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VECTOR SUSCEPTIBILITY TO AFRICAN TRYPANOSOMES

by

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Summary. — Susceptibility of tsetse fly to trypanosome depends on two distinct barriers controling respectively colonization of midgut and, migration to salivary glands. Those barriers are modulated by barely known factors, pertaining to the physiological status of the fly as well as to cytoplasmic and nuclear inheritance. Quantification of colonization (p) and migration (m) rates provides a way to calculate intrinsic vectorial capacity (IVC) as a product IVC = p × m, and to undergo comparative analysis of underlying factors.

Epidemiologists and primary health care professionals facing African trypanosomiasis quite often ask: « What level of parasitaemia is sufficient to infect a fly? How frequent are the infective fly bites? Could man-fly-trypanosome contact be quantified? ». Such questions address two distinct categories of factors, namely environmental factors, which are responsible for the fly-mammal contact rate, and intrinsic factors, which determine the fate of the parasite within the vector.

For African trypanosomes, especially in the case of the human pathogens, ingestion by tsetse is scarcely followed by completion of the parasite life cycle. Actually, very little is known about intrinsic vectorial capacity (IVC) of the tsetse, i.e. its intrinsic capability to develop a metacyclic infection.

This paper would like to pinpoint a few clues from our laboratory cyclical transmission data, in an attempt to identify some of the factors underlying IVC. *Trypanosoma gambiense* and *T. brucei* will be briefly considered and contrasted. A tentative calculation of IVC will be proposed.

1. — IVC does vary depending on the fly and on the trypanosome

Seven colonies of tsetse (2 from the *morsitans* group and 5 from the *palpalis* group) were fed on different isolates of trypanosomes (2 *T. brucei* and 4 *T. gambiense*) (1). Frequency of metacyclic infection in salivary glands varied from 0 to 15 %, even when the same fly (table 1) was fed on different isolates of trypanosomes, or when the same trypanosome (table 2) was offered to various flies (6, 7).

An isolate of *T. brucei* from Bas-Zaire was not transmitted by its putative local vector *G.p.palpalis* but well by East African *G.m.morsitans* (table 3).

TABLE 1

Susceptibility of *G.m. morsitans* (Kariba, Zimbabwe, Bristol colony, wild type) to various isolates of *T. brucei* and *T. gambiense*

Vectorial susceptibility	Trypanosome isolates					
	T. brucei		*T. gambiense*			
	EATRO 1125 (Tanzania)	KIM 1 (Zaire)	MBA (Zaire)	NTUMA (Zaire)	MOER 103 (Zaire)	FRALA (Côte d'Iv.)
n flies	664	176	70	63	54	58
% procyclics	49.9	50	45.7	42.9	48.1	66.1
% metacyclics	14.7	6.8	0	0	0	0

TABLE 2

Susceptibility of various tsetse to the same trypanosome (*T. brucei* EATRO 1125, Tanzania, clone population)

Vectorial susceptibility	Tsetse		
	G.m.m. (Zimbabwe)	*G.m.c.* (Tanzania)	*G.p.p.* (Zaire)
n flies	664	96	85
% procyclics	49.9	22.9	3.5
% metacyclics	14.7	6.2	0

TABLE 3

Susceptibility of tsetse from various geographic origins to a Zaire isolate of *T. brucei* (KIM 1, Kimayala, Bas-Zaire). The putative sympatric *G.p. palpalis* (Mongo-Bemba, Bas-Zaire) is not susceptible

Vectorial susceptibility	Tsetse			
	G.p.p. (Zaire)	*G.p.p.* (Nigeria)	*G.p.g.* (Burkina F.)	*G.m.m.* (Zimbabwe)
n flies	160	106	63	176
% procyclics	0.6	7.5	1.6	50
% metacyclics	0	0	0	6.8

A *gambiense* isolate from Bas-Zaire and its putative sympatric vector (table 4) did not give way to mature infection, which developed in flies from Burkina-Faso and Central African Republic.

TABLE 4

Susceptibility of various tsetse to the same clone population of *T. gambiense* (stock MBA, Bandundu, Zaire). Transmission by Zairian *G.p. palpalis* did not occur

Vectorial susceptibility	Tsetse					
	G.m.m. (Zimbabwe)	*G.p.p.* (Zaire)	*G.p.p.* (Nigeria)	*G.p.g.* (Burkina F.)	*G.t.* (Tchad)	*G.f.f.* (RCA)
n flies	70	160	40	22	46	210
% procyclics	45.7	7.9	5.1	4.5	19.6	1.0
% metacyclics	0	0	0	3.3	0	0.5

Altogether, *brucei* metacyclic development took place in *morsitans* flies only, while *gambiense* metacyclics were restricted to *palpalis* flies. This is in apparent contradiction with the conclusions of Moloo *et al.* (10).

2. — **Fly factors**

Our results evidenced a large variability between gut and salivary glands susceptibility to colonization by trypanosomes and they confirmed previous laboratory observations, *e.g.* Harmsen (5). The development of the trypanosome in the fly takes place through two, apparently independant steps or barriers, *i.e.* colonization of the gut and migration into the salivary glands. Both barriers appear to be modulated by the physiological (nutritional) status of the fly as well as by intrinsic, inherited characters.

Teneral flies are reputedly much more susceptible to trypanosomes. Starved adult flies regain some susceptibility (2, 7), a fact to be taken into account by field surveys (9). Preliminary data from Gooding (3) further suggest an influence of the nature of the bloodmeals taken by adult flies prior to their infecting feed.

Maudin (8) has recently shown evidence for cytoplasmic inheritance of susceptibility to trypanosome by the tsetse. This kind of inheritance appears to be related to intracellular endosymbionts and gut cell receptors in the fly. The character is unstable however as due to its non-mendelian segregation amongst the fly's progeny.

Genomic inheritance too has now proven to modulate both colonization and migration barriers. Sex of the fly and certain mutations influencing the metabolism of the tsetse (such as the *salmon* mutation) affect significantly the establishment of the trypanosome in the midgut as well as in the salivary glands (6, 7). Isogenic lines of flies should help analysis of Mendelian inheritance of IVC (Bushrod, pers. comm.).

Two conclusions could be proposed at this stage:

(i) both extrinsic and intrinsic characters control IVC, and their role in laboratory conditions as well as in field circumstances ought to be precised.

(ii) two parameters — gut colonization rate and migration rate to salivary glands — should be considered separately by studies on factors modulating IVC.

3. — **Mammalian host factors**

Recent success of Moloo *et al.* (10) in transmitting consistently *T. gambiense* through both *morsitans* and *palpalis* flies reconciles conflictory results mentioned here above. It stresses the role of the species of infected mammal subject to the fly bite (table 5) and it could explain the failure of cyclical transmission of *T. gambiense* by using conventional laboratory rodents.

In Moloo's experiments, 2 to 10% *gambiense* metacyclic infection was achieved by maintaining adult flies on chronically infected goats and cattle. This is in sharp contrast with the nul transmission by flies fed on acutely

infected mice. The latter however are most efficient for cyclical transmission of *T.brucei*.

We consider this fact as of relevance for the understanding of the natural transmission of *T.gambiense*. The role of healthy carriers in the epidemiology of sleeping sickness should be evaluated by quantitative studies on vectorial transmissibility of asymptomatic *versus* symptomatic parasitaemia.

4. — Intrinsic vectorial capacity (IVC)

There is a need to quantify the magnitude of the barriers to establishment (gut) and metacyclogenesis (salivary glands) of trypanosomes in the fly, in order to conduct comparative studies on factors underlying IVC. Therefore we propose that intrinsic vectorial capacity of a given fly population be the product of colonization and migration rates:

$$IVC = p \times m$$

— where (p) is the proportion « n' procyclic flies/n fed flies » of tsetse allowing bloodstream trypanosomes to establish as procyclics in the gut, and

— where (m) is the proportion « n'' metacyclic flies/n' procyclic flies » of procyclic-infected tsetse allowing the trypanosomes to migrate to the salivary glands.

Two examples illustrate the interest of this approach. In one case (table 5), two different flies having ingested the same trypanosome may differ in vectorial susceptibility by a factor 2.3 (% metacyclics = 14.7 and 6.2, or IVC = 0.147 and 0.062, respectively). Breakdown of IVC into (p) and (m) show that the difference resides actually at the level of the gut barrier (p = 0.499 and 0.229, respectively) while the migration barrier is of similar magnitude (m = 0.293 and 0.272, respectively).

TABLE 5

Difference in vectorial capacity (IVC) to the same trypanosome (*T.brucei*) may result only from a difference in susceptibility (p) to midgut colonization by procyclic trypanosomes

Fly		procyclics			metacyclics			IVC
subspecies	n fed	n'	%	$p(=\frac{n'}{n})$	n''	%	$m(=\frac{n''}{n'})$	(pxm)
G.m.morsitans	664	331	49.9	0.499	97	14.7	0.293	0.147
G.m.centralis	96	22	22.9	0.229	6	6.2	0.272	0.062

In another instance (table 6), two different fly lines « salmon » and « super » may have a similar vectorial capacity (IVC = 0.254 and 0.283) while they do differ actually at both gut (p = 0.570 and 0.987) and salivary gland (m = 0.455 and 0.289) levels in an opposite way.

TABLE 6

Similar vectorial capacity (IVC) to the same trypanosome (*T.brucei*) may result from opposite susceptibilities to gut (p) and salivary gland (m) colonization

G.m.morsitans		procyclics			metacyclics			IVC
line	n fed	n'	%	$p(=\frac{n'}{n})$	n''	%	$m(=\frac{n''}{n'})$	(pxm)
«salmon»	193	110	57.0	0.570	49	25.4	0.455	0.254
«super»	46	45	98.7	0.987	13	28.3	0.289	0.283

5. — IVC to *T.gambiense*

Preliminary comparison (D. Aerts, unpublished) of our and previous data on vector susceptibility to *T.gambiense* was performed by using the calculation above (table 7).

In acute infections *T.gambiense* was not transmissible, at the contrary of *T.brucei*. Vectorial capacity of *palpalis* flies fed on *T.gambiense* in chronically infected animals ranged from 0.3 to 5.4%. Altogether intrinsic vectorial capacity to *T.gambiense* was found to be on the average 10 to 30 times less than the capacity of transmitting *T.brucei*.

Conclusions

Two main questions should be addressed:

(i) what is the value of conventional laboratory animals (*e.g.* the «acute mouse» model) for studies on cyclical transmission? Mammals acting as natural reservoirs should deserve more attention, especially for *T.gambiense*.

(ii) what would tell studies closer to the field situation, conducted by associating sympatric tsetse and trypanosomes from a given focus?

A more accurate analysis of vector susceptibility to trypanosome is obviously needed for identification of the diverse factors underlying vectorial capacity. Breakdown of the latter into its two main components — gut colonization rate (p) and salivary glands migration rate (m) — and their subsequent product ($p \times m$) are proposed for quantitative studies on intrinsic vectorial capacity (IVC).

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Résumé. — La susceptibilité vectorielle de la glossine vis-à-vis du trypanosome est sous la dépendance de deux barrières distinctes qui contrôlent respectivement la colonisation de l'intestin moyen de la tsétsé par le trypanosome, et la migration de celui-ci vers les glandes salivaires. Ces barrières paraissent modulées par des facteurs multiples et encore mal connus, attribuables tant à l'état physiologique de la glossine qu'à ses caractères génétiques cytoplasmiques et nucléaires. La quantification des taux de colonisation (p) et de migration (m) du trypanosome permet l'analyse quantitative de la capacité vectorielle intrinsèque de la tsétsé en tant que produit

TABLE 7

Intrinsic vectorial capacity (IVC) to *T. gambiense*, recalculated from various authors and expressed as a percentage. *T. brucei* data are mentioned for comparative purpose

Fly	Trypanosome	Host		IVC = (pxm) × 100	Reference
		Species	Infection		
G.m.morsitans WILD TYPE	*T.brucei* (Uganda)	mouse	acute	14.8 %	Makumyaviri (7)
SALMON		mouse	acute	25.4 %	Makumyaviri (7)
SUPER		mouse	acute	28.3 %	Aerts (unpubl.)
G.m.morsitans WILD TYPE	*T.gambiense* (Zaire)	rabbit	chronic	none	Aerts (unpubl.)
G.p.gambiensis	*T.gambiense* (Zaire)	guinea pig	semi-acute	none	Aerts (unpubl.)
G.p.gambiensis	*T.gambiense* (Zaire)	rabbit	chronic	0.7 %	Aerts (unpubl.)
G.p.gambiensis	*T.gambiense* (Ivory Coast) (Liberia)	*in vitro*	/	⩽ 4.1 %	Richner (11)
G.p.gambiensis	*T.gambiense* (Nigeria)	calf	chronic	1.8 %	Moloo (10)
G.p.gambiensis	*T.gambiense* (Nigeria)	goat	chronic	5.4 %	Moloo (10)
G.tachinoides	*T.gambiense* (Nigeria)	dog	chronic	0.9 %	Gray (4)
G.palpalis subsp.	*T.gambiense* (Zaire)	guinea pig	semi-acute	2.4 %	Van Hoof (12)
		pig	chronic	0.3 - 4.0 %	
		man	chronic	3.4 %	

(CVI = p × m) de ces deux barrières, ainsi que l'étude objective des facteurs modulant la susceptibilité vectorielle.

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