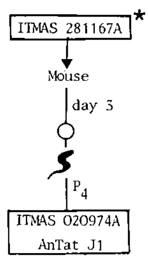

Figure 8

Stabilate pedigree of VAT clone AnTat J1
(★ first stabilate of an old laboratory strain).

Preparation of relapse populations

The patterns of VAT development were studied by immunofluorescence analysis of spontaneous relapse populations appearing in mice inoculated with less than 50 trypanosomes. In some instances pseudo relapse populations were set up from contaminating heterotypes present in clones, using neutralization-infectivity tests (NIT). For this purpose each of 3 mice were inoculated i.p. with 10^6 trypanosomes previously subjected to the lytic action of homotype-specific antiserum.

Results

Serological comparison of VAT clones

The 34 clones were cross-tested by immunolysis and immunofluorescence against 33 clone-specific antisera (antiserum to clone AnTat I5 was not available). In 3 instances 2 clones showed a high degree of cross-reactivity, respectively those of VATs A8 and I3, A11 and B7, F1 and I5. Table 2 shows the cross-reactions observed in trypanolysis tests. In consequence only 31 serologically distinct VATs could be identified among the clone collection.

TABLE 2

Serological cross-reactivities between VAT clones of different trypanosome strains as revealed by immunolysis tests (figures indicate the end-titres of the antisera; 0 = no lysis at the antiserum dilution of 1 : 4)

Clone tested	Antisera used				
	anti-A8	anti-I3	anti-A11	anti-B7	anti-F1
A8	64	128	0	0	0
I3	64	256	0	0	0
A11	0	0	256	256	0
B7	0	0	256	128	0
F1	0	0	0	0	64
I5	0	0	0	0	64

Immunofluorescence analysis of relapse populations

Immunofluorescence analysis of relapse populations revealed further similarities between the VAT repertoires of different strains. The origin of the relapse populations examined and the VATs detected were as follows.

Strain A

Populations examined : a) 10 NIT populations set up from heterotypes present in clones of VATs A1, A4 and A10; b) 13 first spontaneous relapse populations obtained in mice inoculated with VATs A6, A10 or A11. VATs detected : A1, A2, A3, A4, A5, A6, A7, A8/I3, A9, A10, A11/B7 and A12.

Strain B

Populations examined : 2 first spontaneous relapse populations obtained in mice inoculated with VAT B7. Only the VATs of strain A and B were looked up. VATs detected : B2, B3, B4, B5, B6, B7/A11, B10 and B12 (for details see Van Meirvenne *et al.*, 1975b).

Strain C

Eight out of the 31 distinct VATs were detected in spontaneous relapse populations which appeared in 8 mice inoculated with trypanosomes of stabilate ITMAS 160372A (see table 3).

TABLE 3

Variable antigenic types found in spontaneous relapse populations of strain C (mice inoculated with stabilate ITMAS 160372A)

Population examined		Positive findings (per 1,000 trypanosomes)							
Mouse no.	Days p.i.	A5	A6	A13	B3	B10	F1/I5	F2	I1
1	10				0.4				0.4
	17			2					
	23			10					
2	16				0.2				
	21					0.1			
	26	5		0.2			0.4		2
3	19								
4	15	0.4			300				
	21					0.3			
	28							0.4	
5	19	100			0.4				0.6
	26			15		20	5		
6	15	1			0.4	50			
	21		0.2			10			
7	14	2			0.3				5
	21								
	28					0.2			
8	15	10			5	5	10		0.3
	21								
	28								

Strain D

Two out of the 31 distinct VATs were detected in spontaneous relapse populations which appeared in 4 mice inoculated with trypanosomes of stabilate ITMAS 120373A (see table 4).

TABLE 4

Variable antigenic types found in spontaneous relapse populations of strain D (mice inoculated with stabilate ITMAS 120373A)

Population examined		Positive findings (per 1,000 trypanosomes)	
Mouse no.	Days p.i.	A6	F2
1	3		
	11	50	10
2	7		
	16		
3	14	50	
4	7		
	16		

Strain F

Two out of the 31 distinct VATs were detected in 3 NIT populations set up from heterotypes present in the clone of VAT F1, namely the types A8/I3 and H1.

Strain H

Six out of the 31 distinct VATs were detected in spontaneous relapse populations which appeared in 5 mice inoculated with VAT H1 (see table 5).

TABLE 5

Variable antigenic types found in spontaneous relapse populations of strain H (mice inoculated with VAT H1)

Population examined		Positive findings (per 1,000 trypanosomes)					
Mouse no.	Days p.i.	A5	A8/I3	B3	B5	B8	F2
1	14		105				
	17	0.1	55	11		0.2	
	19					0.2	0.7
	21				0.2		0.6
2	15	0.2	85				
3	15	0.05	195				
4	14	0.1	100				
5	14	0.8	45				

Strain I

Eight out of the 31 distinct VATs were detected in spontaneous relapse populations which appeared in 6 mice inoculated with VAT I1 and in 4 mice inoculated with VAT I3 (see table 6).

TABLE 6

Variable antigenic types found in spontaneous relapse populations of strain I (mice nos. 1-6 inoculated with VAT I1, mice 7-10 inoculated with VAT I3)

Population examined		Positive findings (per 1,000 trypanosomes)							
Mouse no.	Days p.i.	I1	I2	I3/A8	I4	I5/F1	B3	B8	H1
1	13		10	400	1				
	15		10	200	0.3				
2	17		500		10				
	20						50		20
	27					0.1			400
3	17		900						0.2
	20						1		100
	22								700
4	17		500						
	20						20		300
	27								2
5	22								
	27					0.2			
	34					20			200
6	17						0.6		0.2
	28					900			
	44								
	50								
	56								
	62							2	
	65							15	
7	16		600						50
	23					1			500
8	14	600	300		100				
	19						20		200
9	19								
	26						1		10
10	16	400			100		0.1		0.5

Strain J

Three out of the 31 distinct VATs were detected in 3 NIT populations set up from heterotypes present in the clone of VAT J1, namely the types A8/I3, B3 and I2.

A complete list of the positive findings of distinct VATs in different strains is given in table 7.

TABLE 7

List of VATs detected in different strains of trypanosomes (clone = isolated as clone population, IF = found in relapse populations by immunofluorescence, figures in brackets = highest proportion observed per 1,000 trypanosomes)

VAT	Strain A	B	C	D	F	H	I	J
A1	clone							
A2	clone							
A3	clone							
A4	clone							
A5	clone		IF (100)		IF (0.8)			
A6	clone		IF (0.2)	IF (50)				
A7	clone							
A8/I3	clone				IF (5)	IF (195)	clone	IF (0.1)
A9	clone							
A10	clone							
A11/B7	clone	clone						
A12	clone							
A13	clone		IF (15)					
B1		clone						
B2		clone						
B3		clone	IF (300)			IF (11)	IF (50)	IF (1)
B4		clone						
B5		clone				IF (0.2)		
B6		clone						
B8		clone				IF (0.2)	IF (15)	
B9		clone						
B10		clone	IF (50)					
B11		clone						
B12		clone						
F1/I5			IF (10)		clone		clone	
F2			IF (0.4)	IF (10)	clone	IF (0.7)		
H1					IF (1)	clone	IF (700)	
I1			IF (5)				clone	
I2							clone	IF (200)
I4							clone	
J1								clone

Protective immunity in rabbits with long-term infections

Of the 47 rabbits used in these experiments 27 were infected with strain A, 13 with strain B, 2 with strain F, 1 with strain H, 3 with strain I and 1 with strain J. Rabbits 259 to 262 were inoculated i.v. with a mixture of clones A1 to A9 whereas rabbits A to L were inoculated s.c. with antigenically undefined relapse populations of strain A (for details see Van Meirvenne *et al.*, 1975a). Rabbits 244 and 246 were inoculated i.v. with 10^6 trypanosomes of antigenically undefined stabilates of strain B, respectively EATRO 38 and EATRO 3. All the other animals were inoculated i.v. with 10^6 trypanosomes of the above-mentioned VAT clones. The results of the trypanolysis tests in which the multi-specific antisera of the rabbits were combined with different VAT clones are summarized in table 8. It may be seen that the animals produced antibodies to most of the antigenic types originating from the infecting (homologous) strain. As could be expected from the foregoing results there was also cross-immunity to some VATs of the heterologous strains.

TABLE 8

Results of trypanolysis tests with VAT clones and multi-specific antisera collected from rabbits with long-term infections (+ = lysis, ± = partial lysis, 0 = negative result, — = not tested)

ANTISERUM TESTED			RESULTS OF LYSIS TESTS WITH 34 CLONES																																	
INFECTING STRAIN	RABBIT	DAY	A													B												F		H	I					J
			1	2	3	4	5	6	7	8	9	10	11	12	13	1	2	3	4	5	6	7	8	9	10	11	12	1	2	1	1	2	3	4	5	1
A	221	30	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	+	0	0	0
	229	30	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	+	0	0	0
	228	30	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	±	0	+	0	0	0
	226	30	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0	0	0	+	0	0	0
	230	35	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	+	0	–	0
	219	33	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	±	+	+	0	0	0	0	0	0	0	+	0	+	0	0	0
	232	29	+	+	+	+	+	+	+	+	0	+	+	+	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0	0	±	0	0	0
	236	29	+	+	+	+	+	+	+	+	0	+	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	+	0	+	0	0	0
	241	32	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	+	+	0	0	0
	242	30	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	+	0	0	+
	266	30	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	+	0	0	+	0	0	0
	268	31	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	+	0	0	0	–	0	0	0	+	0	0	+	0	0	0
		58	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	–	–	0	+	–	–	–	–	–	0	+	+	0	0	+	0	0	+
	259	30	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	–	0	0	+	0	0	0	–	0	0	0	±	0	0	+	0	0	0
	260	30	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	–	0	0	+	0	0	0	–	0	0	0	0	0	0	+	0	0	0
	261	30	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	–	0	0	+	0	0	0	–	0	0	0	0	0	0	+	0	0	0
	262	30	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	–	0	0	+	0	0	0	–	0	0	0	0	0	0	+	0	0	0
	C	46	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	+	0	+	0	0	0	0	0	0	+	+	0	0	+	0	0	0
	D	53	+	+	+	+	+	+	+	±	+	+	+	+	+	0	0	0	0	0	0	+	0	0	0	0	0	0	+	+	0	0	±	0	0	0
	J	28	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	+	+	+	0	0	+	0	0	0	0	0	0	0	+	0	0	0
	K	21	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	+	0	0	0
	L	28	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	+	0	0	0
	A	41	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	±	+	0	0	0	0	0	0	0	+	+	0	+	0	0	+
	B	62	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	+	0	0	0	0	0	0	0	+	0	0	+	0	0	+
	F	39	+	+	+	+	+	+	+	+	+	+	+	±	0	0	0	0	0	0	0	+	0	0	0	0	0	0	+	+	±	0	+	0	0	0
	G	25	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	+	0	0	0	+	0	0	0

B	250	37	0	0	0	0	0	0	0	+	0	0	+	0	0	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	+	0	0	0
	231	35	0	0	0	0	0	0	0	0	0	0	+	0	0	0	+	+	+	±	±	+	0	+	+	0	+	0	0	0	0	0	0	0	0	0
	245	34	0	0	0	0	0	0	0	0	0	0	+	0	0	0	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0
	253	35	0	0	0	0	0	0	0	0	0	0	+	0	0	0	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	+
		49	0	0	0	0	0	0	0	0	0	0	+	0	0	0	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	±	0	0	0
	255	35	0	0	0	0	0	0	0	+	0	0	+	0	0	0	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	+	0	0	0
		47	0	0	0	0	0	0	0	+	0	0	+	0	0	0	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	+	0	0	0
	243	34	0	0	0	0	0	0	0	0	0	0	+	0	0	0	+	+	+	-	0	+	-	-	+	+	+	0	0	0	0	0	0	0	0	0
		57	0	0	0	0	0	0	0	+	0	0	+	0	0	0	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	+	0	0	0
	249	35	0	0	0	0	0	0	0	+	0	0	+	0	0	0	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	+	0	0	0
	254	35	0	0	0	0	0	0	0	0	0	0	+	0	0	0	+	+	+	+	0	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0
	247	34	0	0	0	0	0	0	0	0	0	0	+	0	0	0	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0
	270	30	0	0	0	0	0	0	±	+	0	0	+	0	0	0	+	+	-	-	+	+	-	-	-	-	-	0	0	0	0	0	+	0	0	0
	251	37	0	0	0	0	0	0	0	0	0	0	+	0	0	+	+	+	+	0	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0
	246	34	0	0	0	0	0	0	0	0	0	0	+	0	0	+	+	+	+	+	±	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0
	244	34	0	0	0	0	0	0	0	0	0	0	+	0	0	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	±	0	0	0
F	273	30	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	-	-	0	0	-	-	-	-	-	+	+	-	0	0	±	0	+	+
		61	0	0	0	0	0	0	0	+	-	-	0	-	-	0	0	0	-	-	0	0	-	-	-	-	-	+	+	-	0	0	+	0	+	+
	274	30	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	-	-	0	0	-	-	-	-	-	+	+	-	0	0	+	0	+	0
		61	0	0	0	0	0	0	0	+	-	-	0	-	-	0	0	0	-	-	0	0	-	-	-	-	-	+	+	-	0	0	+	0	+	+
H	272	30	0	0	0	0	+	0	0	+	0	0	0	0	0	0	0	+	-	-	0	0	-	-	-	-	-	0	+	-	0	0	+	0	0	0
I	263	30	0	+	0	0	0	0	0	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	0
		99	0	+	0	0	0	0	0	+	0	0	0	0	0	0	0	+	-	-	0	0	0	0	0	-	0	±	0	±	+	+	+	+	+	+
		152	0	+	0	0	0	0	0	+	0	0	0	0	0	0	0	+	-	-	0	0	-	-	-	-	-	0	0	+	+	+	+	+	+	+
	265	30	0	+	0	0	0	0	0	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	+	0	+	+	+	+	+	+	0
		99	0	+	0	0	0	0	0	±	0	0	0	0	0	0	0	+	-	-	+	0	0	0	0	-	0	+	0	0	+	+	+	±	+	+
	275	32	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	+	-	-	0	0	-	-	-	-	-	+	0	-	+	+	+	+	+	+
J	271	30	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	+	-	-	+	0	-	-	-	-	-	+	0	-	0	+	+	+	+	+
		61	0	0	0	0	0	0	0	+	-	-	0	-	-	0	0	+	-	-	0	0	-	-	-	-	-	+	0	-	0	+	+	+	+	+

Discussion

Previous attempts to differentiate between the subspecies of *T.* (*T.*) *brucei* by comparisons of variable antigenic types have led to equivocal conclusions (Gray and Luckins, 1976). Though only minute parts of the VAT repertoires have been examined during the present experiments, the results suggest that variable antigens are unlikely to be of practical value in differentiating between species and subspecies of the subgenus *Trypanozoon.* At the infra-taxonomic level however, it seems possible to type trypanosomes into serologically different strains by comparing their patterns of VAT development in laboratory animals.

Serologically similar VATs produced by different strains, subspecies and species provide particularly interesting models for study of the genetical aspects of antigenic variation. Taxonomically and geographically widely distributed variable antigens might be used for immuno-diagnostic purposes. Partial cross-immunity to different strains of trypanosomes might play an important role in hosts with mixed infections.

Acknowledgements — *This work was supported by a grant from the « Fonds voor Geneeskundig Wetenschappelijk Onderzoek ». The skilful technical assistance of Mrs Ingrid Lamot is gratefully acknowledged.*

Samenvatting — **Vergelijkende studie van variabele antigeentypen voortgebracht door trypanosomenstammen van het subgenus** *Trypanozoon.*

Het verschijnsel van antigenische variatie werd bestudeerd bij 8 trypanosomenstammen behorend tot verschillende soorten en ondersoorten van het subgenus *Trypanozoon.* In totaal werden 31 verschillende antigeentypen geïdentificeerd. Elke stam produceerde een voor hem tamelijk karakteristieke reeks predominante varianten. Er werden evenwel talrijke serologische gelijkenissen waargenomen tussen varianten van verschillende stammen.

Résumé — **Etude comparative de types antigéniques variables produits par des souches de trypanosomes du sous-genre** *Trypanozoon.*

Le phénomène de la variation antigénique a été étudié dans 8 souches de trypanosomes appartenant à différentes espèces et sous-espèces du sous-genre *Trypanozoon.* Au total 31 types antigéniques différents ont été identifiés. Chaque souche produisait une série plus ou moins caractéristique de variants prédominants. Néanmoins plusieurs similarités sérologiques ont été observées entre variants de souches différentes.

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DISCUSSION

A. R. Gray : The work on the occurrence of common antigenic types over a wide range of trypanosome species, I think is of considerable interest but I don't think we should lose sight of the fact that it's really the differences that are going to count in the longterm rather than the similarities. There is still to my mind not sufficient similarity between these trypanosomes to be useful at the present time.

P. de Raadt : I have a question for Dr Van Meirvenne as well as for Dr Jenni, that is that we are seeing here now overlappings of immune protection but do we know what kind of immune protection we would like to have to protect individuals against trypanosome infection ? Should it be the metacyclics or should it be the forms which are now described by Dr Van Meirvenne ? Can Dr Jenni tell us what happened to those animals who have high titers against metacyclics and you challenge them with a fly, will there be any parasitaemia or not ?

L. Jenni : This is a very important question and it's the key experiment which has to be done now. We have rechallenged immunized mice with infective flies. So far, after three weeks, no infection has occurred.