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中米グァテマラにおけるオンコセルカ症伝搬ブユ

――その研究のあゆみと最近の知見――

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1. は じ め に

オンコセルカ症は，回旋糸状虫（*Onchocerca volvulus*, 以下 *O. v.* と略）が吸血性昆虫ブユ(Simuliidae)によって伝搬されておこる。本症は，皮膚症状・腫瘤形成・眼疾（重篤の場合失明を伴う）を主徴とする慢性疾患で，まだ満足すべき治療法は確立されていない。現在，アフリカ，アラビア半島の一角，および中・南米に広く分布がみられ，全地域あわせて約3,000―4,000万人の罹患者がいると推定されている。

アフリカでは，サハラ砂漠以南のサバンナ地帯と熱帯森林地帯に広大な流行地がある。この流行地における本症の調査研究の歴史は古い。現在，西アフリカのボルタ河流域の流行地では，殺虫剤による媒介ブユのコントロールを中心とした「西アフリカ・ボルタ河流域オンコセルカ症防圧20年計画」(OCP)――1973年開始――が，WHOなどの国際機関によって進められている。一方，西半球においては，オンコセルカ症がかなり以前に発見されている中米のグァテマラおよびメキシコの流行地や，まだ発見後日の浅い南米のベネズエラ，ブラジル，コロンビア，エクアドルの流行地などがみられ，地域によっては臨床症状をはじめとして，流行の様態が一様ではない。さらに，多くの流行地では，伝搬の機構など不明のままである。

筆者は，従来，この西半球の各地域で種々異なる流行の様相を示しているオンコセルカ症の伝搬機構に強い関心を持ってきた。しかるに，1978年8月から1980年1月までの1年半，国際協力事業団派遣専門家として「グァテマラ国オンコセルカ症研究対策プロジェクト」に参加する機会を得，研究の第一歩を踏みだすことができた。本稿は，この研究に関連して西半球において古くから研究のなされてきたグァテマラのオンコセルカ症伝搬ブユについて，これまでの知見をまとめたものである。

さて，この国のオンコセルカ症伝搬ブユに関しては，Dalmat (1955) や Hamon (1974) の総説がある。その中で，いくつかの本質的に重要な知見――*Simulium ochraceum*, *S. metallicum*, および *S. callidum* の3種のブユが媒介者であること，またそれぞれのブユ種の生態学的知見，さらにこれら3種ブユの疫学上の相対的比重など――は，すでに報告されている。一方，媒介ブユ体内での伝搬機構――*O. v.* 幼虫の移行や消長など――については必ずしも満足すべき知見は得られていなかった。これは，一つには，ブユ成虫の室内飼育法などと関連した技術的困難さから止むを得なかった面もあろう。

ところが，最近10年間のグァテマラにおける伝搬ブユの調査研究の進展は目をみはるものがあり，伝搬の本質的な面も少しずつ明らかにされてきた。したがって，本稿では従来の知見に言及しつつも，このような最近の研究によって得られた知見に重点をおいた。

なお，オンコセルカ症の全搬（本病の歴史，病原寄生虫，病理，伝搬，臨床，診断，治療，疫学，および防圧）に関しては，Nelson (1970)，多田(1976)，Sasa (1976) のすぐれた総説があることを付記しておきたい。

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2. 研究のあゆみ

1915年, Dr. R. Robles は, グァテマラにおいて西半球では初めてオンコセルカ症を発見した。まもなく, 彼は, このフィラリアの伝搬者は流行地に数多くみられる吸血昆虫のブユであろうと示唆した（1917, 1919)。これは, 博士自らの流行地における広範にして緻密な疫学調査に裏付けられたものであった。当時, アフリカにおけるオンコセルカ症の媒介者はまだ不明であり, 他のフィラリアについても, やっとバンクロフト糸状虫およびロア糸状虫の媒介者が, おのおの蚊（Manson, 1878）およびアブ（Leiper, 1914）であることしかわかっていなかった。したがって, 博士の推察は大変な卓見に思われる。

しかしながら, この病気が発見されて以来, グァテマラの流行地では臨床的・疫学的調査研究が急速に進められていく一方で, その伝搬に関しては不明のままで過ぎた。この間に, Blacklock (1926) は, アフリカのシエラ・レオネにおいて, *O. v.* のミクロフィラリア（以下 mf と略）保虫者から実験的に吸血させたブユ（*S. damnosum*）の体内で幼虫の第 III 期までの発育を初めて観察した。

グァテマラでは, これに少し遅れて1931・1932年のハーバード大学学術調査隊の現地調査によって, 野外採集ブユに *O. v.* 幼虫の自然感染が初めて見出され, オンコセルカ症の伝搬に *S. ochraceum*, *S. metallicum* および *S. callidum* の3種のブユが重要であると報告された（Strong *et al.*, 1934)。このあと, イタリア人の Dr. M. Giaquinto Mira は, 人吸血嗜好性の強さから, 上記3種のブユのうち *S. ochraceum* が主要媒介者であろうと報告した（1937)。

1940年代に入って, 伝搬ブユの調査は活況を呈した。同国の昆虫学者 Dr. R. De León は, ブユの分類のほか, ブユ成虫と伝搬との関係を調査した。また Pan American Sanitary Bureau から派遣された Dr. H. Elishewitz（1944-1945年の間滞在）は, Dr. De León らの協力を得て, ブユの成虫だけでなく幼虫・蛹期についても生態学的調査を行った。博士の仕事は, ほとんどが業務報告の形でしか発表されていないこともあり, あまり知られていない。その概要は次のごとくである。Dr. Elishewitz の調査対象は, 1)流行地内外に分布するブユの動物吸血嗜好性, 2)人吸血性ブユの周年および日周活動, 3)吸血習性（吸血部位, 時間, また吸血と温度, 湿度, 照度などの気象要因との関係など), 4)流行地の地質学的特性, および水系の類型化によるブユ幼虫の棲息水系選択性, 5)ブユ幼虫発育期間, 6)Vector control, など広い範囲におよんでいる。その結果, 彼は, *S. ochraceum* の分布が流行地の分布と一致すること, 人体のいずれの部位からも吸血すること, 人吸血嗜好性が非常に強いこと, さらに吸血行動が安定しているなどのいわゆる生態学的要因を根拠として, このブユ種がグァテマラのオンコセルカ症の主要媒介種であろうと報告している（Elishewitz, 1953)。

第二次大戦後に, Dr. Elishewitz の後任として赴任した Dr. H. Dalmat（1947-1953年滞在）は, 米国の NIH やグァテマラ国厚生省の援助も受け, 流行地の中心であるチマルテナンゴ県のエポカパに設立されたオンコセルカ症の研究室（ロブレス博士記念研究所）を舞台として, ブユの分類, 生態, ブユと伝搬との関係, などについて精力的な研究を行い, 数多くの論文を発表した。これらの業績はまとめられ,「The black flies (Simuliidae: Diptera) of Guatemala and their role as vectors of onchocerciasis」と題した一冊の本として, 米国の Smithsonian Institution から出版された（1955)。この本は, それ以降, 中米のオンコセルカ症の伝搬に関する研究を志すものにとって, バイブル的存在価値を有している。Dr. Dalmat は, グァテマラ国内各地において行った広範なブユの採集結果をもとに, 多数の新種を報告するとともに, これまでの既知種をあわせて41種のブユについて各発育期の記載, 検索表の作成など, 同国におけるブユの分類学的研究を大きく進展させた。一方, 博士の行った生態学的調査のなかで, ブユ幼虫の水系選択性, 棲息水系の種々の環境要因の

分析，およびブユ成虫の吸血活動に関する結果は，前述の Dr. Elishewitz のと重複するが，各種ブユの分布，成虫の飛翔距離，寿命，休息場所などについては新しい知見を数多く報告した。また，このような生態学的調査と自然感染調査の結果とにもとずいて，*S. ochraceum, S. metallicum, S. callidum* の相対的重要性を詳細に考察した。

Dr. Dalmat と同時期に派遣されていた Dr. C. L. Gibson (1948-1952年滞在) は，グァテマラのブユ体内での *O. v.* 幼虫の発育を初めて実験的に観察した。

このあと，1966年，英国の Dr. B. O. L. Duke は，Dr. De León と協同で，中米およびアフリカの「オンコセルカ－ブユ」complex の差異を検討するため，アフリカのオンコセルカ症患者の移送を伴う画期的な実験をおこなった。これをきっかけとして，1970年代前半には，アフリカのオンコセルカ症調査研究の経験を有する外国人研究者 (Dr. I. Tada(日)，Dr. R. Garms, Dr. M. S. Omar (独)，Dr. O. Bain (仏) など) が相次いで来訪し，中米グァテマラのオンコセルカ症伝搬ブユについての研究に新しい局面が開かれた。最近では，わが国の国際協力事業団による「オンコセルカ症研究対策プロジェクト」(第一次，1975-1980，リーダー高橋弘博士；第二次，1980～，リーダー鈴木猛博士)，および Dr. R. Collins をリーダーとする米国の C. D. C. チーム——1975年から——などによる組織的な研究が展開され，新しい知見が次々と発表されている。

3. *O. volvulus* 媒介ブユ種および媒介可能ブユ種

どのブユ種が，グァテマラのオンコセルカ症を媒介しているのかに関して，これまで自然感染調査および感染実験が数多く行われてきた。そして，すでに述べたように，Strong *et al.* (1934)の報告以来，古くから *S. ochraceum, S. metallicum, S. callidum* が媒介者として一般に知られてきた。しかしながら，その第一の根拠となるべき自然感染の結果は，ごく最近のを別にすれば，ブユ体内で見出されたフィラリアの種および発育期の鑑別に関して必ずしも明確に示されてはいない。この機会に，これまでの調査結果を検討してみた。

ところで，グァテマラでは現在45種のブユが報告されているが，人吸血性が認められているのは

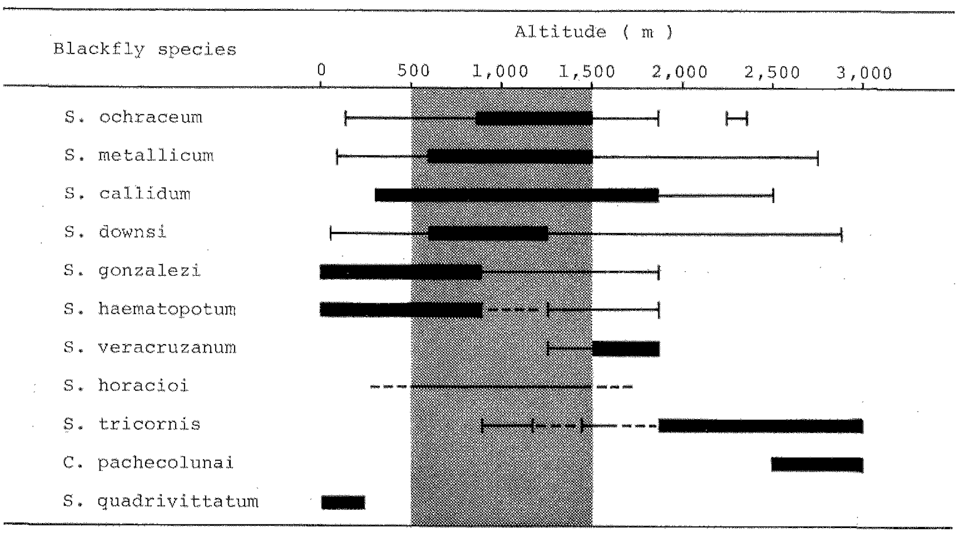

Figure 1 Vertical distribution of the anthropophilic blackfly species in relation to that of the onchocerciasis endemic areas of Guatemala (Distribution range of endomic areas was represented by the shaded column; all data were based on Dalmat (1955) except for *S. horacioi* and *S. quadrivittatum*).

Table 1 Investigations on natural infection of Guatemalan blackflies with *O. volvulus* larvae

Author(s) and year	No. females dissected	No. females with *O. volvulus* larvae			% Females with larvae of any stages	% Females with third-stage larvae
		I	II	III		
			S. ochraceum			
Strong *et al.*, 1934	1,658*		——89——		5.3	?
Gibson, 1951	1,839		—— 7——		0.38	?
Dalmat, 1955	6,815		——30——		0.44	?
De León, 1957	26	0	0	1	3.8	3.8
	70	0	0	1	1.4	1.4
	61	0	0	1	1.6	1.6
Garms, 1975	2,243	23	3	1	1.2	0.04
	294	17	4	2	7.8	0.68
Collins, 1979	4,851	——69——		15	1.7	0.31
Garms & Ochoa, 1979	1,445	80	11	13	7.2	0.90
	154	13	3	5	13.6	3.2
Collins *et al.*, 1981	1,282	—— 9——		5	0.7	0.39
	3,569	——60——		10	1.68	0.28
Takaoka, 1982	1,551	9	0	1	0.6	0.06
			S. metallicum			
Gibson, 1951	1,734		——18——		1.04	?
Dalmat, 1955	7,678		——26——		0.34	?
De León, 1957	40	0	0	3	7.5	7.5
	18	——1——		0	5.6	0.0
	15	——1——		1	13.3	6.7
	339	——2——		4	1.8	1.2
	74	0	0	1	1.4	1.4
	26	0	0	1	3.9	3.9
Garms, 1975	242	1	0	0	0.4	0.0
	679	3	0	0	0.4	0.0
	269	1	0	0	0.37	0.0
	1,826	2	0	0	0.11	0.0
Collins, 1979	1,855	——23——		0	1.2	0.0
Garms & Ochoa, 1979	1,395	16	2	2	1.4	0.14
	570	8	0	2	1.75	0.34
	59	2	0	0	3.4	0.0
Tanaka *et al.*, unpub. cited by Ito *et al.*, 1980	2,638	——10——		2	0.45	0.07
Collins *et al.*, 1981	795	—— 5——		0	0.63	0.0
	1,060	——26——		0	2.45	0.0
Takaoka, 1982	1,663	—— 2——		0	0.12	0.0

Table 1 continued (1)

			S. callidum			
Gibson, 1951	161		——— 1 ———		0.62	?
De León, 1957	1		——1——	0	100	0.0
	?	0	0	1	?	?
Garms, 1975	27	1	0	0	3.7	0.0
Collins, 1979	702		——3——	1	0.57	0.14
Garms & Ochoa, 1979	100		——2——	4	6.0	4.0
Collins *et al.*, 1981	251		——1——	0	0.4	0.0
	451		——2——	0	0.44	0.0
			S. gonzalezi			
Gibson & Dalmat, 1952	463		———12———		2.6	?
Garms, 1975	13	1	0	0	7.9	0.0
	134	2	0	0	1.5	0.0
Garms & Ochoa, 1979	206	8	0	0	3.9	0.0
			S. downsi			
Collins, 1979	503		——1——	0	0.2	0.0
	491		——1——	0	0.2	0.0
			S. haematopotum			
Gibson & Dalmat, 1952	1,276		———2———		0.2	?
			S. horacioi			
Takaoka, 1982	743	0	0	0	0.0	0.0

* *Simulium metallicum* and *S. callidum* are also included.

11種である（Figure 1 参照）。このうち，Table 2 に示すように，8種のブユが自然感染または実験感染について報告されている。

自然感染 これまで *S. ochraceum*, *S. metallicum*, *S. callidum* の3種に *O. v.* 感染幼虫の自然感染が報告されている（Table 1）。

グァテマラにおいて，*O. v.* 幼虫のブユ体内での自然感染を初めて発見したのは，Strong *et al.* (1934) である。上記3種あわせて1,658個体解剖し，89個体の雌に第 III 期幼虫を含む感染を報告した。しかしながら，この3種のいずれのブユに第 III 期幼虫が見られたのかは明らかにされていない。また，一部の第 III 期幼虫は，計測値（長さ，450-1,140 μm）および図示されている形態とその寄生していた部位（マルピーギ管）などから判断して，全く別の不明フィラリア――後にDe León and Duke (1966) によって指摘されたのと同一種――であった可能性が強い。

これら3種のブユのうち，自然感染の例が最も多く報告されているのは *S. ochraceum* である。解剖雌のうち第 III 期幼虫を保有する割合は，報告者および調査地により異なるが，0.02-3.2% と低い。

次に *S. metallicum* についてみると，第 I・II 期幼虫の感染は数多く報告されているが，第 III 期幼虫保有雌は De León (1957)，Garms and Ochoa (1979) および Tanaka *et al.* (unpublished data, Ito *et al.* (1980) に引用）によって観察されているに過ぎない。このうち，De León (1957) の結果は，マルピーギ管寄生幼虫も含めているので，全ての幼虫が *O. v.* であったか疑問がもたれる。これを一応別にすれば，第 III 期幼虫保有率は0.07-0.34% とやはり低率である。

Simulium callidum における第 III 期幼虫の自然感染は，僅かに De León (1957)，Collins

(1979) および Garms and Ochoa (1979) によって発表されているだけである。このブユ種における第III期幼虫保有率は0.14-4.0%であった。

他種においては，Gibson and Dalmat (1952) が，*S. gonzalezi* と *S. haematopotum* の自然感染について報告した。しかし，これは，発育期を区別していないので第 III 期幼虫が含まれていたかどうか不明である。また，*S. downsi* について，Collins (1979) は，第 I・II 期幼虫の感染を報告した。1980年，Okazawa and Onishi によって記載された *S. horacioi* には，745個体解剖されたかぎりでは，*O. v.* 幼虫は見つかっていない (Takaoka, 1982)。

自然感染調査においては，流行地内外にみられる馬や牛のオンコセルカなどの動物由来のフィラリア幼虫による感染との鑑別が重要である。Garms and Ochoa (1979) は，オンコセルカ症流行地内外で採集されたブユの解剖を行ない，両地域におけるブユのフィラリア感染を比較した。その結果，流行地外では *S. ochraceum* 304個体から全くフィラリア幼虫を見出さなかったが，*S. metallicum* の 0.35% (12/3,438) および *S. callidum* の 0.4% (1/242) に *O. v.* 幼虫と形態的に区別されない I-III 期の幼虫をみとめている。この結果は，動物吸血嗜好性も有するブユ種においては，フィラリア幼虫感染が *O. v.* 幼虫だけによるものかどうか一概に判断できないことを示唆している。

実験感染 実験感染においては，これまで，*O. v.* mf を体内で第 III 期幼虫まで発育させうることが報告されたのは *S. ochraceum*, *S. metallicum*, *S. callidum*, *S. gonzalezi*, *S. haematopotum* *S. veracruzanum* および *S. horacioi* の7種類である。

古くは，Strong *et al.* (1934) により自然感染調査と並行して感染実験が試みられたが，ブユ成虫の飼育の困難さから不成功に終っている。

グァテマラのブユの体内で *O. v.* 幼虫の第 III 期までの発育を実験的に観察したのは，1951年および1952年の Gibson の研究が最初である (Gibson, 1965)。すなわち，*S. ochraceum*, *S. metallicum* および *S. callidum* あわせて約15,000個体に *O. v.* mf 保有者を吸血させ，約20°C の条件下で飼育し，13日目まで生存した少数の雌から第 III 期幼虫を得ている。しかしながら，上記3種のブユの全てに第 III 期までの発育がみられたかどうかについては触れられていない。いずれにしろ，用いた成虫数をみただけでもブユ成虫の飼育がいかに困難であったかが想像される。因に，これは，Blacklock (1926) が，アフリカのシエラ・レオネにおいて，*O. v.* 幼虫が *S. damnosum* で体内で発育することを初めて報告してから，実に26年後のことであった。この年月の違いは，両者の実験条件，特に温度条件の違いによる *O. v.* 幼虫の発育速度の差に帰されよう。すなわち，Blacklock は，第 III 期幼虫を得るために，mf をとりこんだブユ成虫を約1週間飼育すればよかったのに対し，Gibson は約2週間も飼育しなければならなかったことによろう。

Gibson は，これに続いて，Dalmat と協同で *S. gonzalezi*, *S. haematopotum*, および *S. veracruzanum* の3種のブユにおいても第 III 期までの発育がおこることを報告した (Gibson and Dalmat, 1952)。

Simulium horacioi には，前述したように，自然感染は見つかっていないが，本種の *O. v.* 幼虫媒介能は実験的には示されている (Ito *et al.*, 1980)。

De León and Duke (1966) は，アフリカでの媒介ブユ成虫飼育の経験を生かして，*S. ochraceum*, *S. metallicum* および *S. callidum* について感染実験を行い，*O. v.* mf とりこみの7，8日後に全ての種に第 III 期幼虫を観察している。これら3種のうち，前2種についてはその後行われた多くの実験でも確認されている (*S. ochraceum*—Collins *et al.*, 1977; Collins, 1979; Monroy, 1979; Matsuo *et al.*, 1980; Ito *et al.*, 1980; Takaoka *et al.*, 1982; *S. metallicum*—Collins, 1979; Ito *et al.*, 1980)。

Table 2 は，自然感染と実験感染の結果の要約である。これからわかるように，*S. ochraceum*, *S. metallicum* および，*S. callidum* の3種がグァ

Table 2 Result of natural and experimental infection of Guatemalan blackflies with *O. volvulus* larvae

Blackfly species	Natural infection		Experimental infection (Development to the third-stage larvae)
	Developing stages	Third stage	
S. ochraceum	+	+	+
S. metallicum	+	+	+
S. callidum	+	+	+
S. gonzalezi	+	?	+
S. haematopotum	+	?	+
S. veracruzanum	−	−	+
S. horacioi	−	−	+
S. downsi	+	−	?

テマラのオンコセルカ症の媒介種，他の *S. downsi* をのぞく4種が媒介可能種と結論づけられよう。ただ，今後の調査で，これら媒介可能種にも自然感染が見出される可能性はある。

4. ブユ体内における *O. volvulus* 幼虫の移行と消長

フィラリア幼虫が媒介者の体内にとりこまれてから感染型幼虫に成長し，次の宿主へ移行するまでの経路および消長は，フィラリアの種類により，また媒介者の形態的（解剖学的）および生理的特異性によって異なる（Lavoipierre, 1958）。

体内移行経路 *O. v.* 幼虫のブユ体内での移行経路は，Blacklock（1926）がアフリカにおいて行った観察で明らかにされた。つまり，とりこまれた mf は中腸から体腔へ脱出して，胸筋に達し，そこで第 III 期幼虫まで発育したあと，頭部の吻部から人体へ移行する。

グァテマラにおいては，Strong *et al.*（1934）が野外採集ブユの剖検によりこのことを確認した。一方，De León (1957) は，*S. ochraceum*, *S. metallicum*, および *S. callidum* あわせて4,639個体の雌を解剖し23個体に第 III 期幼虫を見出したが，その74%がマルピーギ管に幼虫を保有し（残りは胸部と腹部におのおの13%），頭部に幼虫を全く保有していなかったことから，幼虫はマルピーギ管で発育するのではないか，または，胸部で発育したものがこの器官に特異的に移行してくるのではないか，と推測した。彼は，さらに，感染幼虫は吸血のさいブユの腹壁を貫通して吸血部位の皮膚面へ移行するのではないかと考えた。この仮説は，De León (1957) の報告したマルピーギ管にみられる感染幼虫が別種のフィラリアであったことにより，一応否定されている（De León and Duke, 1966）。

胸筋で発育を終えた幼虫がどの経路で頭部へ移行するか，また，頭部のどの部位から宿主に移行するのかについては，詳細な研究は少ない。Collins (1979) は，実験的に感染させた *S. ochraceum* の頭部の組織標本を作り，上唇—上咽頭中に感染幼虫を見出した。このことから，彼は，蚊の媒介するフィラリアとは違って，*O. v.* の感染幼虫は，上唇と上咽頭を両側部でつなぐ膜質部から直接吸血部位へ入るのではないかと推測している。

mf のとりこみ さて，mf とりこみに関して，De León and Duke (1966) は *S. ochraceum*, *S. metallicum*, *S. callidum* いずれのブユ種も同一保虫者から吸血させても0から数百とさまざまな数の mf をとりこみ，また皮膚 mf 密度から推定される期待値以上の mf をとりこむことを観察した。後者の現象——いわゆる「過剰とりこみ」とよばれる——は，特に *S. ochraceum* において顕著であった（これは，同一ブユ種がアフリカの

O. v. 保有者から吸血した場合の mf とりこみ数の20—25倍も多かった）と述べている。

この「過剰とりこみ」については，1934年に Strong *et al.* がすでに報告しており，ブユの唾液中に mf を誘引する物質が存在するのではないかと推測している。また，体外診断法への応用が可能であると述べている。最近では，Collins *et al.* (1977)，Tanaka *et al.* (1980) および Hashiguchi *et al.* (1981) も同様の現象を報告した。

幼虫の移行および発育過程における消長　こうしてとりこまれた mf のすべてが感染型まで発育するわけではない。De León and Duke (1966) は，ブユ体内にとりこまれた mf のうち，*S. ochraceum* で 0.65—2.1%，*S. metallicum* で 1.2-2.5%，また *S. callidum* で4.3%が感染型まで成長することを示した。また，とりこまれた mf 数のこのような減少は中腸でおこることを観察した。Collins *et al.* (1977) は，*S. ochraceum* について 0.2—2.4%の低い発育率を報告した。

Bain *et al.* (1974) は，とりこまれた mf の低い発育率に関して，*S. ochraceum* では，とりこまれた mf のうち僅か 0.87—1.6% しか中腸から体腔へ脱出できず，残りの mf は中腸内に閉じこめられ消化分解されることを観察した。

一方，Omar and Garms (1975) は，組織学的手法を用いて，*O. v.* 幼虫のブユ体内での消長は *S. ochraceum* と *S. metallicum* とでは異なることを明らかにした。つまり，*S. ochraceum* では，多数の mf がとりこまれるが，咽頭部に歯状突起が存在するため，大部分の mf が通過のさい傷つけられて，その結果中腸内で消化分解されてしまい，僅かに2.6%しか胸筋まで到達しない。これと対照的に，*S. metallicum* では，*S. ochraceum* より少数の mf をとりこむ傾向がみられるが，歯状突起という障壁がないので，mf は無傷で中腸へ通過でき，高率（74.5%）に胸筋へ移行できると報告した。また，同著者らは，吸血直後から両種の中腸内に形成されはじめる peritrophic membrane について，mf はこの膜がまだ柔らかい吸血後数分から6時間の間に中腸から体腔へ脱出するので，この膜はとりこまれた mf の減少に大きな役目は果たしていないだろうと述べている。

次に胸筋へ達した mf の感染型への成長について，De León and Duke (1966) は，幼虫発育は *S. ochraceum* 体内ではほぼ同時に円滑に進行し，その成長の過程でほとんど死滅することなく第 III 期幼虫まで発育することを報告した。Collins *et al.* (1977) も同様の結果を報告した。一方，Hashiguchi *et al.* (1981) は，mf とりこみ30および48時間後に胸筋に達した幼虫を調べ，約半数は mf のまま死亡していることを報告した。

これに対して，*S. metallicum* 体内では，幼虫発育に要する時間は *S. ochraceum* 体内におけるのとほぼ同様かまたは1日位の遅れがみられているが，胸筋内で発育遅延や変形した幼虫もしばしば観察されている (De León and Duke, 1966; Collins, 1979; Ito *et al.*, 1980)。

Collins (1979) は，同一保虫者の上半身から *S. ochraceum*, および下半身から *S. metallicum* を吸血させ，その後の幼虫発育を両種ブユで比較した。その結果，吸血後24時間後および8—10日後の両種ブユ1雌当りの幼虫数は，*S. ochraceum* でそれぞれ1.5，2.3隻と差がなかったが，*S. metallicum* では18.9，3.8隻と約1/5に減少することをみている。また，8—10日後にみられた幼虫のうち *S. ochraceum* では90%が第 III 期幼虫であったのに対し，*S. metallicum* では26.3%だけが第 III 期幼虫であった。*Simulium metallicum* 胸筋中の残りの幼虫の半数以上はまだ mf や第 I 期のままで，さらに後者の42%は変形ないし変質していたと述べている。つまり，*S. metallicum* では，胸筋中への mf 移行は *S. ochraceum* にくらべて約12倍高いが，1雌当りの第 III 期幼虫数では，逆に，約半分に減少していることになる。Ito *et al.* (1980) も，同様の実験において，吸血後8—10日に見出された幼虫のうち第 III 期幼虫の割合は *S. metallicum* で60%，*S. ochraceum* で93.5%と両種ブユで異った結果を得ている。このことは，両種ブユの *O. v.* 幼虫に対する生理的親和性の違いを示しているといえよう。

Simulium callidum 胸筋での幼虫発育は順調で

あったと報告されている（De León and Duke, 1966)。

mf とりこみのブユの生存率へ与える影響

このようにブユにとりこまれた *O. v.* 幼虫は，幼虫の発育完了までに生存したブユの体内では，ブユの種類によってその成長のいろいろの段階に応じて，その数に減少がみられ，ほんの少数の幼虫だけしか感染型まで生残れない。

しかしながら，一方では多くの幼虫が媒介ブユの死亡とともに死滅している。De León and Duke (1966) は，*O. v.* mf のとりこみの媒介ブユの生存におよぼす影響について報告した。これによると，*S. ochraceum* では，とりこまれた mf の平均が1雌当り9隻の場合生存率に影響はみられなかったが，とりこまれた mf 平均が170および390隻の場合には，24時間以内におのおの 14.2%, 39.3%のブユが死亡した。一方，*S. metallicum* の24時間以内の死亡率は，5ないし6隻の mf とりこみの場合約12%であったが，190隻と多数の mf をとりこんだときは100%であった。*Simulium callidum* についても，*S. metallicum* と同じく，平均160隻の mf をとりこんだとき100%の早期死亡が観察されている。また，生存したブユの2日目以降の死亡率は非感染ブユと差がなかったと述べている。その後，Omar and Garms (1975), Collins (1979) および Ito *et al.* (1980) も mf とりこみとブユの生存率の関係について同様の結果を報告している。

Ito *et al.* (1980) は，同一保虫者から吸血させた場合 *S. ochraceum* で43.4% (85/196), *S. metallicum* で 12.4% (41/330) の感染幼虫保有率を得，この種間の差は，上述したように，後者のブユが mf 多数とりこみによって早期に死亡したためであると述べている。また，同著者らは，*S. metallicum* が mf とりこみ後24時間を超えて生存できる最高 mf 数を1雌当り168隻と算出している。

後になって，Omar and Garms (1977) は，*S. metallicum* や *S. callidum* に特に顕著にみられる mf 多数とりこみによる死亡の原因を組織学的に追究した。その結果，1)中腸拡大部へとりこまれた mf の前方の管状部への逆戻りとその部位の腸表皮に与える著しい傷害，2)ときに腸内容物の体腔への遺漏を伴う程の mf の腸上皮剝離，3) Peritrophic membrane の形成阻害（特に中腸前・後部）とそのための消化障害，4) mf および血液の後腸への移動による機械的圧迫および障害，5) 種々の器官（腹部神経幹，脳，視神経，眼，平均棍，脂肪体，飛翔筋肉など）への mf の侵入による機能障害，のうち一つまたはいくつかが重なってブユが死亡するのであろうと結論づけた。

一方，*S. ochraceum* の mf 多数とりこみ時にみられる早期死亡の原因については詳細な研究はない。最近 Hashiguchi *et al.* (1981)は，このブユ種においても腸壁を貫いた mf (Cibarial armature によって部分的に傷害をうけた mf も含む）が胸筋以外の組織（頭，脚など）へも侵入することを観察し，早期死亡の原因を考察している。

5. オンコセルカ症の伝搬に及ぼす媒介ブユ集団の生物学的および生態学的要因

フィラリア伝搬に果す媒介者の役割は，媒介者の分布，人吸血嗜好性の強さ，流行地での吸血飛来雌個体群の大きさ，吸血活動の日周および季節変動，吸血習性，Gonotrophic cycle, 経産率，寿命，および飛翔距離などの生物学的および生態学的要因によって大きく異なる。グァテマラのオンコセルカ症伝搬に及ぼすこれらブユ側の要因の大部分については，早くも，Elishewitz (1953), Dalmat (1955) によって検討されている。

地理的分布　グァテマラのオンコセルカ症の主な分布は，この国を北西から南東に走るシエラ・マドレ山脈の太平洋側斜面の山麓部から中腹部の標高約 500—1,500 m に限局されている。何故このような特徴的分布をしているのかについては十分検討されていないが，一つには媒介者の分布と密接に関係していると思われる。

Figure 1 にグァテマラにおける人吸血性ブユ11種の高度分布を Dalmat (1955) の報告をもと

に示した。

Simulium ochraceum, *S. metallicum*, および *S. callidum* は流行地内外に広く分布がみられるが, 主な発生源は流行地の高度分布とほぼ一致する。この3種が早くからオンコセルカ症の伝搬との関わりを指摘されてきた由えんでもある (Elishewitz, 1953; Dalmat, 1955)。

一方, *S. veracruzanum* は流行地の分布の上限近くで, また *S. gonzalezi* と *S. haematopotum* の2種は流行地の下限近くに分布する。発生数が多いところでは, 局地的には伝搬に重要な役割を担っているのではないかと推察されている (Dalmat, 1952, 1955)。

また, そのほかの人吸血性ブユ種では, Figure 1 で明らかなように, 流行地の上, 下限外に分布していることから, オンコセルカ症の伝搬との関係は問題にされない。*Simulium tricornis* は, 流行地内でも分布が報告されているが, 2,000—3,000 m の高地が主な分布地であり伝搬との関係は薄いと思われる。

Simulium horacioi については, まだ広範な調査は行われていないので, 詳細な分布状況は推測の域を出ない。しかしながら, *S. ochraceum* と同様の水系を発生源とすることから (Takaoka, 1982), 高度分布についても類似の傾向を示すことが予想される。

吸血嗜好性 *O. v.* mf が媒介ブユにとりこまれ, また感染幼虫が次の宿主に移行するのは, ブユの吸血時に限られる。したがって, ブユが人だけ吸血するのか, また他の動物をも吸血するのかという性質は, その媒介ブユの伝搬効率に大きく影響する要因である。

グァテマラのブユの吸血嗜好性については, すでに Giaquinto Mira (1937), Elishewitz (1953), および Dalmat (1955) の報告がある。これらの結果を総合すると, *S. ochraceum*, *S. metallicum*, *S. callidum* の3種とも人以外の多くの動物からも吸血する。また, *S. ochraceum* はいずれの動物と比較したときも人に対する嗜好性が圧倒的に強く, 逆に *S. metallicum* と *S. callidum* は牛, 馬などの大動物に強い嗜好性を示す。

別の人吸血ブユ種 *S. horacioi* については, これまでに, 牛から僅か1個体採集された記録しか無い (Okazawa and Onishi, 1980)。ほかのどのような動物から吸血しているのかは不明である。ただ, Garms (1975) が *S. metallicum* 体内にみたのと同様の不明フィラリア幼虫が本種の野外捕集成虫から見出されている (Ito *et al.*, 1980; Takaoka, 1982) ので, 本種が, 人以外の野生の動物を吸血していることは間違いない。一方, 著者の Rio Verde 地区における周年調査では, 同地区で採集された幼虫の種構成比は *S. metallicum* が圧倒的に多く, *S. horacioi* はその1/3, また *S. ochraceum* は1/20であった (Takaoka, 1982)。しかし, 人囮法で捕集された成虫の種構成比では, 逆に *S. ochraceum* が最も多く, *S. horacioi* はその3/4, *S. metallicum* はさらに *S. horacioi* の1/2という結果であった。このことから, *S. horacioi* の人嗜好性は *S. ochraceum* に劣るが, *S. metallicum* よりは強いだろうと推定される (Takaoka, 1982)。

吸血雌個体群の種構成と相対比率 流行地における人吸血雌ブユ個体群の種構成と相対比率に関して, Dalmat (1955) は, 合計3,249回の採集によって得られた総計69,337個体の雌成虫のうち, *S. metallicum* 65.3%, *S. ochraceum* 30.0%, *S. callidum* 4.0%, *S. downsi* 0.1%, *S. gonzalezi* 0.14%, *S. haematopotum* 0.4%および *S. veracruzanum* 0.059% の成績を報告した。この結果は, 一般的には, *S. ochraceum* と *S. metallicum* が最も重要な種であることを示している。

しかしながら, 種構成と相対比率は調査した場所, 時期によって異なることは言うまでもない。たとえば *S. ochraceum* や *S. metallicum* が分布していないか, または分布していても発生量が少なく, 逆に *S. gonzalezi*, *S. haematopotum*, または *S. veracruzanum* のいずれかが優先種である流行地も観察されている (Dalmat, 1955)。したがって, 最終的には, 個々の流行地別にどの吸血性ブユが伝搬に重要な役割を果しているかを調査

することが大切であろう。

吸血活動の日周変動　オンコセルカ症の場合, 人の mf 密度に日内の Periodicity はみとめられていない (Tada and Figueroa, 1974)。したがって, ブユの吸血活動の日内変動は,「夜間強周期型バンクロフト糸状虫 mf の血中出現と媒介蚊の吸血活動時刻との一致」にみられるような関係ほどに厳しい要因とはなっていない。ブユは昼間吸血性であるが, 理論的には1日のうちいつでも mf をとりこむことができる。ただその割合はブユの吸血活動の盛んな時刻と人が野外で吸血にさらされる時間帯の重なり度によって左右されることになる。したがって媒介ブユの種類によって吸血活動の日内変動パターンが異なれば, 各媒介ブユ種の感染率も変ってくることが予想される。

また, 吸血活動の日内変動は, 同時に, 人がブユ体内で発育した感染幼虫をいつ伝搬されるのかという問題とも関連してくる。

ブユ吸血活動の日内変動について, Dalmat (1955) は, *S. ochraceum* および *S. metallicum* の吸血活動は午前中 (8—10時) に最も高く, 午後は低い, しかしながら, *S. callidum* は早朝と夕方に2回活動の山を示し日中は低いことを報告した。*Simulium ochraceum* について, Tada *et al.* (1975) は同様の観察結果を得た。一方, Collins *et al.* (1981) は, 吸血活動は *S. ochraceum* では午前中 (7—9時) に高いが, *S. metallicum* では午後 (2—5時) に高い, と異なる結果を報告した。この成績の差異は調査地点の差異に関連していると思われる。

また, Collins *et al.* (1981) は, 吸血のため飛来した *S. ochraceum* 個体群の極期は午前中だけの一峰性であったが, *O. v.* 幼虫を保有していた個体群だけについてみてみると, 午前 (8—9時) と午後 (0—1時) にピークを持つ明瞭な二峰性パターンがみられたと述べている。このことは, ブユが一日のうち最も感染をうける時刻と人がブユから感染幼虫を伝搬される時刻とが異なる, という点で注目される。

吸血活動の季節変動　グァテマラの気候は5—10月の雨期と11—4月の乾期にはっきり分けられる。このような特徴的気候条件下で伝搬が一年中おこるのか, またある時期だけにおこるのか, という問題に関連して, 媒介ブユの季節消長は大変注目される。Dalmat (1955) は, *S. ochraceum*, *S. metallicum* および *S. callidum* の成虫個体群の季節消長について報告した。これによると, *S. ochraceum* は年間二峰性で第一の大きな山は乾期の1•2月にみられ, 第二の山は雨期でも比較的雨の少ない8月にみられることが示されている。Dalmat (1955) は, このブユ種の第一の山がコーヒーの収獲期に当り, 多くの労働者が吸血されることの疫学的重要さを指摘している。一方, *S. metallicum* の個体群密度は1—4月に比較的高く, 5月に一旦減少し, 6月に再び高くなり, その後12月にかけて漸減の傾向がみられた。すなわち, このブユ種では, *S. ochraceum* にみられるような顕著な周期はみられていない。*Simulium callidum* については, *S. ochraceum* とほぼ同様の季節消長パターンが示されている。

しかしながら, 季節変動パターンは同一ブユ種でも場所によって異なる (Takaoka, 1981)。したがって, Dalmat (1955) の指摘したように, 個体群密度の極期とコーヒー収獲期とが常に一致するわけでなく, 疫学的意味は個々の流行地で違っているであろう。

また, ブユの個体群密度の高い時期が伝搬の適期であるかどうかについては一概に言えない。たとえば, Collins *et al.* (1981) は, *S. ochraceum* の自然感染率 (*O. v.* の I, II, III 期幼虫の総計) は吸血個体群密度の低い3—5月および9月に高く, 個体群密度が極期となる10—11月には非常に低く (感染幼虫は見出されていない) なることを観察した。すなわち, 伝搬は必ずしも吸血雌個体数の多い時期に限らないと言えよう。

吸血部位　グァテマラの *O. v.* mf の人体における分布は一様でない——mf 密度は上半身に高く, 下半身に低い——ということが報告されている (De León and Duke, 1966; Zea *et al.*, 1980)。した

がって，媒介ブユが人体のどの部位に吸血選択性を示すかは，mf のとりこみに関連して重要な要因である。このことに関して，Elishewitz (1953), Dalmat (1955)，および De León and Duke (1966)は，*S. ochraceum* は人体の上半身，また *S. metallicum* と *S. callidum* は下半身を主に吸血することを観察した。一方，Tada *et al.* (1975) は，*S. ochraceum* は裸出している部分のいずれからも吸血し，特に上半身に吸血部位が片寄っているということはなかった，と報告した。*Simulium horacioi* は，*S. metallicum* 同様，下半身を中心に吸血することが観察された (Takaoka, 1982)。

従来，*S. metallicum* および *S. callidum* は，mf 密度の低い下半身皮膚から主に吸血することを理由に，効率のよい媒介者ではないとされていた (Elishewitz, 1953; Dalmat, 1955)。しかし，すでに述べたように，*S. metallicum* の場合少数の mf とりこみでも高い割合で mf が胸筋へ移行する。さらに mf 多数とりこみはブユの早期死亡につながることも明らかにされているので，このブユ種の下半身吸血嗜好性が媒介者として不利な要因とは一概に言えないだろう。これに対し，*S. ochraceum* は mf 密度の高い部位に吸血嗜好性を示す。また，mf 密度の低い部位でも「過剰とりこみ」現象を起こす。しかるに，これらのことは，そのあと，とりこまれた mf の大多数がブユの咽頭部の歯状突起によって決定的な傷害をうけることと考えあわせると，大変興味深く思われる。

吸血習性および時間　ブユの吸血のさいの行動，特に吸血時間は，mf のとりこみだけでなく，感染幼虫のブユから人体への移行へも影響すると思われる。

Simulium ochraceum は，一旦吸血を始めると終りまで安定した行動を示すが，これに対し *S. metallicum* と *S. callidum* は落着きがなく不安定で，部分吸血で飛去ることも多い，と報告されている (Elishewitz, 1953; Dalmat, 1955, および De León and Duke, 1966)。

平均吸血時間は，Dalmat (1955) によると，*S. ochraceum* で4.8分 (1—19分)，*S. metallicum* で4.3分 (1—31分)，*S. callidum* で4.5分(1—15分) であった。Tanaka *et al.* (1980) は，*S. ochraceum* を *O. v.* mf 保有者から吸血させた場合，約70%のブユは3—4分で飽血し，mf とりこみ数もこのときに最大値に達すると述べている。

経産率　吸血のため人に飛来してくるブユのうち，フィラリア幼虫を保有しているのは，原則として少なくとも1回の吸血と産卵の経験をもつ経産雌に限られ，このようなブユの割合のもつ疫学的意味は大きい。

Garms (1975) は，*S. ochraceum*, *S. metallicum* そのほかの2，3の人吸血性ブユについて卵巣の状態——特に follicular dilatation の有無，性状——から経産・未経産の区別を可能にした。彼の，Chimaltenango の4地区，および Quezaltenango の1地区での観察によれば，*S. ochraceum* の31—59%，*S. metallicum* の18—38%，*S. callidum* の26—61%および *S. gonzalezi* の28—67%が経産雌であった。Garms and Ochoa (1979) は，Chimaltenango およびその他の県のあわせて6地区で，これら4種のブユについて，それぞれ，46—79%，23—73%，25—60%および40—75%の経産率を報告した。また Collins *et al.* (1981) によると，Los Tarrales と El Vesuvio では，おのおの *S. ochraceum* で33.8%，37.0%，*S. metallicum* で37.0%，14.3%，および *S. callidum* で39.8%，14.6%の雌が経産であった。これらの結果は，同一ブユ種でもその経産率が，調査の場所，時期によって大きな変動がみられることを示している。

一方，Garms and Ochoa (1979) は，*S. ochraceum* の経産率は午後の早い時刻に少し高くなるものの明瞭な日内変動パターンはみられない，しかし，*S. metallicum* では顕著なパターン——経産率は午前中に高く，午後の中頃に低く，再び午後の遅い時刻に高い——がみられることを報告した。しかし，Collins *et al.* (1981) は，周年調査の結果から *S. ochraceum* の経産率は午前中 (6—11時) は27%以下と低く，11時以後急に上昇し

13—14時には最高64%に達する明白な日内変動パターンを示すが，*S. metallicum*, *S. callidum* および *S. downsi* ではそのようなパターンはみられなかった，と異なる結果を述べている。いずれにしろ，このように経産率に日内変動が認められるブユ種の場合，採集された時間帯によって自然感染率は大きく異ってくることが予想されるので留意する必要があろう。

吸血間隔（Gonotrophic cycle） 吸血から次の吸血までに要する期間（Gonotrophic cycle）は，ブユ体内で発育を完了した感染幼虫が何回目の吸血時に次の人に感染可能であるかを知る上で，大変重要である。Gonotrophic cycle は，吸血後のブユの卵巣の発育期間により，ある程度推定される。Cupp and Collins (1977) は，室内において *S. ochraceum* の卵巣内 follicles の，羽化ならびに吸血後の発育，そして産卵後の変化の過程を経時的に観察し，吸血後成熟卵が完成するまでに 25°C で 48時間を要したと述べている。Watanabe *et al.* (1980) は，22°C の温度条件下で卵巣発育に 4 日要することを観察した。Monroy (1979) および Takaoka *et al.* (1982) は卵巣発育に要する期間は温度条件によって異なり，22°C 以上では2日，温度が 22°C より低くなれば 3—9 日と長くなることを報告した。一方，Garms and Ochoa (1979) は，*S. ochraceum*, *S. metallicum* とも 20—27°C の条件下で吸血後 2，3，4 日目に産卵することを観察した。

また Garms (1975) は，産卵から次の吸血までの期間に関して，午前中に採集される経産雌個体群では dilatation は小さく，午後の個体群では袋状で大きい，という dilatation の大きさに顕著な日周パターンがあることを観察し，おのおの前日および当日産卵を経た個体群であろうと推察した。したがって，Gonotrophic cycle の期間は上述の産卵までの期間とほぼ等しい 2—4 日と推定している（Garms and Ochoa, 1979)。

同時に，Garms (1975) は，大きい dilatation を有する経産雌の頻度が午後に高いことから，*S. ochraceum* の産卵は，Dalmat (1955) の報告したように，午後の早い時刻（0—2 時）におこっているだろうと述べている。

一方，Gonotrophic cycle 期間中のブユの栄養摂取についてはよくわかっていない。Cupp and Collins (1977) は，Anthron テストにより，*S. ochraceum* の未経産および経産雌のおのおの70%, 65%に果糖の摂取を観察した。この結果から，1 回の Gonotrophic cycle 後も，次の吸血源を探すためのエネルギー源として，花蜜（ネクター）が必要であろうと推測している。

吸血から卵成熟までの期間中の成虫の休止場所については あまり調査されていない。Dalmat (1955) は，ブユ成虫が夜間には地表に休止していること，また日中には樹上の葉や枝に休止している——*Simulium ochraceum* 37個体が地上 4—34 m の高さから，*S. metallicum* 11個体が 6—16 m から採集されている——ことを観察した。しかしながら，残念なことにこの休止中の成虫がどのような生理的状態——すなわち未吸血，吸血，卵包蔵など——であったのかについては触れられていない。

生存率 ブユ成虫の日生存率は，*O. v.* mf をとりこんだブユが幼虫の発育完了までどの位の割合で生存でき，さらに感染幼虫保有ブユがどの位生き延びれるかということに関連して，媒介ブユの伝搬能力を左右する重要な要因の一つである。

しかしながら，蛹から羽化させた雌成虫を用いて保虫者から吸血させることが難しいことと，成虫の飼育の困難さから，室内実験の結果をもとに，感染ブユの生存率を直接求めることは現在では不可能である。ブユ成虫飼育法は最近急速に改良された（Figueroa *et al.*, 1977; Matsuo *et al.*, 1978)。しかし，羽化させた成虫を吸血に導くことは，依然として，今後の問題として残されている。ただ，mf をとりこんだ感染ブユがその生存率にどのような影響をうけるかについては，mf 多数とりこみの場合24時間以内の早期死亡がみられること，そしてその影響の程度はブユ種によって大きく異なること，しかし，2 日目以降の生存率は感染ブユと非感染ブユとでは差がないことはすでに述べ

たとおりである。

一方，野外調査の結果から生存率を推定する方法も2，3試みられてきた。Dalmat (1950, 1952) および Dalmat and Gibson (1952) は，マーキング法を用いて，野外でのブユ成虫の寿命に関する実験を行った。これによると *S. ochraceum* で62日，*S. metallicum* で85日および *S. callidum* で63日目に最後のマーク雌個体が回収されている。いずれの種も野外での生存率が予想以上に高いことが推測されるが，残念ながら平均寿命については触れられていない。また *O. v.* mf 保有者を吸血させた雌成虫の場合は，3日以降回収されたマーク個体の中に *O. v.* 幼虫は見出されていない。このことから感染によりブユの生存率が低下したのだろうと推測している (Dalmat and Gibson, 1952)。

Watanabe *et al.* (1980) は，20°C の温度下での *S. ochraceum* の Gonotrophic cycle の期間 (5日)，また，野外で実際求めた経産率 (48.7%) を用いて1日の平均生存率を0.866と算出した。このように Gonotrophic cycle の日数と経産率の値から日生存率を求める方法もある。しかし，これには日生存率が一定と仮定してよいのか，などの前提条件を検討する必要があろう。また，すでに述べてきたように，ブユの卵巣の発育に要する日数は温度条件によって長短があり，さらに経産率も場所，季節，それに時刻によっても変動が大きいことから，これらの値から日生存率を出す場合慎重さが要求されよう。

このほかに，野外条件下でのブユ体内での *O. v.* 幼虫発育観察のデータと，同一地域における野外ブユを解剖して得られる *O. v.* 幼虫の各ステージ別の保有率とから日生存率を出す方法などもあるがまだ試みられていない。

飛翔距離　ブユ成虫の飛翔能力は，流行地の大きさ，拡大，伝搬の場所などに関連する重要な因子である。Dalmat (1950, 1952) および Dalmat and Gibson (1952) は，前述したマーキング法を用いて，最高飛翔距離として *S. ochraceum* で 10.1 km, *S. metallicum* で 11.7 km および *S. callidum* で 11.9 km を記録している。また *O. v.* mf 保有者から吸血させた場合，回収したマーク個体のうち *O. v.* 幼虫を保有していたのは3個体で，おのおの 4.64 km (*S. ochraceum*, 3日目)，4.32 km (*S. ochraceum* と *S. callidum*, 4日目) 地点で再採集されている。このことから感染ブユの飛翔能力はかなり制限されているだろうと推測している。

Annual Transmission Potential　一年間に一人の人が感染幼虫を持っているブユに何回吸血されるか (Annual Infective Biting Density)，または何隻の感染幼虫に感染されるか (Annual Transmission Potential) は流行地の伝搬の動態，特に感染の強さを知るうえで大きな指標になる (Duke, 1968)。すでに，アフリカのある地域では流行地別の AIBD および ATP が計算され住民の mf 保有率，*O. v.* に起因する皮膚症状，腫瘤および失明率などと対比したオンコセルカ症の流行の様相が調査されている (Duke *et al.*, 1972; Thylefors *et al.*, 1978 など)。

一方，グァテマラにおいては最近まで同様の調査は試みられていなかった。Collins (1979a) は Finca Los Tarrales において，一年間の調査を行い，*S. ochraceum* の 0.31% に感染幼虫を見出した。この値と年間の *S. ochraceum* の推定個体群密度からこの Finca の AIBD を試算すると540となる。また，ブユ1個体当りの感染幼虫が2.2隻であったので ATP は1,188と推定される。ただし，この二つの値は，Duke (1968) が計算の基礎とした各月別の自然感染率とブユの個体群密度を用いて計算していないので，アフリカで報告されている値と直接比較はできない。

すでに報告されているように，グァテマラの媒介ブユの自然感染率 (0.02—3.2%) は，アフリカの媒介ブユの場合と比較してきわめて低い。したがって，実際の AIBD および ATP を求めようとすれば，毎月相当数のブユを採集し剖検することが必要となってこよう。

6. *O. volvulus*—媒介ブユ complex におよぼす要因

オンコセルカ症伝搬は，流行地の人側の要因や種々の自然環境要因によっても大きく影響されることは言うまでもない。しかしながら，これらの要因についてはあまり研究されていない。

保虫者の mf 密度　人側の要因の一つとして皮膚 mf 密度は，どのレベルの mf 密度の保虫者が感染源になりうるか，という点で疫学的にも重要である。Collins *et al.* (1977) は先にも述べたように，mf 密度の異なる保虫者10人を選び，*S. ochraceum* による mf とりこみ数，胸筋への移行数，発育した感染型幼虫数について相互の関係を調べた。その結果，mf 密度ととりこまれた mf 数との間にははっきりした関係はみられなかった。一方，前述したように，とりこまれた mf 数と胸部の幼虫数および感染型幼虫数，さらにブユの感染率との間には明らかな相関がみられている。ここで，mf 密度が 0 および 0.5 mf/mg と低い保虫者からでもおのおの1雌当り平均1.7，44.7隻の mf がとりこまれ，さらにそれぞれが平均0.04，0.17隻の感染幼虫に発育していること，また，それぞれの保虫者がおのおの5.3および9.2％のブユに感染を成立させている（感染型幼虫）ことは注目されよう。

この問題は，mf の密度分布は人体各部位で均一でなく変動も大きいこと (Zea *et al.*, 1980)，野外における人体の露出部位，またブユ種による吸血部位選択性などを考慮して，さらに仔細な検討が望まれる。

温度　一方，自然環境要因については，*O. v.*—ブユ complex に対する温度の影響が研究されているだけである (Monroy, 1979; Takaoka *et al.*, 1982)。

Monroy (1979) は種々の恒温条件下で *S. ochraceum* 体内で *O. v.* 幼虫の発育を観察した。その結果，感染型までの発育は 18—30°C の範囲でみられること，発育速度は温度の上昇に比例して速くなることを報告した。また，感染型幼虫のあらわれるまでのブユの生存率にもとづいて，22—24°C の温度範囲が伝搬に好適だろうと述べている。

一方，Takaoka *et al.* (1982) は同様の実験を行い，*S. ochraceum* 体内での *O. v.* 幼虫の発育

Table 3　Comparison of infective fly day period in nine groups of *S. ochraceum* which were fed on a carrier of *O. volvulus* microfilariae and were kept at various constant temperatures or at fluctuating temperatures by day and night (arranged from Takaoka *et al.*, 1982)

Temperature (C)	Duration of larval development (in day) (*n*)	Survival probability on day *n* (1)	% Females with infective larva(e) among survivors through *n* days (2)	Life expectancy with infective larva(e) (in day) (3)	Infective fly day period [10×(1)×(2)×(3)]
20	16 (13)*	0.229 (0.295)	33.3 (26.3)	4.1 (7.1)	31.3 (55.1)
22	8	0.508	43.3	4.5	99.0
25	6	0.501	30.0	4.4	66.1
28	4	0.051	33.3	0.5	0.9
10–25	13	0.249	11.8	3.5	10.3
12–25	17 (13)	0.265 (0.315)	43.8 (38.8)	5.2 (9.2)	60.4 (112.4)
14–25	13	0.505	45.7	5.4	174.6
16–25	13	0.656	47.7	5.8	181.5
18–25	10	0.438	38.1	3.4	56.7

* Figure in parentheses shows the value calculated if the duration (*n*) were 13.

を 20—28°C の範囲で観察している。そして，その発育速度と温度との関係を $y=-0.3760+0.0222x$ (y: 発育速度; x: 温度) の式で表わした。また，温度の伝搬効率におよぼす影響を，mf が感染型幼虫に発育するまでに生存していたブユの割合，生存ブユのうち感染幼虫を保有していた割合，および後者の平均余命の3つの指標の積として測った。その結果，Monroy (1979) の結果とほぼ同じく，22—25°C の温度範囲で伝搬効率が高いことが示された (Table 3)。

Takaoka *et al.* (1982) は，同時に昼と夜間で異なる温度条件の影響も調べた。その結果，昼間の温度を 25°C に保ち，夜間の温度を 10, 12, 14, 16°C と発育限界温度 (17°C) 以下にした場合でも，*O. v.* 幼虫の生長が認められ，約2週間後に感染型幼虫がブユの頭部に見出されている。また，昼夜の温度条件が 25/14°C, 25/16°C, および 25/18°C のとき，ブユの伝搬効率は一定温度条件 (25°C) より高い値が得られている。すなわち，夜間の温度の降下により *O. v.* 幼虫の発育は遅れるが，第 III 期幼虫が発育するまでの *S. ochraceum* の生存率が高くなり，またそれ以降の平均余命も延びるので，全体として伝搬効率の点ではかえって有利になると述べている (Table 3)。Figure 3, 4 には，各温度条件下の伝搬効率と感染幼虫を伝搬可能になるまでの Gonotrophic cycle の回数を模式的に示した。Takaoka *et al.* (1982) は，この結果から，グァテマラのオンコセルカ症流行地が何故昼夜の温度差が著しい (夜間は 17°C 以下) 標高 500—1,500 m の地域に分布しているかを考察している。また，流行地がこれより低い地域へ拡大しないのは媒介ブユ *S. ochraceum* が高温に耐えられないためであり，逆に，流行が標高の高い地域にみられないのは低温のため *O. v.* 幼虫の発育阻止または発育遅延がおこるためであろうと推測している。

一方，流行地内でも標高——したがって温度条件——に差があり，伝搬が一様におこっているとは考えられない。Takaoka *et al.* (1981) は「*S.*

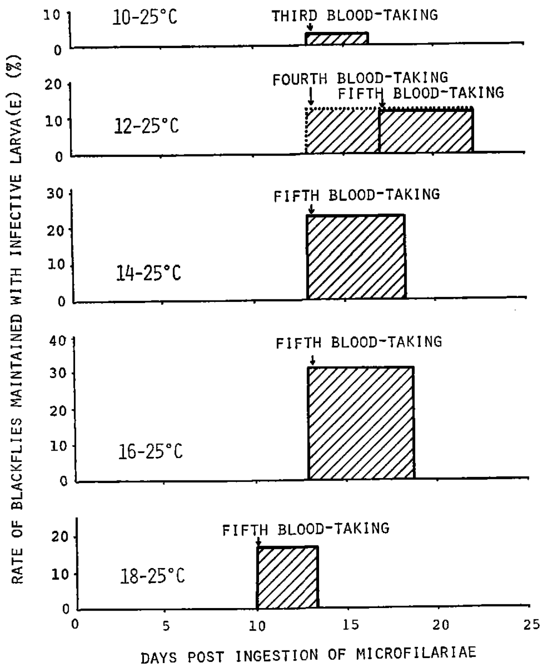

Figure 3 Infective fly day periods in the groups of *S. ochraceum* which were fed on a carrier of *O. volvulus* microfilariae and were kept at different temperatures of night time (14 hr) but under a constant temperature of 25°C during the day time (10 hr), and their relation to the number of gonotrophic cycles.

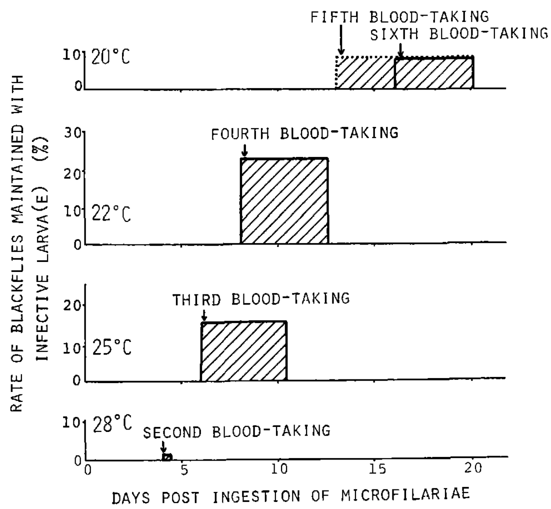

Figure 2 Infective fly day periods in the groups of *S. ochraceum* which were fed on a carrier of *O. volvulus* microfilariae and were kept at various constant temperatures, and their relation to the number of gonotrophic cycles.

ochraceum 体内での *O. v.* 幼虫発育は有効積算温量に左右される」との前の実験結果にもとづいて，350―1,500 m 間の標高の異なる5つの場所で，自記温度計により温度変化を測定しながら，*O. v.* 幼虫の発育を調べた。その結果，有効温量にもとづいた推定発育日数は標高が高くなる程長くなる（4.4―28日）という傾向を報告している。しかしながら，標高 650 m 地点で有効温量から予想される日とほぼ等しい8日目に感染型幼虫を見出した以外は，ほかの4地点ではブユの高い死亡率のため十分なデーターを得ていない。この点に関して，今後，再検討することが望まれる。

7. おわりに

グァテマラのオンコセルカ症伝搬に関連して，その研究の歴史を簡単に紹介し，最近の知見をまとめた。このなかで何よりも特筆されたのは *O. v.* 幼虫の媒介ブユ体内での生活史の模様が明らかにされたことであろう。そして，それは，*S. ochraceum* と *S. metallicum* では幼虫の移行経路は同一であるが，幼虫の減少の大きな要因が前者では咽頭部の歯状突起という解剖学的障壁，後者では胸筋内の生理的不和合性，と媒介ブユの種類によって違うことも明らかにされた。今後は他の媒介種および媒介可能種についても類似の研究がおこなわれ，やがてそれぞれのブユ種の *O. v.* 幼虫に対する伝搬様式および伝搬効率が明らかにされるであろう。

今後の課題の一つとして，牛，馬に強い吸血性を示すブユ種――特に *S. metallicum* や *S. callidum*――が，これら動物に寄生する *O. gutturosa* や *O. cervicalis* などの伝搬に関与しているのかどうかという疑問に答えるために，ブユ体内で見出されるフィラリア幼虫の同定を確実にすること，また各動物寄生のフィラリアの実験的感染を行うことが望まれる。

次に，このような研究を進めていく上で，グァテマラにおけるオンコセルカ症の媒介種および媒介可能種といわれているブユ種の実体をしっかりと見極めておく必要があろう。たとえば，現に，グァテマラ国内だけでも *S. metallicum* グループの構成種として *S. metallicum* s. str., *S. horacioi*, *S. jobbinsi*, *S. puigi* (Takaoka and Takahasi, 1982) の4種の存在が認められている。すでに述べたように，前2種の人吸血性および *O. v.* 幼虫媒介能は知られているが，後者の2種については不明である。最近の調査で，*S. horacioi* の吸血飛来雌数は，場所によっては *S. metallicum* s. str. よりも多いことが示された (Takaoka, 1982)。今後の調査では，このグループに関しては，*S. metallicum* s. l. としてでなく各構成種を区別して対応することが必要となろう。このほかの媒介種や媒介可能種については十分検討されてはいないが，ほとんどの種が中米から南米まで広く分布している状況をみただけでも，各地域の個体群が同一種であるかどうか素朴な疑問が湧いてくる。おそらく，今後の研究によって，アフリカの媒介ブユ種 *S. damnosum* s. l. の例でみられるように，細胞遺伝学的，形態学的，ならびに生態学的な差異や *O. v.* 幼虫に対する親和性の差などが明らかにされ，従来同一種とみなされていたものが細分化されてくる可能性が強い。

将来，このような寄生虫と媒介昆虫種との関係，媒介昆虫の生態学的要因のほかに，個々の流行地の複雑な生態系を構成する他の要因――すなわち人側の要因や自然環境要因――を解明することによって，オンコセルカ症伝搬の本質および流行の模様が次第に明らかにされてこよう。

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文 献

1) Bain, O., Durette-Desset, M. C. and De León, R. (1974): Onchocercose au Guatémala: l'ingestion des microfilaires par *Simulium ochraceum* et leur passage dans l'hémocéle de ce vecteur, Ann. Parasit. hum. comp., 49, 467–487

2) Blacklock, D. B. (1926): The development of *Onchocerca volvulus* in *Simulium damnosum*, Ann. Trop. Med. Parasitol., 20, 1–48

3) Collins, R. C. (1979a): Onchocerciasis transmission potentials of four species of Guatemalan Simuliidae, Am. J. Trop. Med. Hyg., 28 (1), 72–75

4) ——— (1979b): Development of *Onchocerca volvulus* in *Simulium ochraceum* and *Simulium metallicum*, Am. J. Trop. Med. Hyg., 28 (3), 491–495

5) Collins, R. C., Campbell, C. C., Wilton, D. P. and Newton, L. (1977): Quantitative aspects of the infection of *Simulium ochraceum* by *Onchocerca volvulus*, Tropenmed. Parasitol., 28, 235–243

6) Collins, R. C., Merino, M. E. and Cupp, E. W. (1981): Seasonal trends and diurnal patterns of man-biting activity of four species of Guatemalan black flies (Simuliidae), Am. J. Trop. Med. Hyg., 30 (3), 728–733

7) Cupp, E. W. and Collins, R. C. (1979): The gonotrophic cycle in *Simulium ochraceum*. Am. J. Trop. Med. Hyg., 28, 422–426

8) Dalmat, H. T. (1950): Studies on the flight range of certain Simuliidae, with the use of aniline dye marker, Ann. Entomol. Soc. America, 43, 537–545

9) ——— (1952): Longevity and further flight range studies on the black flies (Diptera, Simuliidae), with the use of dye marker, Ann. Entomol. Soc. America, 45, 23–37

10) ——— (1955): The black flies (Diptera, Simuliidae) of Guatemala and their role as vectors of onchocerciasis, Smithsonian Institution, Washington D. C.

11) De León, J. R. (1957): Simuliid vectors of onchocerciasis in Guatemala, Bull. Wld Hlth Org., 16, 523–529

12) De León, J. R. and Duke, B. O. L. (1966): Experimental studies on the transmission of Guatemalan and West African strains of *Onchocerca volvulus* by *Simulium ochraceum, S. metallicum* and *S. callidum*, Trans. Roy. Soc. Trop. Med. Hyg., 60, 735–752

13) Duke, B. O. L. (1968): Studies on factors influencing the transmission of onchocerciasis. IV. The biting cycles, infective biting densitity, and transmission potential of "forest" *Simulium damnosum*, Ann. Trop. Med. Parasitol., 62, 95–106

14) Duke, B. O. L., Moor, P. J. and Anderson, J. (1972): Studies on factors influencing the transmission of onchocerciasis. VII. A comparison of the *Onchocerca volvulus* transmission potentials of *Simulium damnosum* populations in four Cameroon rain-forest villages and the pattern of onchocerciasis associated therewith, Ann. Trop. Med. Parasitol., 66, 219–234

15) Elishewitz, H. (1953): A summary of the results of entomological investigations on the onchocerciasis complex carried out in Guatemala between August 1944 and July 1945, Unpublished mimeographed document WHO/Onchocerciasis/16

16) Figueroa M., H., Collins, R. C. and Kozek, W. J. (1977): Postprandial transportation and maintenance of *Simulium ochraceum* infected with *Onchocerca volvulus*, Am. J. Trop. Med. Hyg., 26, 75–79

17) Garms, R. (1975): Observations on filarial infections and parous rates of anthropophilic blackflies in Guatemala, with reference to the transmission of *Onchocerca volvulus*, Tropenmed. Parasitol. 26, 169–182

18) Garms, R. and Ochoa A., J. O. (1979): Further studies on the relative importance of Guatemalan blackfly species as vectors of *Onchocerca volvulus*, Tropenmed. Parasitol., 30, 120–128

19) Giaquinto Mira, M. (1937): Contributo agli studi sul problema della transmissione della onchocercosi nel Guatemala, Ann. Igiene, 47, 109–124

20) Gibson, C. L. (1951): Parasitological studies on onchocerciasis in Guatemala, Doctoral dissertation, University of Michigan

21) ——— (1965): Experimental studies on the parasite and vectors of onchocerciasis in Guatemala, Bol. Sanit. Guatemala, 36, 246–287

22) Gibson, C. L. and Dalmat, H. T. (1952): Three new potential intermediate hosts of human onchocerciasis in Guatemala, Am. J. Trop. Med. Hyg., 1, 848–851

23) Hamon, J. (1974): The onchocerciasis vectors in the Western Hemisphere. Vector biology and vector-parasite relationships, In: Pan American Health Organization, Proceedings of the International Symposium on Research and Control of Onchocerciasis in the Western Hemisphere, Washington D. C. PAHO Scientific Publication No. 298, 58–68

24) Hashiguchi, Y., Kawabata, M., Ito, S. and Recinos C., M. M. (1981): Limited fly load and development of *Onchocerca volvulus* microfilariae in Guatemalan *Simulium ochraceum*, J. Helminth., 55, 189–196

25) Ito, S., Tanaka, I. and Ocha A., J. O. (1980): Comparative studies on the affinities of two black flies, *Simulium metallicum* and *S. ochraceum* for the larvae of *Onchocerca volvulus* in Guatemala, Jap. J. Sanit. Zool., 31 (4), 261–270

26) Lavoipierre, M. M. J. (1958): Studies on the host-parasite relationships of filarial nematodes and their arthropod hosts II. — The arthropod as a host to the nematode: A brief appraisal of our present knowledge, based on a study of the more important literature from 1878 to 1957, Ann. Trop. Med. Parasitol., 52, 326–345

27) Leiper, R. T. (1914): Report of the helminthologist for the half year ending 30 April 1913, In: Report of the Advisory Commitee for the Tropical Diseases Research Fund for the year 1913, London, 86–87

28) Manson, P. (1878): Further observations on *Filaria sanguinis hominis*, In: Medical Reports, China Imperial Maritime Custums, Shanghai, No. 14, 1–26

29) Matsuo, K., Okazawa, T., Onishi, O. and Ochoa A., J. O. (1978): Maintenance of the adults of Guatemalan black fly, *Simulium ochraceum*, in the laboratory, Jap. J. Sanit. Zool., 29 (3), 251–254

30) ———, ———, ——— and ——— (1980): Experimental infection of black fly, *Simulium ochraceum*, with microfilariae of *Onchocerca volvulus* in Guatemala, Jap. J. Parasitol., 29 (1), 13–17

31) Monroy, M. C. (1979): Infección experimental de *Simulium ochraceum* con microfilarias de *Onchocerca volvulus*, Informe de Tesis, Escuela de Biología, Universidad de San Carlos de Guatemala, Guatemala

32) Nelson, G. S. (1970): Onchocerciasis, In: Advances in Parasitology 8, Academic Press, London, 173–224

33) Okazawa, T. and Onishi, O. (1980): Description of a new species of *Simulium* (*Simulium*) Latreille and redescription of *Simulium* (*Simulium*) *metallicum* Bellardi from Guatemala (Diptera: Simuliidae), Jap. J. Sanit. Zool., 31 (3), 167–179

34) Omar, M. S. and Garms, R. (1975): The fate and migration of microfilariae of a Guatemalan strain of *Onchocerca volvulus* in *Simulium ochraceum* and *S. metallicum*, and the role of the bucco-pharyngeal armature in the destruction of microfilariae, Tropenmed. Parasitol., 26, 183–190

35) ——— and ——— (1977): Lethal damage to *Simulium metallicum* following high intakes of *Onchocerca volvulus* microfilariae in Guatemala, Tropenmed. Parasitol., 28, 109–119

36) Robles, R. (1917): Enfermedad nueva en Guatemala, La Juventud Medica, 16

37) ——— (1919): Onchocercose humaine au Guatémala produisant la cécité et l'érysipéle du littoral, Bull. Soc. Path. exot., 12, 442–460

38) Sasa, M. (1976): Human filariasis, University of Tokyo Press, Tokyo

39) Strong, R. P., Sandground, J., Bequaert, J. C. and Ochoa, M. M. (1934): Onchocerciasis, with special reference to the Central American form of the disease, Contr. No. 6, Dept. Trop. Med. and Inst. Trop. Biol. and Med., Harvard Univ.

40) 多田 功 (1976)：オンコセルカ症，金沢医科大学雑誌 1, 227–245

41) Tada, I. and Figueroa M., H. (1974): The density of *Onchocerca volvulus* microfilariae in the skin at different times of the day in Guatemala, Jap. J. Parasitol., 23, 220–225

42) Tada, I., Figueroa M., H. and Takaoka, H. (1975): Epidemiological studies on Robles' disease (American onchocerciasis) in Guatemala, Jap. J. Trop. Med. Hyg., 2 (1), 35–51

43) Takaoka, H. (1981): Seasonal occurrence of *Simulium ochraceum,* the principal vector of *Onchocerca volvulus* in the southeastern endemic area of Guatemala, Am. J. Trop. Med. Hyg., 30, 1121–1132

44) ——— (1982): Observations on the bionomics of larval and man-biting female populations of *Simulium horacioi,* a new potential vector of *Onchocerca volvulus* in Guatemala, Jap. J. Trop. Med. Hyg., 10(1), 49–62

45) Takaoka, H., Hansen, K. M., Takahasi, H., Ochoa A., J. O. and Juarez, E. L. (1981): Development of *Onchocerca volvulus* larvae in *Simulium ochraceum* at various altitudes in Guatemala with special reference to the ambient temperature, Jap. J. Trop. Med. Hyg., 9 (3/4), 187–197

46) Takaoka, H., Ochoa A., J. O., Juarez, E. L. and Hansen, K. M. (1982) Effect of temperature on development of *Onchocerca volvulus* in *Simulium ochraceum,* and longevity of simuliid vector, J. Parasitol., 68(2) (in press)

47) Takaoka, H. and Takahasi, H. (1982): A new species of black fly (Diptera: Simuliidae) from upland areas of Guatemala, J. Med. Entomol. 19 (1), 63–67

48) Tanaka, I., Hashiguchi, Y., Okazawa, T., Ochoa A., J. O. and Tada, I. (1980): Duration of blood feeding of *Simulium ochraceum* in relation to intake of *Onchocerca volvulus* microfilariae, Jap. J. Sanit. Zool., 31 (3), 209–214

49) Thylefors, B., Philippon, B. and Prost, A. (1978): Transmission potentials of *Onchocerca volvulus* and the associated intensity of onchocerciasis in a Sudan-Savanna area, Tropenmed. Parasitol., 29, 346–354

50) Watanabe, M., Tanaka, I., Okazawa, T., Yamagata, Y. and Ochoa A., J. O. (1980): Notes on the age determination, ovariole changes and gonotrophic cycle of *Simulium ochraceum* in Guatemala, Jap. J. Sanit. Zool., 31 (3), 215–222

51) Zea, G., Hashiguchi, Y., Kawabata, M., Tada, I., Yoshimura, T., Flores, O. and Recinos C., M. M. (1980): Guatemalan onchocerciasis: Skin snipping methods and microfilarial densities in a given minute area of the skin, Jap. J. Trop. Med. Hyg., 8 (1), 23–32

REVIEW ON THE BIOLOGY AND ECOLOGY OF THE ADULT BLACKFLIES IN RELATION TO THE TRANSMISSION OF ONCHOCERCIASIS IN GUATEMALA

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The present paper was read as a special presentation at the 31st annual meeting of Southern Region of the Japan Society for Sanitary Zoology held at Kumamoto on November 7, 1981. It reviews chiefly the recent advances of knowledge on the biology and ecology of the adult blackflies in relation to the transmission of onchocerciasis in Guatemala and also outlines the history of the research work carried out during the period from 1915 when Dr. R. Robles discovered onchocerciasis in his country, to the present time. The contents are summarized as follows: 1) *Simulium ochraceum, S. metallicum* and *S. callidum* were incriminated as the vectors of onchocerciasis in Guatemala on the basis that they were naturally infected with the third-stage larvae of *Onchocerca volvulus*, whereas *S. gonzalezi, S. haematopotum, S. veracruzanum* and *S. horacioi* were regarded as potential vectors due to their capability of supporting the larval development to the third stage experimentally. 2) With regard to the fate and migration of microfilariae of *O. volvulus* larvae, it was reported that the numbers of microfilariae taken up by the three vector species mentioned above were usually larger than might be expected considering the microfilarial density in the skin. The percentage of the microfilariae developing to the third stage per individual females was very low, ranging from 0.2 to 4.3%. The decrease in the number of the larvae was caused by various factors differing between the blackfly species. In *S. ochraceum* most microfilariae ingested were damaged and killed by the toothed buccopharyngeal armature when they passed through, and only a few larvae could reach the thorax where most of them developed to the third stage. Whereas, in *S. metallicum* which had no such armature as a barrier, about 70% of the microfilariae ingested could penetrate through the wall of the midgut, but in the thoracic muscles inefficient development occurred with many undeveloped and/or abnormally formed larvae still remaining even after the normal period required for the completion of the larval development. The proportion of *S. ochraceum* fed on an *O. volvulus*-microfilariae carrier becoming infected with the third-stage larvae was three times higher than that of *S. metallicum.* This difference was mainly due to the latter vector's high mortality within 24 hours after ingestion of microfilariae usually observed when large numbers of microfilariae were ingested. 3) Concerning biological and ecological aspects of the vectors, it was shown that *S. ochraceum* was highly anthropophilic but *S. metallicum* and *S. callidum* were more zoophilic. The most predominant anthropophilic species were *S. ochraceum* and *S. metallicum*, although their relative proportion differed between localities, by months and even within a day. The proportions of the parous females of these two vectors caught on man also varied greatly depending on the situation. The duration of the gonotrophic cycle of *S. ochraceum* was estimated as two to

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four days in nature. 4) Among the factors influencing the transmission, various degrees of microfilarial densities in the skin were assessed in terms of the infection rate of the blackflies by the third-stage larvae, and it was shown that even a person with very low microfilarial density such as 0.5 mf/mg in the skin could become a source of infection, yielding a low percentage of *S. ochraceum* females harbouring the infective larvae. It was reported that the temperature greatly influenced the development of *O. volvulus* larvae in *S. ochraceum* and also the latter's longevity. The development of larvae to the third stage took place between 18 and 30°C, and its duration ranged from four to 13 days increasing with the decrease in temperature. Temperatures fluctuating by day and night to simulate natural conditions did not adversely affect the completion of the larval development nor the vector's survival even when the temperature during the night was set below 17°C (predicted developmental zero point).

AN AUTOPSY REPORT OF LASSA FEVER IN ONITSHA/NIGERIA/1974

Kiichi Sato,* Silvanus E. Ikerionwu and Kenneth C. Katchy

Recieved for publication September 24 1981

Abstract: The present report is one of the autopsy report of Lassa fever in Nigeria, 1974. Hospital epidemic of Lassa fever occurred in Onitsha of Nigeria. A 29 year old German physician, E.S. working at the St. Charles' Borromeo Hospital in Onitsha complained abdominal pain on February 10, 1974. At that time intermittent high fever up to 40°C, headache, malaise and vomiting were also noticed. On Feb. 15, sore throat and high fever lasted. On February 17, sore throat got worse and temporary fall of temperature. On February 19, severer sore throat involving tonsillitis, ulceration in the soft palate and cervical lymphadenopathy were found. Tracheotomy was performed because of dyspnea and resulted in profuse bleeding, clotting time 17 minutes. Some petechiae developed on the face. On February 20, 1974 there came convulsion and coma, then death. Blood culture was negative. Isolation of Lassa virus was successfully performed from the pleural and ascites fluid of the cadaver. Histopathologic examination of the organ specimens was performed in Tokyo and disclosed following findings. (1) Viral hepatitis. (2) Acute splenitis. (3) Focal glomerulonephritis. (4) Localized fibrosis of myocard. (5) Marked congestion and edema of both lungs. (6) Lymphadenitis. (7) Meningoencephalitis. According to these findings, we obtained following comments: Necrosis of nerve cells in the brain and liver cells, and focal necrosis of glomeruli may be attributed to primary damage by the virus. However, pathognomotic change in the spleen, acellular eosinophilic necrosis around follicle and in Billroth cord (Edington 1972) seems to be deposition of hyaline like substance resulting from increased permeability of capillary in the marginal zone of follicle and Billroth cord.

Introduction

Lassa fever is an acute febrile viral disease first observed at Lassa in Northern Nigeria in 1969 (Frame et al. 1970). The new virus was first isolated by Buckley et al. in the same year of 1970. Up to 1973, four autopsies have been performed out of a total of 19 deaths recorded in 4 outbreaks. The first and second cases were performed at Lassa in 1969 (Frame et al. 1970). The third and the fourth cases were performed at Jos in 1970 (Troup et al. 1970). However no histological findings of the first case were published (Frame et al. 1970). Troup et al. (1970) had reported the third and the fourth autopsy findings in 1970 and Edington et al. (1972) had described detailed pathological findings of the same autopsies in 1972, but the changes in the brain were not described by any of these authors.

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Authors had an autopsy case of Lassa fever in hospital epidemic that occurred in St. Charles' Borromeo Hospital/Onitsha in the East Central State of Nigeria in February 1974. The present report described the autopsy findings of our own.

Clinical Abstracts

Dr. E. Saurwald., a 29 year old German Missionary Medical Doctor in St. Charles' Borromeo Hospital became ill on February 10th 1974 with generalized abdominal pain and loose stool for one day. Later he developed intermittent fever (40°C) associated with headache, general malaise and vomiting. Ampicillin 250mg 6 hourly was given from the 5th day, but was ineffective. On the 6th day, he developed sore throat and his fever continued. On the 7th day, his therapy was changed to Chloramphenicol following a diagnosis of typhoid. On the 8th day, his sore throat got worse and he was given to start on 60 mg of Predonisolone. His temperature now fell and remained steady untill the 10th day, but in the meantime his throat infection became severer with involvement of tonsills and ulceration of the soft palate. He also had cervical lymphadenopathy and developed respiratory obstruction, for which tracheotomy was performed by a colleague, Dr. Mandrella. Following this, the patient developed profuse bleeding from the operative wound. A clotting time of 17 minutes was recorded and he also developed some petechie on the face. A few hours after the operation he started having convulsions and went into coma. He died 10 hours later on February 20th, 1974.

Laboratory investigations showed normal haemoglobin throughout the illness. W.B.C. varied from 4,100/cmm. to 7,500/cmm. with a differential count showing relative lymphocytosis. The blood culture grew contaminants only. The isolation of the virus was not performed clinically. An autopsy was performed later at the University of Nigeria Hospital.

Macroscopic findings

External examination of the body showed several petechie on the forehead and on the scalp. There was tracheotomy wound at the 3rd tracheal ring which appeared clean. The peritoneal cavity contained 1000 m*l* of straw coloured fluid and the pleural cavities together contained about 500 m*l* of haemorrhagic fluid. The brain weighed 1600 gm. The surface of the brain and the cut surface showed evidence of oedema. The tongue was normal and both tonsills were enlarged and congested. No evidence of any ulceration of the stomach or of the rest of the gut was noticed. Liver (1,500 gm) was normal in colour and surface showed a few distended bile ducts. The gall bladder was distended with bile. The tracheal lumen was patent. The lungs which weighed left 750 gm and right 900 gm were markedly congested massive and subcrepitant, especially in the lower lobes. The pleurae were also congested. The spleen (240 gm) appeared normal. There was no lymphadenopathy. The pericardial sac contained a few mililitres of straw coloured fluid. The heart (400 gm) contained post mortem clots in all chambers. Both kidneys (150 gm each) appeared normal with normal corticomedullary differentiation.

Microscopic findings

Section from the brain showed meningoencephalitis. There were oedema, congestion, scattered necrosis and neuronophagia of the nerve cells in the cerebral cortex (Fig. 1, 2). Occasional perivascular infiltration (cuffing) of lymphocytes and histiocytes (Fig. 3, 4). Meninges showed congestion, oedema, slight infiltration of lymphocytes and histiocytes (Fig. 5, 6). The liver showed viral hepatitis. There were scattered eosinophilic necrosis of individual or small groups of the hepatic cells without cell infiltration. Periportal round cell infiltration (up to moderate degree) and slight fibrosis (Fig. 7, 8, 9). And neither bile stasis nor fatty changes were seen. The spleen showed slight fibrinous perisplenitis. There were marked congestion and deposition of hyaline-like substance around follicles and Billroth's cords (Fig. 10, 11). Marked deposition of lymphocytes and reticular cells and occasional intrafollicular bleeding were seen. In addition, there were pavementing and subendothelial infiltration of lymphocytes, plasma cells and monocytes of trabecular vein (Fig. 12, 13). In the kidney, there were scattered focal necrosis of the glomeruli, interstitial oedema and slight fibrosis (Fig. 14). Scattered granular and

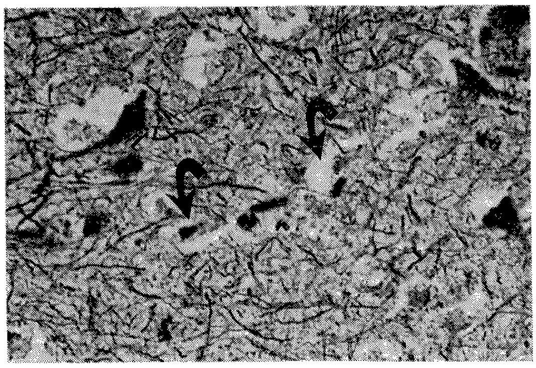

Figure 1 Neuronophagia of necrotic nerve cells (Bodian's protargol method, ×400).

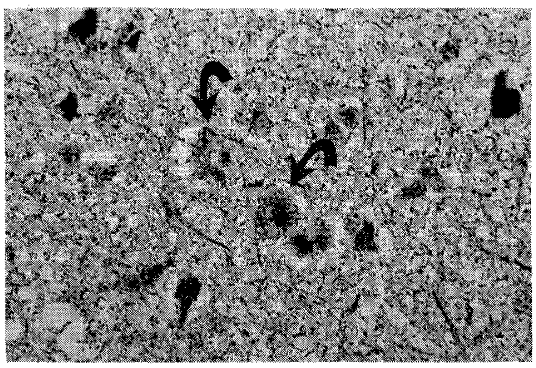

Figure 2 Necrosis of nerve cell with fairly prominent nucleolus. (Bodian's protargol method, ×400).

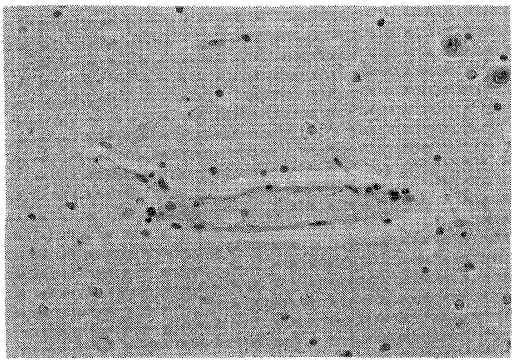

Figure 3 Perivascular infiltration of lymphocytes. (H.E., ×100).

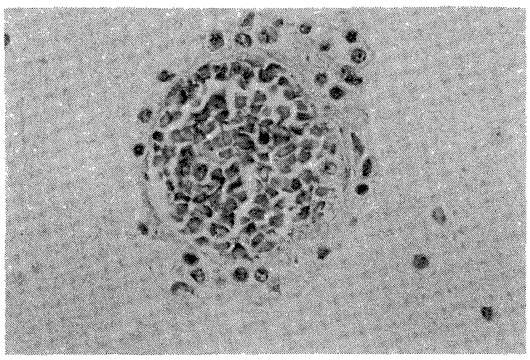

Figure 4 Perivascular infiltration (Cuffing) was not so remarkable. Lymphocyte and monocyte. Congestion. (H.E., ×400).

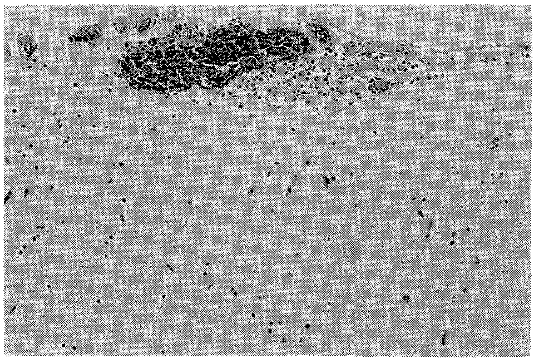

Figure 5 Meningitis, slight infiltration of lymphocyte and histiocyte in congested meninges. Congestion of grey matter. (H.E., ×100).

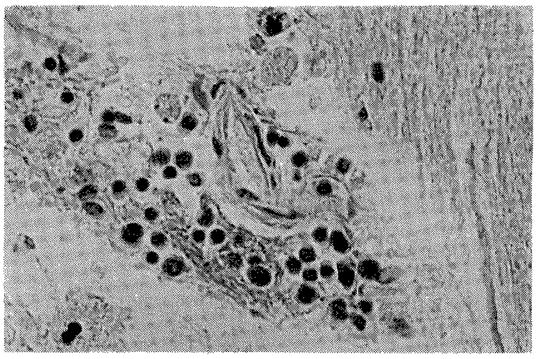

Figure 6 High magnification of Fig. 5.; Infiltration of lymphocyte, plasma cell and histiocyte. (H.E., ×400)

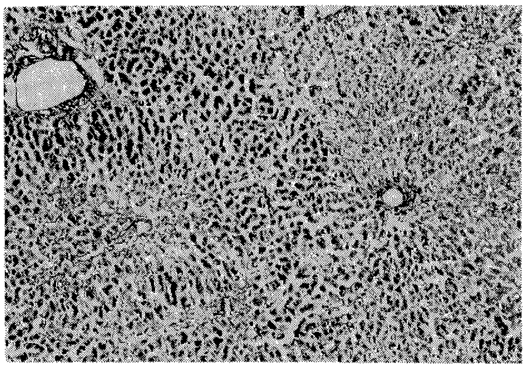

Figure 7 Slight fibrosis of periportal area of the liver. (Pap's silber stain., ×40)

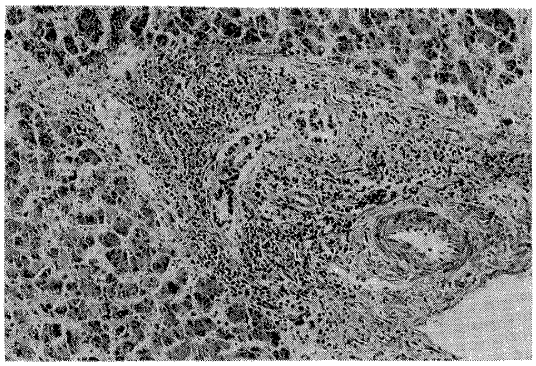

Figure 8 Infiltration of lymphocytes, plasma cells and histiocytes in Glisson's sheath. No bile stasis. (H.E., ×100)

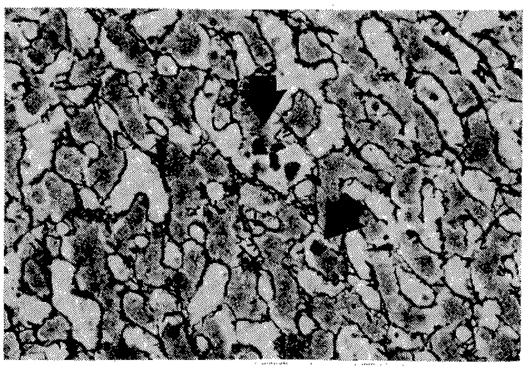

Figure 9 Necrosis of individual and a small group of the hepatic cell. (Pap's silber stain., ×200).

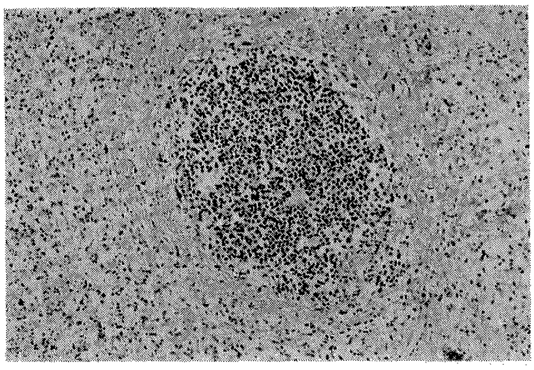

Figure 10 Pericapillary deposition of PAS positive substance in marginal zone of follicle. Scattered reticular cells, in spleen. (PAS., ×100)

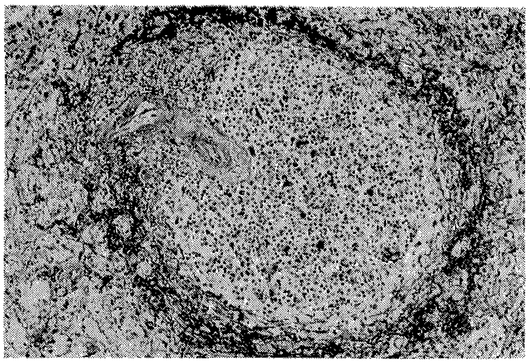

Figure 11 Perifollicular deposition of hyaline-like substance, in spleen. Lymphocyte depletion of follicle. (Masson., ×100)

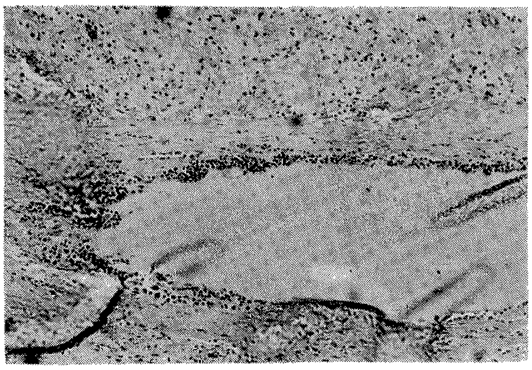

Figure 12 Pavementing and subendothelial infiltration of lymphocytes of trabecular vein. Lymphocytic infiltration is seen in media. (PAS., ×100)

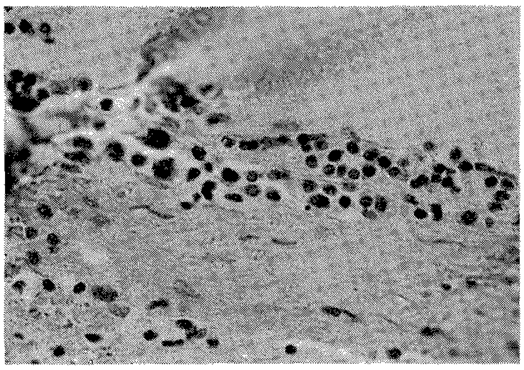

Figure 13 Subendothelial infiltration of lymphocytes, plasma cells, eosinophile leucocyte and others in spleen. (PAS., ×400)

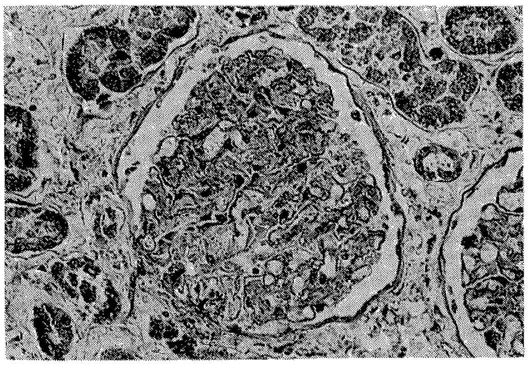

Figure 14 Focal necrosis and destruction of glomerular tuft of kidney. Interstitial oedema. Advanced post mortem change of tubular epithelia. Slight thickening of Bowmans' capsule. (PAS., ×200)

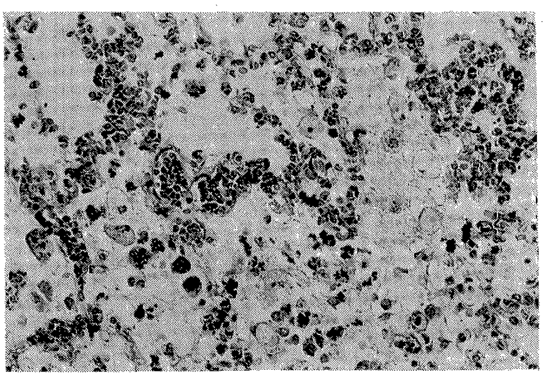

Figure 15 Marked congestion and oedema of the lower lobe of the right lung. (H.E., ×400)

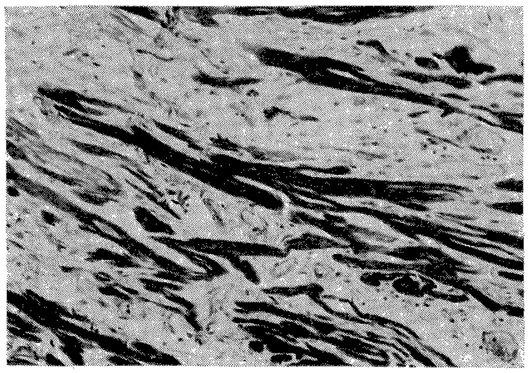

Figure 16 Circumscribed fibrosis of myocardium and degeneration of muscular fiber of the heart. Interstitial oedema. (Azan., ×400)

hyaline casts were seen in the tubules. The lungs showed marked congestion and oedema (Fig. 15). Slight bleeding in the lower lobe of the left lungs and scattered heart failure cells in the alveolar lumen (Fig. 15). No interstitial pneumonitis was seen. The hilar lymphonode showed congestion, lymphocyte depletion and slight anthracosis. No remarkable reticulosis of the lymph node was seen. The cervical lymph node showed lymphadenitis. There were congestion, marked depletion of lymphocytes and reticulosis. The heart showed interstitial oedema, circumscribed fibrosis and degeneration of the myocard (Fig. 16). No remarkable change in the valvae was seen. No ulceration in the gastrointestinal tracts were seen. Peritonitis was present. Congestion and atrophy of the thyroid were seen.

Isolation of Lassa virus

During our autopsy, the pleural and peritoneal fluid were obtained and had been kept in isolated refrigerator at outside of our hospital. Later, authors had brought this specimen, which was kept in absolute ice box, to Prof. Fabiyi who was in virus labotatory of Ibadan University/Nigeria. On 29th June 1974, authors received the result of specimen, which was surely described "positive of Lassa virus" (Fig. 17).

COPY:

29th June, 1974.

Thro: Dr. I. O. K. Udeozo
Head, Department of Pathology.

to: Dr. S. E. Ikerionwu,
Registrar in Pathology
University of Nigeria Teaching Hospital
Enugu. E.C.S.

Dear Dr. Ikerionwu:

Re: Dr. Egon Saurwald's Specimens
VRL Ref. Nos. H79145 and H79146

Thank you for your letter of June 14, 1974 re the above specimens (Pleural and peritoneal fluids). I can confirm that these have been shown to be positive for the presence of Lassa virus (by isolation).

There are no serological reports as you did not send us any serum from this patient.

Several strains of the Lassa Virus have been isolated from rodent, *Mastomys natalensis*, only. Most of these strains were from rodents caught in the households occupied by Lassa fever patients (Monath, T.P., C.D.C. Morbidity and Mortality Weekly Report Vol. 22, No. 24, June 16, 1973). However these isolations, important as they are, do not necessarily indicate that *Mastomys natalensis* is the reservoir for Lassa virus. Work on this aspect is in progress.

If I can be of further service please do not hesitate to get in touch.

Yours sincerely,

(Sgd.) Akinyele Fabiyi
Professor & Director.

cc: Dr. I. O. K. Udeozo.
Prof. K. Sal.

Figure 17

Hospital epidemic in St. Charles' Borromeo Hospital

There was no detailed report of hospital epidemic in St. Charles' Borromeo Hospital/Onitsha of Nigeria in February 1974. However, authors had been given some document about the hospital epidemic by Dr. Mandrella of St. Charles' Borromeo Hospital, before autopsy. According to his document, a boy of 19 year old had got common cold with high fever and was admitted to this hospital, suspecting malaria. However, high fever of this patient did not decreased and continued. In this time, nursing doctor for this patient was Dr. Egon Sauerwald, whose clinical document had already described. In the late stage, Dr. Sauerwald was received tracheotomy by Dr. Mandrella. Dr. Mandrella had been also infected with Lassa fever, after ten days our autopsy. Fortunately, Dr. Mandrella was transferred to his mother country, West Germany and recovered after the antiserum therapy by Dr. Casals.

Discussion

According to the literature, there have been four outbreaks of Lassa fever, two in Nigeria, one in Liberia and one in Sierra Leone before our autopsy. Of these, the first outbreak was seen at Lassa, a town in the North Eastern State of Nigeria in January, 1969 (Frame et al). The second outbreak of Lassa fever was at Jos in January, 1970 (Troup et al. 1970). The third outbreak was in Zorzor area in Liberia in March, 1972 (Mertens et al. 1973, Monath et al. 1973). And the fourth outbreak was in Sierra Leone in September/October, 1972 (Monath et al. 1974).

Four autopsies were performed out of the 19 deaths that occurred in these outbreaks. However, in the first necropsy, only macroscopic findings were described. The second autopsy was performed by Prof. Stoerk, who described the histological findings as marked congestion of the heart, lungs, liver, spleen and kidneys (Frame et al. 1970). Another significant lesion found was in the submucosa of the intestine which showed severe oedema. The third and the fourth autopsies were performed by Dr. Troup, who, unfortunately, got the infection herself through an accidental cut while performing the autopsy and died in February, 1970. Later, the organs of the third and fourth autopsy cases were handled by Prof. Edington who reported that the findings were somewhat similar to the pathology of Thai and Bolivian haemorrhagic fever and he considered Lassa fever to be a new haemorrhagic fever (Edington and White, 1972). No autopsy was performed on any of the deaths that occurred in the outbreaks in Liberia and Sierra Leone.

According to the detailed microscopic findings of Prof. Edington, the characteristic lesion of Lassa fever is in the spleen and consists of an eosinophilic necrosis surrounding the depleted white pulp associated with pavementing and subendothelial lymphocytic infiltration of the splenic veins. However, in our case, we did not find the typical eosinophilic coagulative necrosis around the depleted white pulp in the spleen. But we did recognize the deposition of hyaline-like substance around follicles and Billroth's cords (Fig. 11). These findings seemed to be deposition resulting from increased permeability of capillary in the marginal zone of follicle and Billroth's cord (Sato and Akiyoshi, 1975). And we also found marked widening of the perivascular space of the brain associated with perivascular cuffing with lymphocytes (Fig. 3), which is suggestive of encephalitis. These histological features of the brain are the first to be described in Lassa fever.

The clinical history of our case was very similar to those previously published (Frame et al. 1970, White 1972, and Mertens et al. 1973). The isolation of the virus from the blood or tissue fluids is the important criterion to the diagnosis of Lassa fever. In our case, Lassa virus was reported to be isolated from both pleural and peritoneal fluids by Prof. Fabiyi (Fig. 17).

Acknowledgment

We would like to thank Dr. B. Mandrella of St. Charles' Borromeo Hospital, Onitsha, who supplied us with clinical history; Prof. G. M. Edington for his kind advice on the slides, Prof. M. Akiyoshi for his kind advice on histopathologic examinations and Prof. A. Fabiyi of the Virus Research Centre, Ibadan University, for isolating the virus. Finally, we wish to express our thanks to the Japanese International Cooperation Agency for supporting our cooperation.

References

1) Buckley, S. M. and Casals, J. (1970): Lassa fever, a new virus disease of man from West Africa. (III) Isolation and characterization of the virus. Am. J. Trop. Med & Hyg., 19; 680–691.

2) Edington, G. M. and White H. A. (1972): The pathology of Lassa fever. Trans. Royal Soc. Trop. Med. & Hyg. 66: 381–389.

3) Frame, J. P., Baldwin, J. M. and Troup, J. M. (1970): Lassa fever, a new virus disease of man from West Africa. (I) Clinical description and pathological findings. Am. J. Trop. Med. & Hyg., 19: 670–676.

4) Mertens, P. E., Patton, R., Baum, J. J. and Monath, T. P. (1973): Clinical presentation of Lassa fever cases during the Hospital epidemic at Zorzor, Liberia. March-April 1972. Am. J. Trop. Med. & Hyg., 22: 780–784.

5) Monath, T. P., Mertens, P. E., Patton, R., Moser, C. R., Baum, J. J., Pinneo, L., Cray, G. W. and Kissling, R. E. (1973): A hospital epidemic of Lassa fever in Zorzor,; Liberia, March-April 1972. Am. J. Trop. Med. & Hyg., 22: 773–779.

6) Monath, T. P., Newhouse, V. F., Kemp, G. E., Setzer, H. W. and Cacciapuoti, A. (1974): Lassa virus isolation from Mastomys natalensis rodents. during an epidemic in Sierra Leone. Science, 185: 263–265.

7) Sato, K. and Akiyoshi, M. (1975): An autopsy case of Lassa fever in Nigeria. Acta Pathol. Jpn. 64: 94–95.

8) Troup, J. M., White, H. A., Fom, A. L. D. and Carey, D. E. (1970): An outbreak of Lassa fever on the Jos Plateau, Nigeria, in January-February 1970, a preliminary report. Am. J. Trop. Med. & Hyg., 19: 695–696.

9) White, H. A. (1972): Lassa fever a study of 23 Hospital cases. Trans. Royal Soc. Trop. Med & Hyg. 66: 390–401.

1974年ナイジェリア国のオニッチャ市において，ラッサ熱で死亡した医師の剖検報告

佐藤喜一*・Silvanus E. IKERIONWU, Kenneth C. KATCHY

1974年2月，ナイジェリア国のオニッチャ市にあるボロメオ病院内で，ラッサ熱の流行があった。この時期に，この病院で働いていた29歳の西ドイツ人医師（男）が入院中の患者から，ラッサ熱に感染し死亡した。著者らはナイジェリア大学病院（Enugu 市：Onitsha 市より東へ約 70 km）の病理解剖室で本例の病理解剖を行なった。剖検体は，1974年2月10日に 40°C の高熱と腹痛を訴えた。そして嘔吐があった。抗生物質の投与に抗して高熱は5日間継続した。10日目には激しい咽頭痛を訴え，扁桃と軟口蓋に潰瘍がみられ，頸部リンパ腺腫脹も現われた。呼吸困難が現われたために気管切開術を行なったが，間もなく意識喪失し死亡した。

病理組織学所見のうち，主たる所見は以下の如くであった。(1) 脳（1500 g）：脳膜炎と脳炎の所見を認めた（図1～6）。すなわち脳膜はうっ血を示し，組織学的には軽度のリンパ球や組織球の浸潤と浮腫がみられた。脳実質にもうっ血と浮腫がみられた。また神経細胞の壊死と Neuronophagia が考えられた（図7～9）。肝細胞と肝細胞の小塊には好エオジン性壊死像がみられた。(3) 脾臓（240 g）：脾周囲炎と脾炎がみられた。脾白色髄の辺縁帯と脾索内に硝子様物質の沈着がみられた（図10～13）。(4) 腎臓：巣状糸球体腎炎（図14）。(5) 胸水と腹水をイバダン大学ウイルス研究所へ送り，ラッサ熱ウイルスの同定試験で陽性であった（図17）。(6) その他として肺のうっ血と浮腫，心筋の限局性線維症などがみられた。これらの所見から，ラッサウイルスが直接障害したと考えられる所見として大脳皮質の神経細胞の壊死，肝細胞の散在性壊死，腎糸球体の巣状壊死などにみられるような毛細血管内皮細胞の障害などがあげられる。

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BACTERIOLOGICAL AND CHEMICAL STUDY OF THE DRINKING WATER IN INDONESIA[1]

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Abstract: We carried out the examination of drinking water in Indonesia for past 13 years. The present paper reports the resutls of bacteriological and potassium permanganate consumed test conducted from July to August in 1980.

Bacteriological examination revealed that *Klebsiella pneumoniae* was isolated most frequently from the drinking water in Indonesia, and *Acinetobacter calcoaceticus*, *Aeromonas hydrophila*, *Pseudomonas* sp. and *Pseudomonas aeruginosa* were found next to it. *Klebsiella penumoniae* and *Acinetobacter calcoaceticus* were found most frequently from drinking water in Java, while *Aeromonas hydrophila* was isolated most often in Kalimantan and Sumatra. From the samples of the tap water, *Acinetobacter calcoaceticus* and *Hafnia alvei* were found most frequently, while from the well water, *Klebsiella pneumoniae*, *Acinetobacter calcoaceticus* and *Aeromonas hydrophila*, and from rain water, *Acinetobacter calcoaceticus* and *Chromobacterium violaceum* were respectively found.

From the results of the potassium permanganate consumed test, 62% of the drinking water from Java were shown positive in this test, while 90% from Kalimantan, 100% from Sumatra as well as Sulawasi were proved positive respectively. The correlation between isolated bacteria and results of potassium permanganate consumed test was following; All the drinking water, in which such bacteria as *Escherichia coli*, *Edwardsiella tarda*, *Enterobacter aerogenes* were found, showed positive also in potassium permanganate consumed test, while only 33% of the drinking water contained *Hafnia alvei* was proved positive in this test.

Introduction

Indonesia lies in the tropical zone, and with high temperature and humidity, its climate is most favorable for the propagation of microorganisms. In most parts of the country, sanitary conditions are far from desirable. Diseases like typhus, cholera, dysentery, epidemic hepatitis, and various parasitosis are still high frequent under the existing circumstances.

We focus our attention to the drinking water, as it plays as important role in outbreak and transmission of these diseases. With the aid of Japan Association for

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Tropical Medicine, we started to examine the drinking water in each place of Indonesia since 1968 and have conducted the study for more than ten years.

The results were shown that the drinking water in Indonesia contained a large amount of microorganisms, and chlorinization of drinking water was never carried out in any places in Indonesia (Fujita, Ikeda and Matsumoto, 1974). For example, *Escherichia coli* was found in more than 10% of samples of the drinking water supplied by Jakarta city, in spite that the base line of the drinking water of Indonesia was prescribed more strictly by law than that of WHO (World Health Organization, 1971), and of Japan (Takeuchi, 1980).

In the present paper, we report the microbiological and some chemical study of drinking water in Indonesia based on the survey in 1980.

Period, Area, Materials and Methods

During the period from 4 July to 2 August 1980 in Jakarta and other main cities of Java, Kalimantan, Sulawasi, and Sumatra, we collected samples from the drinking water with cleansed containers, after washing them thoroughly with it (Table 1). Each sample of 0.1 m*l* water was dropped into 0.2% agar added to Brain heart infusion broth to make semi solid medium with a sterilized pipet, and on our return to Japan, it was cultivated with modified Drigalski medium, SS agar, TCBS agar, NAC agar and Manitol salt agar. The isolated colonies were discriminated in the procedure of Fig. 1–1 and Fig. 1–2, and their species were determined (Buchanan,

Table 1 Drinking water classified by regions and sources

	JAVA	KALIMANTAN	SULAWASI	SUMATRA
Tap water	9	2	0	0
Well water	35	7	5	10
Rain water	2	5	1	0
Total points	46	14	6	10

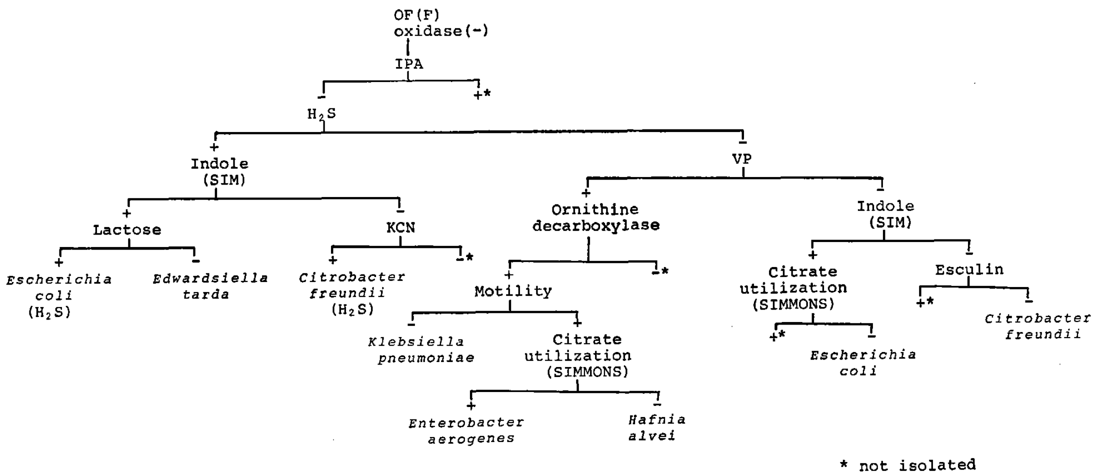

Figure 1–1 Procedure of discrimination of Gram negative rods.

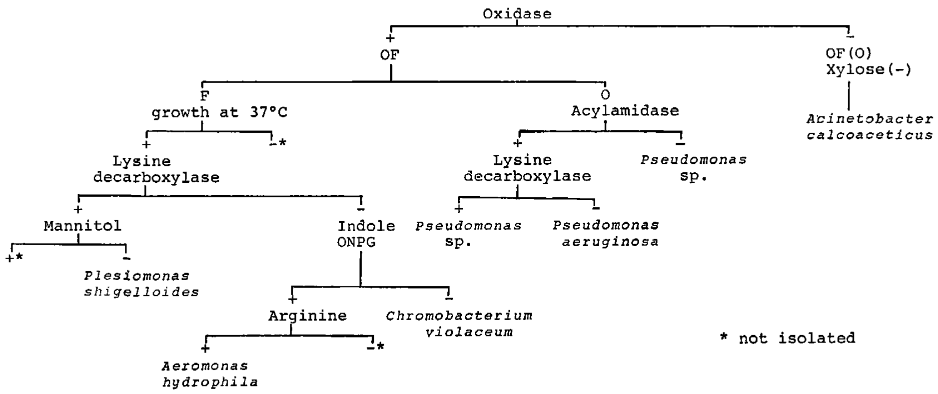

Figure 1–2 Procedure of discrimination of Gram negative rods.

R. E. and Gibbons, N. E., 1975; Kozakai, 1974).

On the same time, each of 50 m*l* sample water was taken to sterilized polyethylene vessels, and potassium permanganate consumed test was carried out with Shibata 8052–05 type water gauge (Shibata Chem. Co., Ltd. Tokyo, Japan).

Results

Seventy six samples of the drinking water from Java, Kalimantan, Sumatra and Sulawasi of Indonesia were examined first by the potassium permanganate consumed test, which is regarded as the most important index among various kinds of chemical examinations in connection with the water pollution. Results of the test revealed that 62% of the drinking water from Java was shown positive in potassium permanganate consumed test, while 90% from Kalimantan, 100% from Sumatra as well as Sulawasi were proved positive, as shown in Table 2. When the positive rate classified by the source, 17% of tap water, 82% of well water and 100% of rain water became positive (Table 3).

Table 2 Results of potassium permanganate consumed test from drinking water classified by regions

	Total points	positive (%)
JAVA	46	62
KALIMANTAN	14	90
SULAWASI	6	100
SUMATRA	10	100

Table 3 Results of potassium permanganate consumed test from drinking water classified by sources

	Total points	positive (%)
Tap water	11	17
Well water	57	82
Rain water	8	100

Table 4 shows the results of bacteriological examinations of drinking water in Indonesia. It was *Klebsiella pneumoniae* and *Acinetobacter calcoaceticus* that were isolated most often, and *Aeromonas hydrophila, Pseudomonas* sp. and *Pseudomonas aeruginosa* were found next to it. Contrary to our expectation, *Escherichia coli* was found only in seven samples. When the strains isolated were classified by regions, *Klebsiella pneumoniae* and *Acinetobacter calcoaceticus* were most frequently found in Java, *Pseudomonas aeruginosa* and *Pseudomonas* sp. followed them. In Kalimantan, *Aeromonas hydrophila* and *Pseudomonas* sp. were frequent, and *Escherichia coli* was found in four samples. In Sumatra, *Aeromonas hydrophila* was isolated most often, and *Escherichia coli* in three samples. In Sulawasi, *Acinetobacter calcoaceticus* and *Chromobacterium violaceum* and others were found.

Table 4 The number of strains isolated from drinking water classified by regions

Strains	JAVA	KALIMANTAN	SULAWASI	SUMATRA	Total No. of strains
Escherichia coli	0	4	0	3	7
Klebsiella pneumoniae	19	0	2	3	24
Edwardsiella tarda	0	2	0	0	2
Enterobacter aerogenes	1	1	0	0	2
Hafnia alvei	3	1	0	1	5
Citrobacter freundii	3	0	0	2	5
Aeromonas hydrophila	2	4	0	7	13
Chromobacterium violaceum	1	2	3	0	6
Acinetobacter calcoaceticus	16	0	4	3	23
Plesiomonas shigelloides	3	0	0	0	3
Pseudomonas aeruginosa	5	3	0	2	10
Pseudomonas sp.	4	4	0	3	11

When classified by the source of the water, the results are shown in Table 5. From the samples of the tap water, *Acinetobacter calcoaceticus* and *Hafnia alvei* were found frequently. But in case of 57 samples of the well water, various kinds of species were

Table 5 The number of strains isolated from drinking water classified by sources

	Tap water	Well water	Rain water	Total No. of strains
Total points	11	57	8	
Escherichia coil	0	7	0	7
Klebsiella pneumoniae	0	22	2	24
Edwardsiella tarda	0	2	0	2
Enterobacter aerogenes	0	0	2	2
Hafnia alvei	3	1	1	5
Citrobacter freundii	2	3	0	5
Aeromonas hydrophila	0	11	2	13
Chromobacterium violaceum	1	2	3	6
Acinetobacter calcoaceticus	4	15	4	23
Plesiomonas shigelloides	0	3	0	3
Pseudomonas aeruginosa	1	7	2	10
Pseudomonas sp.	2	8	1	11

found, but among them, *Klebsiella pneumoniae* was most frequently found (in 22 samples) and *Acinetobacter calcoaceticus* was next (in 15 samples). From the samples of the rain water, *Acinetobacter calcoaceticus, Chromobacterium violaceum* and others were found.

Table 6 shows the correlation between isolated bacteria and results of potassium permanganate consumed test. Among the samples in which *Hafnia alvei* or *Chromobacterium violaceum* was found, only 40% and 33% of drinking water was proved positive in the potassium permanganate consumed test. In case of *Plesimonas shigelloides*, this correlation was next lowerst value of 67%. But, 100% of drinking water which contained such bacteria as *Escherichia coli, Edwardsiella tarda, Enterobacter aerogenes, Aeromonas hydrophila* and so on showed positive also in potassium permanganate consumed test.

Table 6 Correlation between the results of potassium permanganate consumed test and the isolated bacteria

Strains	negative sample (%)	positive sample (%)
Escherichia coli	0	100
Klebsiella pneumoniae	4	96
Edwardsiella tarda	0	100
Enterobacter aerogenes	0	100
Hafnia alvei	60	40
Citrobacter freundii	20	80
Aeromonas hydrophila	0	100
Chromobacterium violaceum	67	33
Acinetobacter calcoaceticus	9	91
Plesiomonas shigelloides	33	67
Pseudomonas aeruginosa	5	95
Pseudomonas sp.	9	91

Discussion

We have conducted the examination of drinking water in Indonesia for 13 years, and we found that the sanitary conditions have never changed during these periods, as already reported by Fujita, et al. in 1974. Furthermore, according to the last year's survey, 30 among 54 samples (56%) of drinking water in Indonesia contained more than 10^2 coli-form bacilli per m*l* (Fujita, Okuwaki, Ikeda, Tsukidate, Sugiyama and Iwami, 1980).

Mohammedanism, which forms a basis for the life in this country, seems to have a greatest influence to the sanitary conditions. Cities and villages are situated along large or small rivers, which also serve as traffic routes. Peoples are attached to the rivers, and use the water for drinking, washing vessels or clothes, bathing and washing away faecal matters. An Islamic way of thinking that "flowing water is always clean" brings about such a situation. People cannot even imagine that the water possibly contains pathogenic microorganisms. Therefore, cholera, dysentery, typhus or epidemic hepatitis became prevalent every year in each locality.

We have considered that the drinking water plays a most important part of the causes of these diseases in Indonesia. Therefore, we carried out the more detailed bacteriological as well as chemical tests of the drinking water in Indonesia in the present study. In our bacteriological survey of the drinking water in Indonesia, *Escherichia coli*, which suggests the existence of fecal matters, was isolated not very often. But, bacteria such as *Klebsiella pneumoniae*, and *Aeromonas hydrophila* were found regardless of the locality. As these bacteria are normally distributed in the realm of natural and river water, we can infer that the admixture of bacteria by the bursting of the water pipe from outside water occurs in almost all parts of Indonesia.

From the results of the potassium permanganate consumed test, 100% of the drinking water in Indonesia, in which *Escherichia coli* was found, was proved positive also in this test, and over 62% of drinking water from each place of Indonesia became positive in the potassium permanganate consumed test. These results will suggest that the drinking water in Indonesia is polluted by fecal matters with relatively high probability. It is hoped that the efforts to maintain the drinking water clean will be realized in the near future in Indonesia.

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References

1) Buchanan, R. E. and Gibbons, N. E. (1975): Bergey's manual of determinative bacteriology 8th ed., 290–531, The Williams and Wilkins Co., Baltimore
2) Fujita, K., Ikeda, S. and Matsumoto, M. (1974): Examination of drinking water in Indonesia — A retrospective study for past 8 years, Tropics, 9, 11–22
3) Fujita, K., Okuwaki Y., Ikeda, T., Tsukidate, S., Sugiyama, M. and Iwami, K. (1980): Hygienic study of the drinking water in tropical regions; Microbiological survey of the drinking water in Indonesia, Tropics, 13, 1–4
4) Kozakai, N. (1974): Modern medical technology, 7, 208–248, Igakushoin, Tokyo
5) Takeuchi, K. (1980): Hygienic control of water, 190–192, Chuo Hoki Publishers, Tokyo
6) World Health Organization (1971): International standards for drinking-water, WHO, 3rd ed. Geneva, Switzerland

インドネシア各地の飲料水の細菌学的および化学的研究[1]

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われわれは，過去13年間にわたりインドネシア各地の飲料水について，その水質に関する調査を実施してきた。その成績とインドネシア各地に発生する疾病との因果関係に考察を加えると同時に，熱帯地における衛生上の問題点についても言及してきた。

今回，1980年7月から8月にかけての約1カ月間，インドネシア各地の飲料水について，細菌学的検索を行い，併せて過マンガン酸カリウム消費量を測定し，興味ある知見を得たので報告する。

インドネシア各地で採取した総計76検体の飲料水について，細菌学的検査を行ったところ，*Klebsiella pneumoniae* が最も多く検出され，次いで *Acinetobacter calcoaceticus*, *Aeromonas hrdrophila*, *Pseudomonas* sp. および *Pseudomonas aeruginosa* などの細菌類が検出された。飲料水を地域別に分けた場合，ジャワ島では *Klebsiella pneumoniae* や *Acinetobacter calcoaceticus* が多く検出されたのに対して，カリマンタン島やスマトラ島での飲料水には *Aeromonas hydrophila* が最も多く検出された。また，飲料水を水源別にした場合では，水道水からは *Acinetobacter calcoaceticus* と *Hafnia alvei* が量も多く検出されたのに対して，井戸水からは *Klebsiella pneumoniae*, *Acinetobacter calcoaceticus* と *Aeromonas hydrophila* が，一方，雨水からは *Chromobacterium violaceum* がそれぞれ多く検出された。

さらに，水質検査のうちで，有機物混入の最も良い指標とされている過マンガン酸カリウム消費量を測定し，検出された細菌類との地域別および水源別についての関係を調べた。ジャワ島での飲料水は62%が過マンガン酸カリウム消費量が陽性となった。一方，カリマンタン島での飲料水は90%が，スマトラ島およびスラウェシ島での飲料水では実に100%が同反応で陽性となった。また，検出された菌種との関係をみると，*Escherichia coli*, *Enterobacter aerogenes* などが多く検出された飲料水は過マンガン酸カリウム消費量の反応が100%陽性であったが，*Hafnia alvei* が検出された飲料水は33%にしか同反応で陽性を示さなかった。

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CORRELATION OF INFECTION RATE OF INTESTINAL HELMINTHS IN JAPANESE INHABITING TROPICAL COUNTRIES WITH DEGREE OF POLLUTION OF DRINKING WATER[1]

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Abstract: Many Japanese inhabit tropical countries, and some of them are suffering from infectious diseases. In order to know the relationship between the prevalence of these diseases and the degree of faecal pollution of the drinking water, we have examined the drinking water in these countries of the world, and at the same time we have studied the infection rate of intestinal helminths of the Japanese who stayed 6 months or more in these countries.

In total over 300 samples of drinking water from countries of South East and South West Asia, Middle East, East Africa and Central and South America were tested with potassium permanganate consumed test, and number of coliform bacilli from the drinking water was counted after cultivation. Relatively high proportion of samples appeared to be polluted by faecal matters. Then, we checked parasite eggs in the stool samples of Japanese who used the water samples in the tropical countries actually. Eggs of *Ascaris lumbricoides, Trichuris trichiura, Taenia saginata* and *Clonorchis sinensis* were found among the Japanese. Infection rate of intestinal helminths was proved to be highest among Japanese in Indonesia, and the rate was followed by those of people in South West Asia, in East Africa and in Central and South America. The relationship between the infection rate of intestinal helminths and the degree of pollution of the drinking water was studied. Significant relationship was observed between the infection rate of intestinal parasites of the Japanese inhabitants and the rate of water containing 10^2 or more/ml of coliform and of total micro-organisms ($r=0.957$). However, no relationship between the infection rate of *Enterobius vermicularis* among the Japanese children there and the degree of the pollution of drinking water was found.

INTRODUCTION

At present time, over 440 thousand Japanese inhabit tropical countries, and

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the number of Japanese stayed in these countries is increasing, as the Japanese technical cooperation with the countries is improved. The diseases from which Japanese suffers in the tropical countries are infectious hepatitis, especially type A, amoebic as well as bacillary dysentery, typhoid fever, various kinds of intestinal helminthiasis and so on. These diseases are thought to be orally infected, and among sources of infection, the drinking water is considered to be most important as the main route of infection.

In 1968, we started to examine the drinking water from tap as well as deep-well at various places in Indonesia. Results were shown that the drinking water in Indonesia contained a large amount of micro-organisms. For example, *Escherichia coli* was found in more than 10% samples of the drinking water supplied by Jakarta city (Fujita, Ikeda and Matsumoto, 1974). The examination continued to be conducted by us from 1968 until recently, but no good progress in the results of the examination of drinking water was seen in the past 13 years (Fujita, Okuwaki, Ikeda, Tsukidate, Sugiyama and Iwami, 1980).

From 1979, we have newly opened inquiry of the drinking water in many tropical countries in the world including Indonesia. Results also revealed that comparatively large proportion of drinking water contained *E. coli*, and it was thought to be polluted by faeces and sewage. On the same time, we conducted the survey of the intestinal helminth infection rate among the Japanese who live in tropical countries and use the drinking water in these countries. The present paper describes the relationship between the infection rate of intestinal helminths among the Japanese and the degree of pollution of drinking water in the tropical developing countries.

Materials and Methods

From April 1979 to December 1980, we have visited various tropical countries, as shown in Table 1, and collected samples from drinking water with cleansed containers, after washing them throughly with it. The drinking water from tap as well as deep-well was dropped with a sterilized pipet into MacConky and CLED agar (Uricult Set, Daiichi Kagaku, Co. Ltd. Tokyo) and cultivated according to the previous paper (Okuwaki, Fujita, Sugiyama, Yanai, Yutaka, Tsukidate and Asakura, in press). To determine the number of bacteria/m*l* of the sample, the density of colonies appearing on each of the slide was compared with the densities on the model chart, according to our previous paper (Fujita et al., 1980).

Potassium permanganate consumed test, total hardness and other chemical tests of drinking water were also carried out with Shibata 8052–05 type water gauge (Shibata Co. Ltd., Tokyo).

On the same time, we have collected stool samples from the Japanese who inhabited in tropical countries for at least over 6 months and were using the same water we tested for drinking. The stool samples were put into the containers with 10% formaline, and on our return to Japan, they were examined by Formaline-Ether (MGL) method whether the Japanese suffered from intestinal helminths. Especially the children staying in these countries were tested to determine the infection rate of *E. vermicularis* by Scotch Tape method (Yoshida, 1977).

RESULTS

Potassiun permanganate consumed test of drinking water.

The greatest danger associated with drinking water in tropical countries is that it has been contaminated by sewage or by human excrement. Therefore, we examined first the potassium permanganate consumed test which is regarded as the most important index among various kinds of chemical examinations in connection with these kinds of water pollution. In total, 383 samples of the drinking water from tropical countries of South-East and South-West Asia, Middle East, Africa and Central and South America were tested with potassium permanganate consumed test. When the drinking water showing over 10 mg per litre in the test was regarded as positive, the positive rate was shown in the left column of Table 1. The positive rates of the drinking water from Argentina, Venezuela and India were proved to be highest. The values of drinking water from Brazil, Taiwan, Iraq, Tanzania and Bolivia were found next to them.

Determination of the coliform group and the total content of micro-organisms from the drinking water.

The organisms most commonly used as indicators of faecal pollution are *E. coli* and the coliform group. In order to know the degree of faecal pollution of the drinking water in tropical countries, we picked up the samples containing 10^2 or more per millilitre of coliform and of the total content of micro-organisms on the same time. Among 298 samples tested, the drinking water from Malagasy and Indonesia showed the highest degree of contamination with micro-organisms, and the values were followed by the samples from Tanzania, Ecuador, Panama, India and Mexico, as shown in the central column of Table 1. It is intresting that percentage of drinking water containing micro-organisms from Indonesia was astonishingly high, in spite of that the relatively low positive rate was found in the potassium permanganate consumed test, as already mentioned.

Total hardness of the drinking water.

Total hardness of the drinking water was assayed, and results were indicated as (1) to (6) according to the degree of the hardness, as shown in the right column of Table 1. Relatively soft drinking water was available in areas of South-East Asia and Central America, while harder water was obtained from Middle East.

Infection rate of the intestinal helminths in Japanese inhabiting tropical countries.

We have collected the stool samples from the Japanese who stayed 6 months or more in these tropical countries, and examined parasite eggs. Eggs of *A. lumbricoides, T. trichiura, T. saginata* and *C. sinensis* were found, and the infection rates of these intestinal helminths were calculated in Japanese inhabiting Indonesia, South-West Asia (India and Pakistan), Middle East (Qatar), East Africa and Central

Table 1 Examination of the drinking water from tropical countries (1979 to 1980)

Area	Name of country	Potassium*[1] permanganate consumed test (%)	Coliform*[2] bacillus (%)	Total hardness
South East Asia	Indonesia	6/74 (8.1)	53/74 (71.6)	(2)–(3)*[3]
	Philippine	5/13 (38.5)	N.T.*[4]	(1)–(3)
	Taiwan	4/7 (57.1)	N.T.	(1)
South West Asia	India	16/24 (66.7)	10/24 (41.7)	(3)–(6)
	Pakistan	6/17 (35.3)	1/17 (5.9)	(4)
	Bangladesh	0/2 (0.0)	0/2 (0.0)	(2)
Middle East	Iraq	3/6 (50.0)	N.T.	(6)
	Qatar	0/12 (0.0)	1/12 (8.3)	(5)
	Egypt	1/8 (12.5)	0/8 (0.0)	(4)–(5)
Africa	Kenya	10/34 (29.4)	3/16 (18.8)	(1)–(3)
	Zambia	0/9 (0.0)	0/9 (0.0)	(4)–(5)
	Tanzania	7/14 (50.0)	7/14 (50.0)	(1)–(2)
	Malagasy	1/7 (14.3)	6/7 (85.7)	(1)–(5)
	Mozambique	0/17 (0.0)	6/17 (35.3)	(1)–(2)
	Neigeria	0/2 (0.0)	N.T.	(2)–(3)
Central America	Mexico	4/29 (13.8)	12/29 (41.4)	(2)
	Panama	3/14 (21.4)	6/14 (42.9)	(1)
	Guatemala	0/4 (0.0)	1/4 (25.0)	(1)
	Trinidad	0/5 (0.0)	N.T.	(4)
South America	Venezuela	14/18 (77.8)	N.T.	(3)
	Colombia	3/8 (37.5)	3/8 (37.5)	(1)
	Ecuador	1/9 (11.1)	3/7 (42.9)	(1)
	Peru	4/13 (30.8)	5/13 (38.5)	(5)
	Chile	1/8 (12.5)	1/8 (12.5)	(5)
	Argentina	6/7 (85.7)	N.T.	(2)
	Paraguay	1/5 (20.0)	N.T.	(2)
	Bolivia	1/2 (50.0)	N.T.	(2)
	Brazil	9/15 (60.0)	3/15 (20.0)	(1)

*[1] Number of samples showing 10 mg/l or more in the potassium permanganate consumed test / Number of total samples tested

*[2] Number of samples showing 10^2/ml or more of coliform bacilli and of total content of micro-organisms / Number of total samples tested

*[3] (1) mean value of total hardness of below 50 mg/l (2) 50–100 mg/l (3) 100–150 mg/l (4) 150–250 mg/l (5) 250–350 mg/l (6) over 350 mg/l respectivily

*[4] N.T.: not tested

Table 2 Correlation of infection rate of intestinal helminths in Japanese inhabiting tropical countries with the degree of pollution of drinking water

Area	South East Asia (Indonesia)	South West Asia (India, Pakistan)	Middle East (Qatar)	East Africa	Central and South America
Number of stool samples tested	212	80	48	89	226
Number of parasite positive samples	17 (8.0%)	2 (2.5%)	0 (0.0%)	2 (2.2%)	3 (1.3%)
*Ascaris lumbricoides**[1]	12 (5.7)	2 (2.5)	0	2 (2.2)	0
Trichuris trichiura	4 (1.9)	0	0	0	2 (0.8)
Taenia saginata	1 (0.5)	0	0	0	0
Clonorchis sinensis	0	0	0	0	1 (0.4)
Number of Scotch tape tested	48	91	N.T.*[4]	75	105
Enterobius vermicularis	4 (8.3%)	6 (6.6%)	N.T.	5 (6.7%)	7 (6.7%)
Number of drinking water tested	76	43	12	63	98
Potassium permanganate consumed test*[2]	6 (8.3%)	25 (58.1%)	0 (0.0%)	15 (23.8%)	25 (25.5%)
Coliform bacillus*[3]	53 (71.6)	11 (25.6)	1 (8.3)	22 (34.9)	34 (32.4)

*[1] Number of stool samples having eggs of *A. lumbricoides*

*[2] Number of drinking water showing 10 mg/*l* or more in the potassium permanganate consumed test

*[3] Number of drinking water showing 10^2/m*l* or more of coliform bacilli and of total content of micro-organisms

*[4] N.T.: not tested

and South America. The Japanese we selected in each of tropical countries were composed of persons from the same environmental as well as economical status; they were members of the first class of manufacturing trading firms of Japan and their families. Also, there were almost no differences in sex ratio and age of persons composed among these areas except in the case of Qatar. As shown in Table 2, the infection rate of the intestinal helminths among the Japanese was found highest in Indonesia, and those of South-West Asia, of East Africa and of Central and South America followed it. The infection rate of the Japanese in Qatar of Middle East was proved to be null.

The infection rate of *E. vermicularis* was also examined with their children of these areas by Scotch Tape method. The infection rate was also highest among children in Indonesia, but the rate was not so different from those of the other areas, and the infection rates of SouthWest Asia, East Africa and Central and South America showed same value, as shown in Table 2.

Relationship between the infection rate of intestinal helminths among the Japanese and the degree of pollution of drinking water.

In order to know relationship between the infection rate of intestinal helminths and the degree of pollution of drinking water, the potassium permanganate consumed test and the examination of number of coliform bacilli as well as total number of other micro-organisms were carried out and they were compared with the parasite infection rate of the Japanese who were using water for drinking actually in these tropical countries.

The positive rate of potassium permanganate consumed test and of water samples containing 10^2 or more/m*l* of coliform and of total content of micro-organisms were shown in the lower column of Table 2. Significant relationship was observed between the infection rate of intestinal parasites and the rate of water containing 10^2 or more/m*l* of coliform and total micro-organisms. Its correlation coefficient was $r=0.957$, as shown in Fig. 1. However, no relationship was observed between the infection rate of parasites and the positive rate of potassium permanganate consumed test. Then the relation between the infection rate of *E. vermicularis* and the degree of pollution of drinking water was examined, but no significant relationship was found between them.

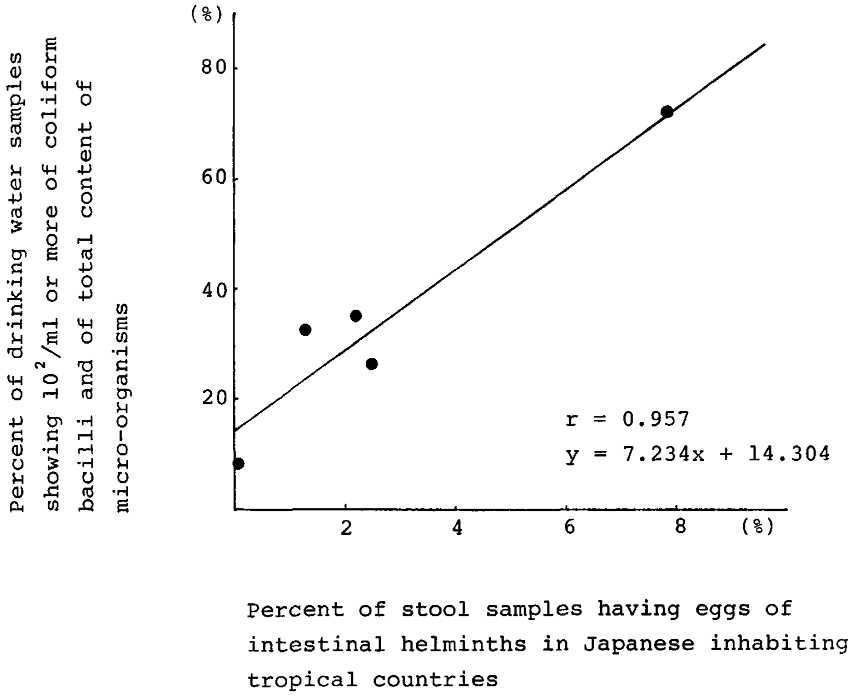

Figure 1 Correlation of infection rate of intestinal helminths in Japanese inhabiting tropical countries with the rate of drinking water samples having coliform bacilli.

Discussion

Water intended for human consumption must be free from organisms that may be a hazard to health. Some countries have established national standards of quality for drinking water and have achieved to get a certain degree of clean water (Takeuchi, 1980). Others, however, still lack official standards of quality (World Health Organization, 1971), and many tropical countries have no recognized methods for keeping good quality of drinking water. Our previous study for 13 years in Indonesia revealed that *E. coli* was always found in more than 10% sample of drinking water and the situation have never changed during these periods (Fujita et al., 1980).

On the other hand, outbreaks of water-borne diseases are still prevalent in these tropical countries and the Japanese inhabiting these countries are often suffering from these diseases. Some one says that they use water for drinking after boiling it completely, so the prevalence of diseases is not ascribed to the drinking water itself. However, it does not seem to be true. Present study clearly demonstrated that the Japanese who used the water containing coliform group for drinking were inclined to be infected with intestinal helminths. The result will suggest us that the drinking water still plays important parts in the prevalence of the water-borne diseases, even if people use the drinking water after boiling it completely.

At regional and international conferences sponsored by the World Health Organization, the problems of establishing standards of quality for safe and acceptable water supply in these countries have been discussed by groups of experienced hygienists and engineers. Outbreaks of water-borne diseases will be avoided through stricter control by the responsible health authorities of the quality of the water distributed for drinking purposes. If the drinking water continued to be kept free from faecal pollution in these countries, people in tropical countries including the Japanese inhabiting there would not contact these diseases.

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Grateful acknowledgment is made to Prof. M. Kikuchi, Department of Hygiene, Juntendo University School of Medicine and to Prof. M. Hashimoto, Department of Microbiology, Kagawa Nutrition College, for their constant interest and guidance in the investigation. Thanks are tendered to Mr. S. Kitano, Executive Director of the Tropical Forestry Association of Japan for his help.

References

1) Fujita, K., Ikeda, S. and Matsumoto, M. (1974): Examination of drinking water in Indonesia — A retrospective study for past 8 years, Tropics, 9, 11–22
2) Fujita, K., Okuwaki, Y., Ikeda, T., Tsukidate, S., Sugiyama, M. and Iwami, K. (1980): Hygienic study of the drinking water in tropical regions; Microbiological survey of the drinking water in Indonesia, Tropics, 13, 1–4
3) Okuwaki, Y., Fujita, K., Sugiyama, M., Yanai, H., Yutaka, K., Tsukidate, S. and Asakura, T.

(in press): Bacteriological and chemical study of the drinking water in Indonesia, Jap. J. Trop. Med. Hyg.
4) Takeuchi, K. (1980): Hygienic control of water, 190–193, Chuo Hoki Publisher, Tokyo.
5) World Health Organization (1971): International standards for drinking-water, WHO, 3rd ed. Geneva, Switzerland
6) Yoshida, Y. (1977): Illustrated human parasitology, Nansando, Tokyo

世界各地の発展途上国に在留する邦人の腸管寄生虫感染率と飲料水水質検査成績との相関[1]

藤田紘一郎[2]・杉山雅俊[3]・月舘説子[2]・奥脇義行[4]
池田照明[5]・小田　力[2]・朝倉健夫[6]

世界各地の，いわゆる発展途上国に在留する邦人の数は著しく増加している。それと同時に，各地で A 型肝炎，腸チフスやアメーバ赤痢など重篤な経口感染症に罹患する邦人の数も増加している。我々は，その主な原因が飲料水の汚染にあると考え，世界各地の発展途上国の飲料水について，特に糞便汚染との関連について調査してきた。今回，飲料水の汚染と在留邦人の腸管内寄生虫感染率との関連について調べたので報告する。

我々は，1979年4月から1980年12月までの間に東南アジア，南西アジア，中近東，東アフリカおよび中南米の種々の国々を訪問し，飲料水を採取し，細菌学的および化学的水質検査を行なった。

総数383検水について，まず過マンガン酸カリウム消費量が測定され，ついで一般細菌数および大腸菌群の数が調べられた。その結果，多かれ少なかれ，熱帯発展途上国の飲料水が糞便系汚染にさらされていることが判明した。

そこで，水質検査と同時にこれらの飲料水を利用している邦人について，彼らの腸管内寄生虫感染率を調査した。各地に6カ月以上滞在している邦人の成人から小指頭大の糞便を採り，10%ホルマリン加バイアル瓶に入れて日本に持ち帰り，MGL 集卵法にて糞便内寄生虫卵を検出した。また邦人の子供達を対象に，セロファン2回法による蟯虫検査を施行した。

糞便からは主として蛔虫卵が見出され，その他鞭虫，無鉤条虫，肝吸虫などの卵が検出された。腸管内寄生虫感染率はインドネシア在留邦人に最も高く，次いで南西アジア，東アフリカ，中南米の順になり，中近東のカタールでは全員陰性となった。在留邦人のうちで，腸管寄生虫に感染している割合は，同地域の飲料水のうちで大腸菌群が検出された検水の割合と高い正の相関を示した。すなわち，大腸菌群の検出頻度の高い飲料水を使用している在留邦人ほど，腸管寄生虫に多く感染しており，両者の相関係数は r=0.957 であった。しかし，糞便内寄生虫卵陽性率と飲料水の過マンガン酸カリウム消費量との間には相関関係は見出せなかった。また，在留邦人の子供を対象に施行した蟯虫卵の陽性率は飲料水の汚染とは全く無関係であった。

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OBSERVATIONS ON THE BIONOMICS OF LARVAL AND MAN-BITING FEMALE POPULATIONS OF *SIMULIUM HORACIOI*, A NEW POTENTIAL VECTOR OF *ONCHOCERCA VOLVULUS* IN GUATEMALA

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Abstract: Larval and man-biting female populations of *Simulium horacioi*, a new member of the *metallicum* group and a new potential vector of onchocerciasis in Guatemala were investigated as compared with *S. metallicum* s. str. and *S. ochraceum* in three different localities of the endemic zones. Larvae of this species occurred throughout the year and tended to prefer small permanent streams as a habitat; they colonized many temporary streams too. Female *S. horacioi* was shown to be one of the main anthropophilic species, although its biting rates varied from 0.06 to 29.0% depending on the localities studied. Seasonal change in its biting activity was distinct, exhibiting a peak in either September or October (during the end of the rainy season). Dissections of 743 *S. horacioi* yielded no *Onchocerca volvulus* larvae, while the first-stage larvae were observed in *S. metallicum* s. str. (2/1, 663) and *S. ochraceum* (9/1, 551), and the third-stage larva only in the latter species (1/1, 551). On the other hand, females of this species were sporadically infected with unknown filaria, mermithid and fungus.

In Guatemala, Onishi *et al.* (1977) found the unknown blackfly species belonging to the *metallicum* group (n. stat.) which was readily distinguishable at the larval stage from the other known Guatemalan members of the same species group, i.e., *Simulium metallicum* Bellardi s. str. and *S. jobbinsi* Vargas, Martínez Palacios and Díaz Nájera. Subsequently, Okazawa and Onishi (1980) described it and gave a new specific name, *S. horacioi.*

Since this species has been shown to bite man (Okazawa and Onishi, 1980) and to support the development of *Onchocerca volvulus* larvae to the infective stage experimentally (Ito *et al.*, 1980), whether this species is involved in the transmission of onchocerciasis in this country has become a matter of epidemiological importance.

The present investigation was conducted to determine the relative abundance of *S. horacioi* in larval and man-biting female populations and to assess its natural infection with *O. volvulus* larvae, as compared with *S. metallicum* s.str. and *S. ochraceum* Walker, the known onchocerciasis vectors in Guatemala.

Materials and Methods

Investigations were carried out in three different localities: 1) in the upstream

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sector of the Rio Verde River, 2) in the upper reach of the Guachipilín River (both localities were situated in the southern area of endemic onchocerciasis in Department of Escuintla), and 3) in the Finca Rincón near Lake Amatilán, Department of Guatemala, where the disease is also present. Detailed informations on the topography and the water system of these three districts were already given in the previous paper (Takaoka, 1981).

As for preference for a preimaginal site, larval populations of *S. horacioi* were observed at fixed stations of various types of streams. These sites were: section O of the main channel (permanent, 1.0–1.2 m wide), tributary G (permanent, 0.2–0.4 m wide) and tributary H (temporary, 0.3–0.6 m wide) in the Guachipilín River (fortnightly from September 1978 to January 1980); at the 1,300 m level of the main stream (permanent, 0.8–1.2 m wide) and 1,200 m level of a tributary (permanent, 0.3–0.5 m wide) in the Finca Rincón (once a month from January 1979 to January 1980). Each collection was made for 10 minutes by two persons.

On the other hand, in the upper reach of the Rio Verde River, all the seven streamlets as well as stretches of the main channel (all temporary water courses) were monthly inspected from April 1979 to January 1980, although several streamlets were preliminarily investigated during September to December 1978. Larval collections in this area were made in each water segment by two persons for five to 20 minutes. Data on the appearance and persistence of the temporary water segments, as well as those of *S. ochraceum* therein in this region were also presented previously (Takaoka, 1981).

Collections of adult blackflies were made monthly in the Rio Verde district (from January 1979 to January 1980), and fortnightly in the other two districts (from September 1978 to January 1980). Females landing on a human attractant with lower parts of the legs and upper parts of the body (above the waist) being exposed were collected by aspirator from 09.00–12.00 hr.

With regard to biting preference of *S. horacioi* on human body parts, a supplementary collection was made in October 1979 in the Guachipilín River area.

All larval and female samples collected were brought to the laboratory (Guatemala City) and preserved in vials with 70% alcohol. These specimens were identified according to the keys of Dalmat (1955). The identification of *S. horacioi* was based on the original description (Okazawa and Onishi, 1980). This species was readily distinguished from its senior Guatemalan members of the *metallicum* group (i.e., *S. metallicum* s. str. and *S. jobbinsi*) at the larval stage by the characteristic body color and in the female by the color of the scutal pubescence as well as the shape of the anal lobes and the presence or absence of the sclerotized seventh sternite.

In order to make the female's identification sure, two steps were applied in this study: First, among females belonging to the *metallicum* group, *S. horacioi* was separated from the other two members by the color of the scutal pubescence which was yellow in this species but brown in the latter two species (This procedure was made before samples were preserved in 70% alcohol). Secondly, on dissection in the laboratory (Medical College of Oita) for *O. volvulus* larvae and other parasites, the identity of *S. horacioi* was confirmed by observing the shape of the anal lobes which was in lateral view much produced ventrally under the cerci in *S. metallicum* s. str.

and *S. jobbinsi* but not in this species.

On the other hand, secure criteria on which a distinction at all stages between *S. metallicum* s. str. and *S. jobbinsi* was based have not yet established, although Dalmat (1955) and Vargas and Díaz Nájera (1957) claimed certain morphological features for separation of these species. For this reason, it is probable that, although it was not known whether anthropophilic or not, *S. jobbinsi* might have been included in the specimens identified as *S. metallicum* s. str. in the present study, because the former species was reported to have a wide distribution within and outside the endemic regions (Dalmat, 1955).

Simulium puigi Vargas, Martínez Palacios and Díaz Nájera, which was originally described from Mexico, has been very recently reported from this country (Takaoka and Takahasi, 1982), and has become a fourth member of the *metallicum* group. The spindle-shaped hind basitarsus of the male easily separates this species from the other members mentioned before. However, the female of this species closely-resembles that of *S. horacioi* in having the sclerotized seventh sternite, the pale base of the hind tibiae and the similar shape of the anal lobes, despite a slight difference in the latter two features. This may create some confusion in identifying the female of *S. horacioi* among the *metallicum* group. However, *S. puigi* was not taken into consideration in the present study because of its possible restricted distribution in upland streams at an elevation of 2,000–3,000 m (Takaoka and Takahasi, 1982).

Natural infections with *O. volvulus* or other parasites were observed by dissecting alcoholic female specimens captured in the region of the Guachipilín River. Each female was divided into head, thorax and abdomen in a drop of Giemsa solution on slide glass under a binocular microscope. Each part of the body dissected was searched for parasites under a microscope at 100 x. The number of larvae in each part of the body was counted and their stages of development were determined by size and morphological features defined by Bain (1969) and Duke (1968).

Results

Relative abundance of S. horacioi *in larval populations found in various types of streams.*

Simulium horacioi, like *S. metallicum* s. str. and *S. ochraceum*, were found to occur in both permanent and temporary streams (Table 1). However, this species tended to prefer small permanent streams (e.g., tributary G of the Guachipilín River) where *S. ochraceum* dominated too. Contrarily, larvae of this species were very few in number in medium-sized streams (i.e., the main channel in the Guachipilín River and the main stream in the Finca Rincón) which were, though, the most favourable site for larvae of *S. metallicum* s. str. In the Rio Verde River area where all streams flew temporarily *S. horacioi* made up 8.7% of 5,681 larvae collected, its monthly variations ranging from 2.0 (in May) to 20.7% (in October). Whereas, *S. metallicum* s. str. consisted of 22.2% of the total, being about six times as abundant as that of *S. horacioi*. In the same area, only 75 larvae (1.3% of the total) of *S. ochraceum* were collected.

Table 1 Overall results of collections of *Simulium* and *Cnephia* spp. larvae in three localities of onchocerciasis endemic zones in Guatemala

Species	No. (%) of larvae collected					
	Rio Verde*[1]	Guachipilín			Rincón	
		Tributary H*[1]	Tributary G*[2]	Main stream*[2]	Tributary*[2]	Main stream*[2]
S. horacioi	428 (7.5)	15 (0.3)	1,250 (38.6)	2 (0.06)	190 (8.2)	0 (—)
S. metallicum	1,259 (22.2)	2,379 (54.6)	506 (15.7)	2,159 (62.7)	30 (1.3)	4,577 (50.9)
S. ochraceum	75 (1.3)	468 (10.7)	1,395 (43.1)	64 (1.9)	2,045 (87.8)	2,399 (26.7)
S. callidum	426 (7.5)	391 (9.0)	5 (0.2)	465 (13.5)	63 (2.7)	1,493 (16.6)
S. downsi	0 (—)	0 (—)	0 (—)	2 (0.06)	0 (—)	110 (1.2)
S. haematopotum	0 (—)	0 (—)	0 (—)	0 (—)	0 (—)	2 (0.02)
S. paynei	1,410 (24.8)	750 (17.2)	78 (2.4)	711 (20.6)	0 (—)	382 (4.3)
S. rubicundulum	0 (—)	123 (2.8)	0 (—)	28 (0.8)	0 (—)	0 (—)
S. nigricornis	0 (—)	101 (2.3)	0 (—)	13 (0.4)	0 (—)	23 (0.3)
S. parrai	55 (1.0)	35 (0.8)	0 (—)	0 (—)	0 (—)	0 (—)
S. jacumbae	1 (0.02)	0 (—)	0 (—)	0 (—)	0 (—)	0 (—)
C. aguirrei	2,027 (35.7)	100 (2.3)	0 (—)	0 (—)	0 (—)	0 (—)
Total	5,681	4,361	3,234	3,445	2,328	8,986

*[1]. Temporary stream. *[2]. Permanent stream.

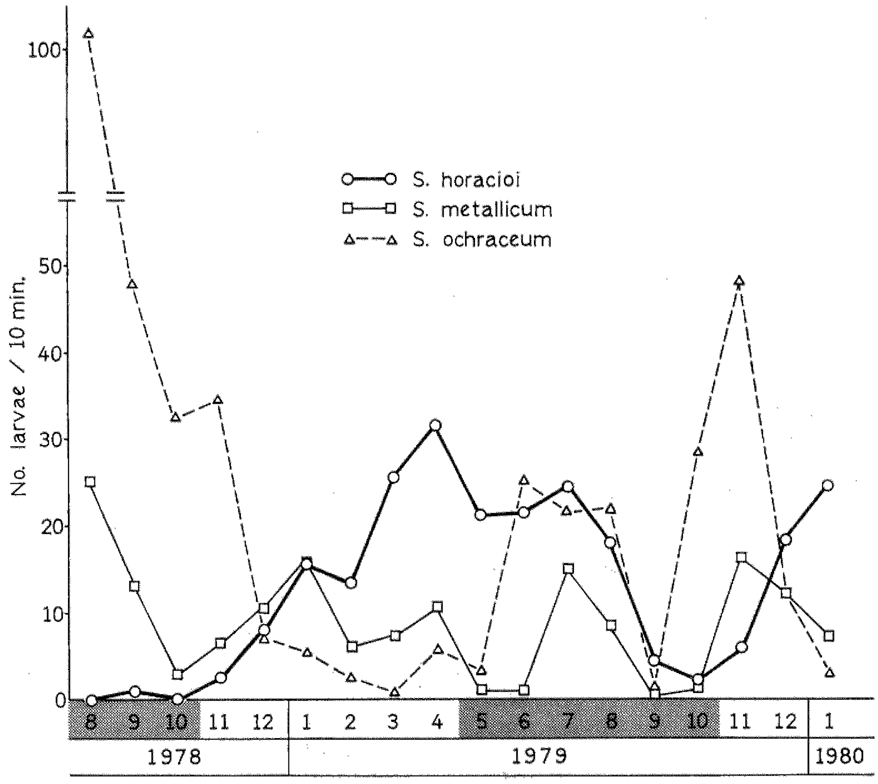

Figure 1 Seasonal trends in larval abundance of three anthropophilic blackfly species in the tributary G (small permanent stream) in the upstream sector of the Guachipilín River. (Shaded portion indicates the rainy season).

Seasonal variations in larval population densities of S. horacioi.

Fig. 1 illustrates the seasonal trend in larval population densities of *S. horacioi* in the tributary G, together with those of *S. metallicum* s. str. and *S. ochraceum*. In this small permanent stream, flow rates were nearly constant during the period observed with range of 0.34–0.67 litre/sec. *Simulium horacioi* larvae occurred throughout the year except for in August and October 1978, and its population densities were tended to be relatively high during the period from January to August in 1979, with the peak in April (the end of the dry season). By contrast, *S. ochraceum*, another dominant species in the same stream, had two peaks a year — the higher peak in October–November and the other less pronounced peak in June–August. The population densities of *S. metallicum* s. str. were low and seemed to have fluctuated every three or four months.

Seasonal variations in larval abundance of the three anthropophilic blackfly species in the upper reach of the Rio Verde River are presented in Fig. 2 as the grand total number of larvae collected. Larvae of *S. horacioi* initiated to colonize in the temporary streams soon after the water began to flow in May, continued to increase in number and reached the peak in July; thereafter, the total number of larvae dropped to some extent in August and September, but slightly increased again in October and then declined to nearly zero in December. *Simulium metallicum* s. str.

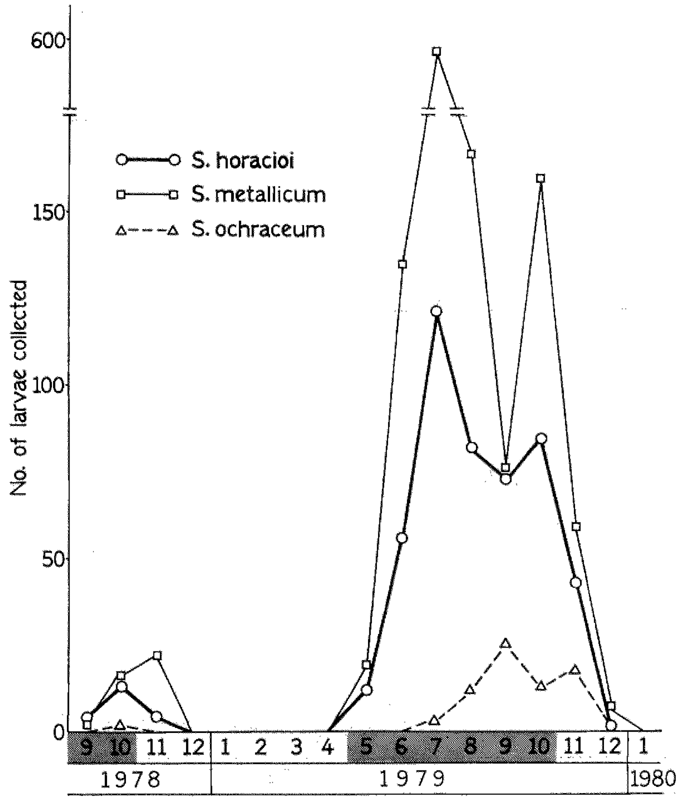

Figure 2 Seasonal trends in larval abundance of the three anthropophilic blackfly species in the upper reach of the Rio Verde River. (Shaded portion indicates the rainy season).

showed the pattern almost similar to that of *S. horacioi*, but the number of larvae of this species was consistently higher than that of the latter species in each month. On the other hand, *S. ochraceum* larvae which were outnumbered by the former two species were found only during the period from July to November.

For further analysis of the variations in larval abundance between blackfly species and by months, the changes in number of larval positive sites (nearly equal to the number of water segments positive for larvae) and the number of larvae per positive site in the same area are shown in Fig. 3. The total number of sites positive for any blackfly species fluctuated in proportion to that of the water segments which appeared and persisted during the rainy season and the subsequent dry months (Takaoka, 1981). It increased gradually during the early rainy months from May

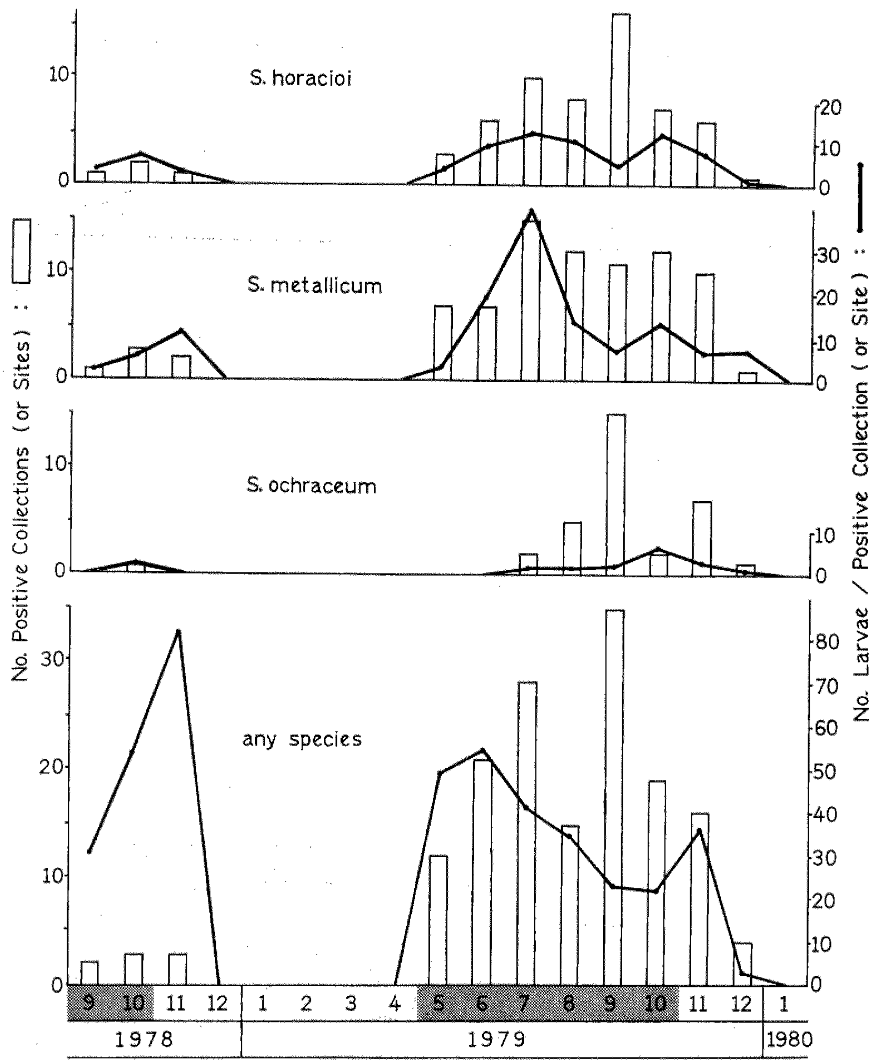

Figure 3 Seasonal trends in the number of larval positive sites and the number of larvae per positive site for three anthropophilic blackfly species. (Shaded portion indicates the rainy season).

to July (from 12 to 28 sites), fell to 15 in August (during the lull of the rainy season), peaked in September (at the climax of the rainfalls) and thereafter decreased during the following months of the dry season to become zero in January. *Simulium horacioi* followed the similar pattern in the number of the preimaginal sites, occupying 25.0–53.5% of the total sites positive for any species. The preimaginal sites of *S. metallicum* s. str. made up 25.0–80.0% of the total larval sites, being consistently higher than that of *S. horacioi* except for in September. *Simulium ochraceum* occupied 7.1–43.8% of the total preimaginal sites during the months from July to December.

The larval colonization of any blackfly species began at a considerably high densities (49 larvae/site) in May. Its densities peaked in July (54.4) and then continued to decrease gradually until December except in November when it rose again. The high densities observed during the first three months of the rainy season were mainly due to the dominancy of *Cnephia aguirrei,* a probable univoltine species, which always occurred in a great number only immediately after the water began to flow in the temporary streams. The larval density of *S. horacioi* was low in May but gradually increased up to the maximum of 12.2 in July, then continued to be almost at the same level (7.2–12.1 larvae/site) in each month except for in September and December when it fell to 4.6 and 1.0, respectively. On the other hand, *S. metallicum* s. str. showed the obvious fluctuation in larval densities with the marked peak in July. The density of this species which ranged from 2.7 to 39.7 larvae/site was also consistently higher than that of *S. horacioi* in each month except in May and November. *Simulium ochraceum* had always low larval densities ranging from 1.0–6.5 larvae/site.

Consequently, although the overall abundance of larval populations of these three blackfly species was determined by the product of the two factors, the number of preimaginal sites and the larval density therein, it was well demonstrated that the proportionate importance of either varied by blackfly species and also by months. Thus the highest peak in larval abundance observed in July for *S. horacioi* and *S. metallicum* s. str. was characterized by the large number of the preimaginal sites and the highest larval density of both species in this month. It was also indicated that *S. horacioi* was inferior to *S. metallicum* s. str. in regard to the ability of extending preimaginal sites in the temporary streams, and of aggregating in one habitat.

Relative abundance of S. horacioi *in the man-biting female populations collected in three different localities.*

Simulium horacioi was shown to be one of the main anthropophilic species although its biting rates varied remarkably between localities (Table 2). In the upper reach of the Rio Verde River, where its total number of females per man per three hours was greatest of all, *S. horacioi* comprised 29.0% of the total catches, being three quarters as abundant as *S. ochraceum* but almost twofold that of *S. metallicum* s. str. In the two other areas, this species was ranked as the third and fourth in order of abundance, occupying 11.0% and 0.06%, respectively, whereas *S. ochraceum* was also the dominant species (51.8% and 67.0%), followed by *S. metallicum* s. str. (33.8% and 32.7%).

Table 2 Species composition and relative abundance of the man-biting populations of blackflies in three localities of the onchocerciasis-endemic zones in Guatemala

Species	No. of females collected* (% of total catches)		
	Rio Verde	Guachipilín	Rincón
S. horacioi	126 (29.0)	113.6 (11.0)	1.0 (0.06)
S. metallicum	65 (14.9)	349.7 (33.8)	530.3 (32.7)
S. ochraceum	176 (40.5)	535.2 (51.8)	1,087.4 (67.0)
S. callidum	33 (7.6)	21.1 (2.0)	4.8 (0.3)
S. downsi	35 (8.0)	13.7 (1.3)	0 (0.0)
S. haematopotum	0 (0.0)	0 (0.0)	0.5 (0.03)
Total	435	1,033.3	1,624

* Numbers represent the total number of females collected (using a human attractant for 3 hours) during January to December 1979; for comparison, the numbers at the Guachipilín River and the Finca Rincón districts were experessed as the sum of the mean number of each month per man per 3 hours, since collections at these two places were made fortnightly.

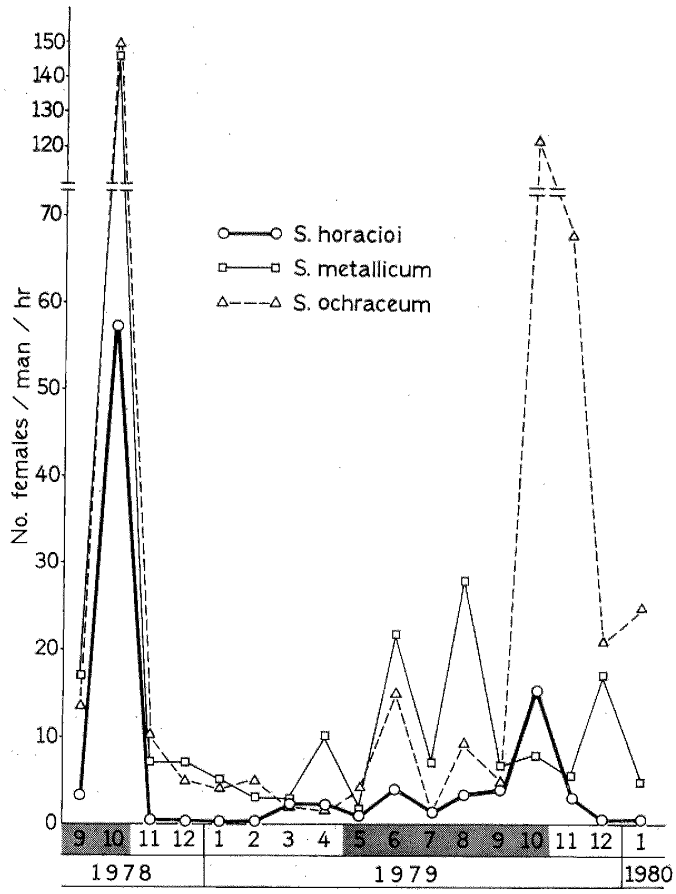

Figure 4 Seasonal trends in abundance of man-biting populations of three anthropophilic blackfly species in the upper reach of the Guachipilín River. (Shaded portion indicates the rainy season).

Seasonal fluctuation in the man-biting activities of S. horacioi.

Fig. 4 illustrates the monthly man-biting densities of the three anthropophilic species at the upstream sector of the Guachipilín River.

Female *S. horacioi* were captured throughout the year. The population density of this species peaked in October, although the peak in 1979 was much smaller than that in 1978, but continued to be low during the remaining part of the year. Similarly, *S. metallicum* s. str. showed the high peak in October in 1978 but its pattern observed in 1979 was different (i.e., having a much lower peak during the rainy season).

On the other hand, biting activity of these three species in the upper reach of the Rio Verde River was evident only during the rainy season and two or three subsequent months (Figure 5). The biting density of *S. horacioi* was higher in September and October than those in the other months. In contrast with this, *S. metallicum* s. str. had two small peaks — one in July and the other in December, although both peaks were much smaller than that of *S. horacioi*. On the other hand, the biting density of *S. ochraceum* was maintained relatively low during the rainy season but increased remarkably in December.

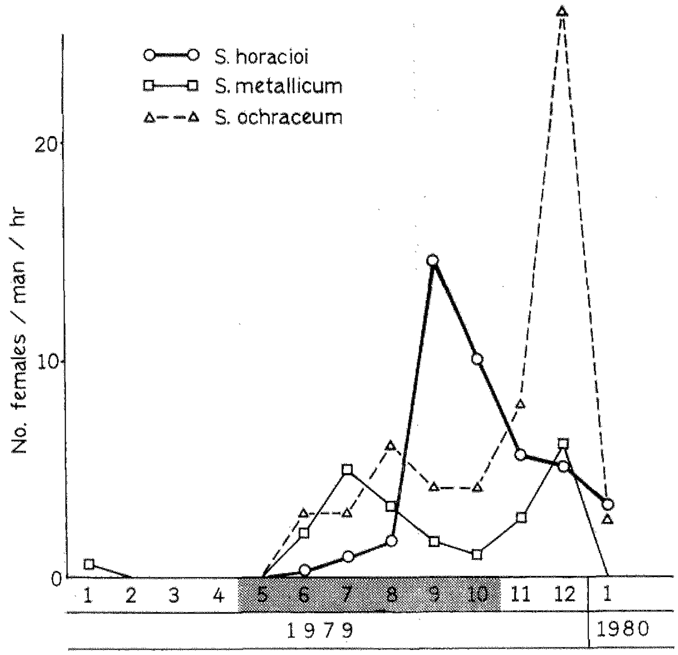

Figure 5 Seasonal trends in abundance of man-biting populations of three anthropophilic blackfly species in the upstream sector of the Rio Verde River. (Shaded portion indicates the rainy season).

Natural infection of S. horacioi *with* O. volvulus *larvae and other parasites.*

The results of dissection (Table 3) showed that no third-stage larvae of *O. volvulus* were found in any of the three blackfly species except for in one *S. ochraceum* which harboured one larva (441.5 μm long × 20.0 μm wide) indistinguishable from *O. volvulus* (0.17% or 1/600 dissected from October collection in 1978). In *S. horacioi* even the first-stage larvae were not discovered. While nine of 1,551 *S. ochraceum*

Table 3 Infections with *O. volvulus* and other parasites in females of *S. horacioi, S. metallicum* s. str. and *S. ochraceum* captured on human attractant during September 1978 to January 1980 in the upper reach of the Guachipilín River

	S. horacioi	*S. metallicum*	*S. ochraceum*
No. flies dissected	743	1,663	1,551
No. flies with larvae indistinguishable from *O. volvulus*:			
1st stage	0	2 (1, 2)*1	9 (1–9)
2nd stage	0	0	0
3rd stage	0	0	1*2(1)
No. flies with other filariae	1*3(4)	4*3(2–14)	0
No. flies with other parasites:			
Nematodes	0	0	1 (1)
Mermithids	2 (1)	20 (1)	0
Fungi	1	0	1
Mites	0	52 (1–3)	4 (1, 2)

*1. The number(s) in parenthesis represents the range in the number of parasites per infected female.

*2. Only one third-stage larva was found in the thorax of this female.

*3. These females harboured only third-stage larvae in their head, thorax and abdomen.

dissected (5/600 from October collection in 1978, and 2/329 and 2/231 from October and November collections in 1979, respectively) had first-stage larvae indistinguishable from *O. volvulus* in their thorax. No larvae of second stage were found in this study.

As to infections with other parasites, it was notable that one *S. horacioi* (1/502 from October collection in 1978) and four *S. metallicum* s. str. (1/958 from October collection in 1978 and 3/58 from April collection in 1979) harboured third-stage larvae of the unknown filaria with three caudal protuberances (637.5–1,113.1 μm long × 20.4–23.3 μm wide). These larvae were identical to that previously reported by Garms (1975). In addition, two females of *S. horacioi* were infected with mermithid larva (1/502 and 1/110, each from October collection in 1978 and 1979). In the popu-

Table 4 Distribution of bites of *Simulium* spp. on a man sitting in a shade exposing the upper portion of the body (above the waist) and the lower part of the legs (below the knees)

Species	No. of females collected	Percentage of body parts bitten		
		Lower part of legs	Back	Arms
S. horacioi	43	86.1	11.6	2.3
S. metallicum	30	83.3	6.7	10.0
S. ochraceum	7	28.6	57.1	14.3
S. callidum	11	90.9	9.1	0.0
S. downsi	3	100.0	0.0	0.0

lation of *S. metallicum* s. str., mermithid infections took place throughout the year (except for the period from April to July). Fungal infection in the ovaries was found in one *S. horacioi* (1/150 from October collection in 1978) and one *S. ochraceum* (1/329 from October collection in 1978). Larval mites were encountered parasitizing *S. metallicum* s. str. and *S. ochraceum*. However, 743 females of *S. horacioi* examined were free from this ectoparasite.

Distribution of bites of S. horacioi *on human body.*

Simulium horacioi preferred the lower parts of the legs to the upper parts of the body, as in the case of its ally, *S. metallicum* s. str.

Discussion

A clear morphological difference between *S. horacioi* and the other members of the *metallicum* group enabled us to investigate its larval and man-biting female populations, although further studies are yet to be required on a distinction between *S. metallicum* s. str. and *S. jobbinsi* at both larval and female stages.

The results of the present study indicated that *S. horacioi* tended to prefer small, permanent streams (less than 0.5 m wide), as briefly noted by Okazawa and Onishi (1980). This species was also shown to colonize temporary streams which flew only during the rainy season and a few subsequent dry months, although it seemed to be inferior to *S. metallicum* s. str. in regard to the ability of extending preimaginal sites and of aggregating in one habitat. However, this species was very rare in medium-sized streams. This was in remarkable contrast with *S. metallicum* s. str. which was shown to inhabit various types of streams from tiny trickles to large streams (even in rivers), as already reported by Dalmat (1955).

The larvae and pupae of this species were already recorded in other endemic areas by occasional collections — Departments of Santa Rosa, Chimaltenango, Suchitepéquez and Sololá (Okazawa and Onishi, 1980) and Huehuetenango (Takaoka, unpublished data). On the other hand, this species has not as yet been found in both lowland (below 400 m above sea level) and upland areas (2,000–3,000 m in elevation) (Takaoka, 1980). Therefore, it may be suggested that, like *S. ochraceum*, *S. horacioi* restricts its own distribution within the intermediate altitudes approximately between 500 and 2,000 m where onchocerciasis is known to be prevalent (Dalmat, 1955).

The present study showed that *S. horacioi* was one of the main anthropophilic blackfly species. However, there was a remarkable variation in biting rates between localities studied as shown in Table 2. The man-biting population of this species in the Finca Rincón was very low when compared with that in the two other areas. This difference may be explained at least in part by the quantity of the preimaginal sites for *S. horacioi*, which were confined to the permanent streams in the Finca Rincón but were greatly extended in the temporary streams in the latter regions.

In the upstream sector of the Rio Verde River, the most abundant species was *S. ochraceum* (40.5%), followed by *S. horacioi* (29.0%) and *S. metallicum* s. str. (14.9%). This was in reverse order when compared with the larval abundance of these three species in the same locality. This discrepancy between larval and man-biting female populations may reflect the degree of anthropophily of these three species, if adults of each species were assumed to have emerged in proportion to the larval abundance. It may be thus suggested that the anthropophilic nature of *S. horacioi* is not as high as that of *S. ochraceum* but is higher, to some extent, than that of *S. metallicum* s.str.

The seasonal change in the biting activity of *S. horacioi* was evident, with the peak in either September or October, although as shown in Figs. 4 and 5, its pattern differed between two localities studied. This fluctuation in biting density may be attributable, in part, to the rainfalls influencing both the seasonal availability and the stability of preimaginal sites for this species.

In regard to the distribution of bites on human body parts, *S. horacioi* showed the definite pattern almost similar to that of *S. metallicum* s. str. in preferring the lower parts of the legs.

In the present study, third-stage larva of *O. volvulus* was encountered in *S. ochraceum* only. This may suggest that *S. ochraceum* is the most significant vector and the other two species are of less importance in the onchocerciasis transmission in and near the area studied. However, this can not rule out the possiblity that *S. horacioi* may be involved in the transmission. It should be noted that females of this species dissected in this study were not sufficient in number to assess the natural infections on monthly basis, and all the samples were derived only from the morning segment of its biting population.

The third-stage larvae of the unknown filaria identical to that reported by Garms (1975) were already recorded in *S. horacioi* by Ito *et al.* (1980) who found them more frequently in this species (5/17) than in *S. metallicum* s. str. (1/100). This filaria was also seen in the present study, wherein infection rate was 0.13% in *S. horacioi* and 0.24% in *S. metallicum* s. str. These results indicate that, like *S. metallicum* s. str., *S. horacioi* feeds wild animal(s) and serves as a transmitter at least to one unknown filaria in nature. However, its biting preference for animals other than humans remains unknown, although a single female was caught on cow (Okazawa and Onishi, 1980).

Overall, it should be mentioned that in future studies on the onchocerciasis-transmission potential of the Guatemalan anthropophilic blackfly species, *S. horacioi* should not be overlooked due to its capacity of supporting the development of *O. volvulus* larvae to the infective stage, relative abundance of biting females, and probable coincidence of its geographical distribution with the endemic regions of onchocerciasis.

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References

1) Bain, O. (1969): Morphologie des stades larvaires d'*Onchocerca volvulus* chez *Simulium damnosum* et redescription de la microfilaire, Ann. Parasitol., 44, 69–81

2) Dalmat, H. T. (1955): The black flies (Diptera, Simuliidae) of Guatemala and their role as vectors of onchocerciasis, Smithson. Misc. Coll., No. 125, 425 pp

3) Duke, B. O. L. (1968): Studies on factors influencing the transmission of onchocerciasis. V: The stages of *Onchocerca volvulus* in wild 'forest' *Simulium damnosum*, the fate of the parasites in the fly, and the age-distribution of the biting population, Ann. Trop. Med. Parasitol., 62, 107–116

4) Garms, R. (1975): Observations on filarial infections and parous rates of anthropophilic blackflies in Guatemala, with reference to the transmission of *Onchocerca volvulus*, Tropenmed. Parasitol., 26, 169–182

5) Ito, S., Tanaka, I. and Ochoa, J. O. (1980): Comparative studies on the affinities of two black flies, *Simulium metallicum* and *S. ochraceum* for the larvae of *Onchocerca volvulus* in Guatemala, Jpn. J. Sanit. Zool., 31, 261–270

6) Okazawa, T. and Onishi, O. (1980): Description of a new species of *Simulium* (*Simulium*) Latreille and redescription of *Simulium* (*Simulium*) *metallicum* Bellardi from Guatemala (Diptera: Simuliidae), Jpn. J. Sanit. Zool., 31, 167–179

7) Onishi, O., Okazawa, T. and Ochoa, J. O. (1977): GJCRCPO-MENSAP Report, No. 2, pp. 1–8, Guatemala-Japan Cooperation Research and Control Program of Onchocerciasis, Malaria Eradication National Service and Adjoined Program, Guatemala

8) Takaoka, H. (1980): Pathogens of blackfly larvae in Guatemala and their influence on natural populations of three species of onchocerciasis vectors, Am. J. Trop. Med. Hyg., 29, 467–472

9) Takaoka, H. (1981): Seasonal occurrence of *Simulium ochraceum*, the principal vector of *Onchocerca volvulus* in the southeastern endemic area of Guatemala, Am. J. Trop. Med. Hyg., 30, 1121–1132

10) Takaoka, H. and Takahasi, H. (1982): A new species of black fly (Diptera: Simuliidae) from upland areas of Guatemala, J. Med. Entomol., 19 (1), 63–67

11) Vargas, L. and Díaz Nájera, A. (1957): Simúlidos mexicanos, Rev. Inst. Salubr. Enferm. Trop. Mexico City, 17, 143–399

グァテマラにおける *Onchocerca volvulus* の媒介可能ブユ種, *Simulium horacioi* の幼虫および成虫個体群の生態調査

高　岡　宏　行

中米グァテマラにおいて *Onchocerca volvulus* の媒介可能種と報告された *Simulium horacioi* (*metallicum* グループ) の幼虫と成虫の生態を明らかにするため, オンコセルカ症浸淫地の3地区で周年調査を行った。*Simulium horacioi* の幼虫は, 年間を通じて発生がみられ, 特に年中恒常的に流れる小さな水系に多かった。また, 雨期 (5—10月) にのみ出現する季節的水系にも発生することが観察された。人おとり法により捕集されたブユ雌個体群のなかで *S. horacioi* は, 地区により異なるものの, 0.06—29.0%を占め, *S. ochraceum* や *S. metallicum* s. str. とともに主要人吸血性ブユであることが判明した。この種の吸血活動には, 9月ないし10月に極期となる明らかな季節変動が認められた。*Onchocerca volvulus* 幼虫の自然感染は *S. ochraceum* (第 I, III 期とも) および *S. metallicum* s. str. (第 I 期のみ) にみられたが, *S. horacioi* では743個体を解剖した限りではいずれの発育期の幼虫も見出せなかった。しかしながら, 不明フィラリアの第 III 期幼虫, *mermithid* の幼虫およびカビに感染されていた雌個体が少数みられた。今回の調査で明らかにされた知見を考慮して, 今後のグァテマラにおけるオンコセルカ症の伝搬調査では, *S. ochraceum* や *S. metallicum* s. str. と同様, *S. horacioi* も検討の対象とする必要があろう。

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