Onchocerca ochengi: epidemiological evidence of cross-protection against *Onchocerca volvulus* in man

G. WAHL*[1], P. ENYONG[2], A. NGOSSO[3], J. M. SCHIBEL[4], R. MOYOU[2], H. TUBBESING[5], D. EKALE[1] *and* A. RENZ[1]

[1] *Institut für Tropenmedizin, Wilhelmstrasse 27, D-72074 Tübingen, Germany*
[2] *Medical Research Station, Kumba, Cameroon*
[3] *Hôpital Central, Yaoundé, Cameroon*
[4] *Lehrstuhl für Populationsgenetik, Universität Tübingen, Germany*
[5] *Hôpital Protestant, Ngaoundéré, Cameroon*

(*Received 21 October 1996; revised 12 February 1997; accepted 12 February 1997*)

SUMMARY

In North Cameroon, the vector of *Onchocerca volvulus* (causative agent of human onchocerciasis) also transmits 2 filariae of animals: *O. ochengi* from cattle and *O. ramachandrini* from wart hogs. In order to assess the qualitative and quantitative roles of these 'animal filariae' in the epidemiology of *O. volvulus*, the transmission of the 3 parasites was measured in 2 villages and related to the endemicity of human onchocerciasis. In Galim, a cattle-farming Guinea savanna village where wild animals are rare, the overwhelming majority of all filarial infections found in the *Simulium damnosum* s.l. vectors throughout the year were *O. ochengi* (89%). The remaining infections were mainly *O. volvulus* (10·5%), and a few *O. ramachandrini* (0·5%). In Karna, a crop-farming Sudan savanna village where cattle are rare, but wild animals common, flies were also more frequently infected with animal filariae than with the human parasite. In the dry season, when nomadic cattle are present, 54% of all infections were *O. ochengi*, 36% *O. volvulus* and 10% *O. ramachindrini*. In the rainy season, when the cattle move away, flies were mainly infected with *O. ramachandrini* (52% of all infections) and secondly with *O. volvulus* (48%). In Karna, the relationship between the Annual Transmission Potential (ATP) of *O. volvulus* and its prevalence in the human population conformed to other onchocerciasis foci, in that a moderate ATP led to hyperendemic onchocerciasis. In Galim, however, a 7-fold higher *O. volvulus*-ATP (caused by a very high biting rate of the flies) contrasted with a strikingly low endemicity of onchocerciasis. Since, at the same time, in Galim the transmission of *O. ochengi* (measured on man) was very high (15000 L3/fly collector/year), we hypothesize that the reduced endemicity of onchocerciasis in Galim is due to 'natural heterologous vaccination' by the large annual number of *O. ochengi*-L3, inoculated into man by anthropo-boophilic *S. damnosum* s.l. The importance of micro-epidemiology for the understanding of the interlinkage of human and animal onchocerciasis is discussed.

Key words: *Onchocerca volvulus*, *Onchocerca ochengi*, epidemiology, cattle.

INTRODUCTION

In North Cameroon evidence had accumulated over the years that a considerable proportion of the infective filarial larvae found in the *Simulium damnosum* vectors of human onchocerciasis (river-blindness) did not belong to the human parasite, *Onchocerca volvulus*. Since these larvae did not correspond to any of the known filarial parasites of man, they were thought to belong to filariae of animals. Duke (1967) described 3 types of infective larvae (Type D, E and F) which were morphologically clearly different from *O. volvulus*, of which, however, only Type D occurred frequently. Later, Renz *et al.* (1989) discovered that among those filarial larvae which had since been considered morphologically indistinguishable from *O. volvulus* there commonly occurred a second type of 'not *O. volvulus*' larva, which closely resembled *O. volvulus*, but which was slightly longer and more slender; they termed it Type G. The present field study was designed to identify the adult worms of Type D and Type G larvae, to assess their qualitative and quantitative role in the transmission of human onchocerciasis and to search for other animal filariae potentially occurring in *S. damnosum* s.l.

In preceeding papers we reported the identification of Type G as *Onchocerca ochengi* from cattle (Wahl *et al.* 1991; Wahl & Schibel, 1998) and Type D as *Onchocerca ramachandrini* from the wart hog (Wahl, 1996*a*) and showed that it is unlikely that other unknown larvae resembling *O. volvulus* occur in significant numbers in North Cameroon (Wahl, 1996*b*; Wahl & Schibel, 1998). In the present article we studied the interlinkage of the epidemiology of *O. volvulus*, *O. ochengi* and *O. ramachandrini* in North Cameroon by measuring their Annual Transmission Potentials in a crop-farming Sudan savanna village

* Corresponding author: Unter den Birken 21, 56154 Boppard, Germany. Tel: +49 6742 3949.

https://doi.org/10.1017/S003118209700228X Published online by Cambridge University Press

and in a cattle-farming Guinea savanna village and relating them to the endemicity of the parasites in their animal and human hosts in each study area.

MATERIALS AND METHODS

Study sites

Two easily accessible villages near perennial *S. damnosum* s.l. breeding sites, which were at least 5 km from any other village, were chosen in the vicinity of our field station in Ngaoundéré (7° 13′ N, 13° 34′ E): (i) *Galim* (7° 12′ N, 13° 35′ E; 1100 m altitude; total autochthonous population: 120), 10 km south of Ngaoundéré, near a large cattle farm (9000 cattle of the Gudali race), and (ii) *Karna* (7° 46′ N, 13° 32′ E; 400 m altitude; 500 authochtones), about 60 km north of Ngaoundéré in the Sudan savanna (Fig. 1). In Karna there are no local cattle, but during the dry months from January to April some nomad families (Mbororo tribe) bring their Fulani cattle from the north (approximately 500 every year) in search of better grazing grounds. Game animals are common in the sparsely populated surroundings of Karna but rare near Galim, where they are driven back by intensive cattle-farming. Galim is situated at 0·2–3·2 km from the Vina du Sud, a big fast-flowing river, the water debit of which decreases only slightly during the dry season. Karna is situated at 1·0–2·4 km from the upper course of the Benoué, which is a strong and fast-flowing river in the rainy season, when it drains the edge of the Adamawa highlands, but is small and slow-flowing in the dry season. In both sites the rainy season is from May to October, the dry season from November to April.

Entomology

Identification of the S. damnosum *s.l. populations.* The fly populations at the 2 study sites were identified by cytotaxonomy of the larvae in the nearby breeding sites (Vajime & Dunbar, 1975) and by the colour (first 3 segments) and compression (segments 4+5) of the antennae of adult flies (Garms, 1978; Renz, 1988). Since larvae of *S. yahense* and *S. sirbanum* were either absent or very rare, respectively (Table 1), all flies with pale and compressed antennae were considered '*S. damnosum* s.str.', while those with dark and uncompressed antennae were '*S. squamosum*'.

Biting rates

All entomological parameters were calculated from flies caught on the riverbanks nearest to the villages. In Galim catching on cattle was done about 1 km downstream from the site where flies were caught on humans. The biting rate of *S. damnosum* s.l. on man and cattle was assessed by catching all blood-searching flies coming to land on a human- or a cattle-bait standing near the river from 7.00 h to 18.00 h. The fly-catching was carried out by teams of 2 paid volunteers (one working from 7.00 h to 12.30 h, the other from 12.30 h to 18.00 h), using pooters ('sucking tubes') and wearing long-sleeved shirts, trousers, socks and shoes when catching on cattle and exposing one calf when catching on themselves. Fly-catching on 'human bait' was carried out at regular intervals over 2 years in both villages; on cattle bait during 1 year in Galim, but only during 2 days in Karna (the nomadic cattle present in Karna are too wild to be approached, so that a tame ox had to be brought from the Adamawa highlands). The biting rate on cattle was calculated by doubling the catch of 1 fly collector (who only collected flies from 1 side of the cattle).

Parous- and infection rate

At the end of each catching day the flies were brought live to our laboratory in Ngaoundéré (flies from Galim) or to a nearby mission (flies from Karna) and stored in a deep freezer (−20 °C) until dissection. The daily catches were either dissected in total, or, if very many flies were caught, in random subsamples. Head, thorax and abdomen of each fly were dissected separately in demineralized water, using a Wild stereo-microscope (10–50× magnification). The parous state of the flies was assessed by the form and texture of the ovaries, the contents of the malpighian tubes and the abundance of fat body. *Onchocerca* larvae found in the flies were classed into sausage stage, 2nd-stage larva (L2) and infective larva (L3 = mature 3rd-stage larvae from any body part of the fly). 'Not-*O. volvulus*' L3 were morphologically separated from *O. volvulus* by the criteria described for *O. ramachandrini* by Duke (1967) and Wahl (1996*a*) and for *O. ochengi* by McCall & Trees (1989), McCall, Townson & Trees (1992) and Wahl & Schibel (1998). A subsample of the morphological identifications was verified by specific DNA probes (Table 3).

Annual transmission potentials (*ATP*)

The transmission potentials were calculated separately for each *Onchocerca* species, for flies caught on man and cattle, and for the dry and rainy season. In each season the daily transmission potential (DTP, Table 2, line k) was calculated by multiplying the mean daily biting rate (DBR, Table 2b) with the arithmetic mean of L3 (from all body parts!) per dissected fly (Table 2i divided by Table 2d). The annual transmission potential (ATP, Table 2l) was calculated by multiplying the mean DTP of each

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1 = Ngaoundéré, 2 = Karna, 3 = Galim, 4 = Garoua, 5 = Maroua, 6 = YAOUNDE, 7 = Douala.
I = Sudan Savanna, **II** = Guinea Savanna (Adamawa Highlands)
A = Benoué, B = Vina du Sud

Fig. 1. Map showing study sites in Cameroon.

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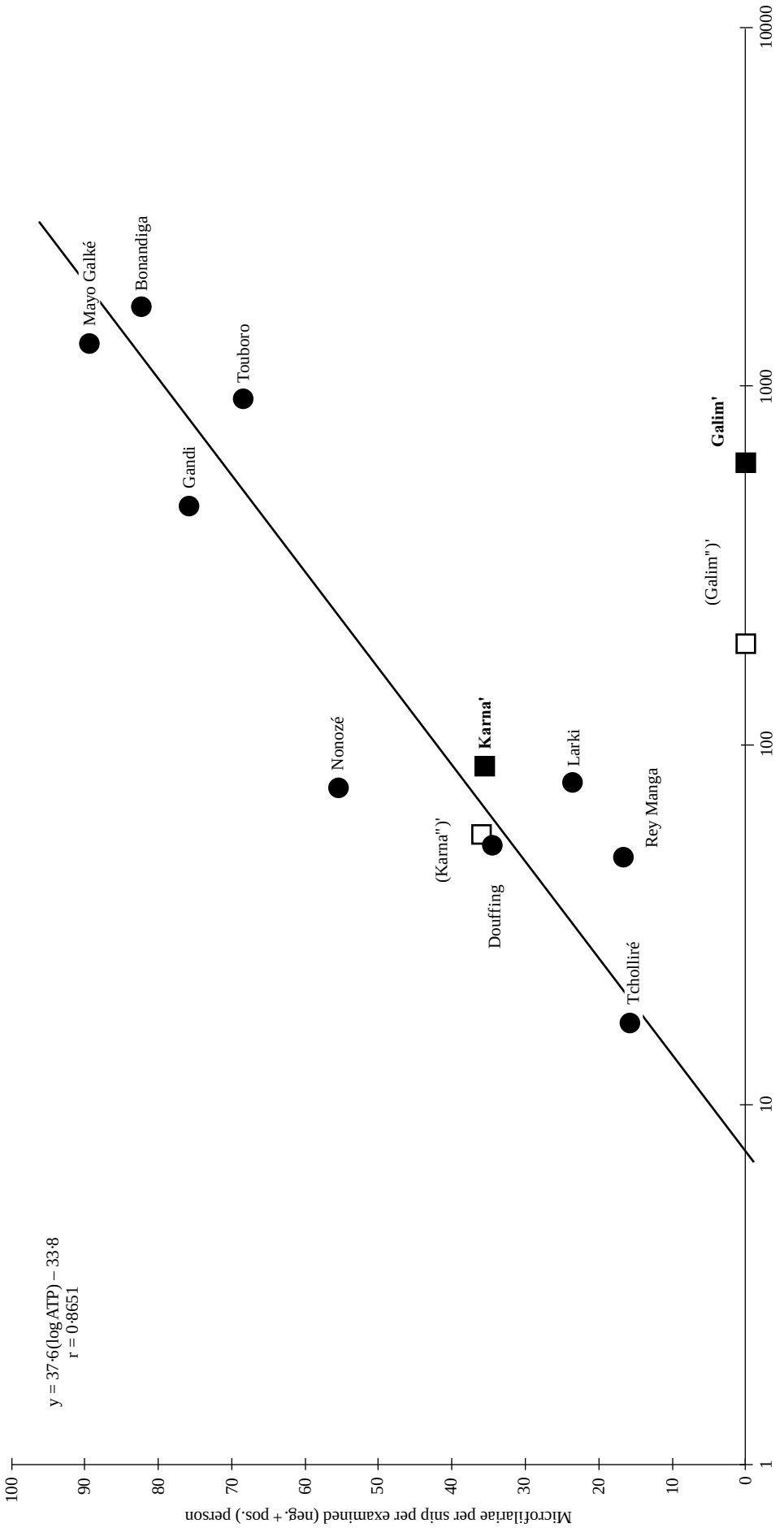

' Actual ATP divided by 2 for reasons of comparability with Renz *et al.* (1987), " calculated from head-L3 only

Fig. 2. *Onchocerca volvulus* annual transmission potential (ATP) and endemicity of onchocerciasis in 11 villages (except Karna and Galim from Renz *et al.* (1987)).

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season with the theoretical number of days in each season (182·5) and adding the two resulting figures. For *O. volvulus* we additionally calculated (as some authors do) the ATP^{head}, which only takes into account the L3 found in the head of the flies (Fig. 2). To furthermore compare these *O. volvulus*-ATPs with those of Renz *et al.* (1987*a*), which were a mean of ATPs assessed at different distances from the river, they were divided by 2 in Fig. 2.

Human onchocerciasis

More than 80 % of the autochthonous population in each village (5 years and older) was examined parasitologically by (a) taking 2 skin snips from the iliac crest with a Walser corneal punch, incubating them in physiological NaCl for 24 h, counting the emerged microfilariae and calculating the proportion of positive subjects and the arithmetic mean number of microfilariae/skin snip/examined subject and (b) by searching for palpable nodules. Of the parasitologically examined subjects 32–42 % were also examined ophthalmologically, by leaving them with the head lowered for 20 min (accumulation of microfilariae in the centre of the anterior chamber) and then examining them with a slit lamp. 'Ocular onchocerciasis' was defined as the presence of 1 or several of the following clinical signs: sclerosing keratitis, chorioretinitis, ocular microfilariae (in the cornea and/or anterior chamber). The examined population roughly corresponded to the standard population sensu Moreau, Prost & Proud'hon (1978) in the Onchocerciasis Control Program (OCP) area (Table 4).

RESULTS

Entomology

The S. damnosum *s.l. populations at the 2 study sites.* In Galim, practically all *S. damnosum* s.l. found in the river, caught on man and caught on cattle throughout the year were *S. squamosum* (Table 1). In Karna, on the other hand, the fly population consisted of 2 species: *S. squamosum* and *S. damnosum* s.str. Whereas among the larvae identified in the river near Karna, *S. squamosum* was the prevailing species in both seasons (about 2 thirds), among adult flies the proportion of *S. damnosum* s.str. was significantly elevated (83 %) in the dry season: χ^2: $P = 7{\cdot}6 \times 10^{-18}$ in comparison to the aquatic stages collected during the same season and $P = 1{\cdot}0 \times 10^{-196}$ in comparison to the adult flies collected in the rainy season (invasion of *S. damnosum* s.str. from areas further north?). In both seasons in Karna and in Galim, the *S. damnosum* s.str. females caught on human or cattle bait had a higher parous rate than those of *S. squamosum* (Table 1, χ^2: $P < 0{\cdot}02$). *S. squamosum* and *S. damnosum* s.str. females were both found regularly infected with *O. volvulus*, *O. ochengi* and *O. ramachandrini*, proving that in North Cameroon both species feed on humans, cattle and wart hogs (Table 1). No obvious difference in host preference was seen between the 2 species, since both were shown to be roughly 3 times more boophilic than anthropophilic in direct experiments (Wahl, Ekale & Schmitz, 1998) and no significant difference was seen in the infection rates with any of the *Onchocerca* species (Table 1, χ^2 $P > 0{\cdot}05$). In view of these similarities, the 2 species are grouped together as '*S. damnosum* s.l.' in all following entomological results.

S. damnosum *s.l. biting rates.* The annual biting rate on man (ABR, measured on the riverbank nearest to the village) was 8 times higher in Galim than in Karna (Table 2, line c). Whereas in both sites, the daily biting rate (DBR) on man was not different in the dry and rainy season, in Galim the DBR on cattle was considerably lower in the rainy than in the dry season (Table 2 b). The biting rate on cattle was between 1·6 (rainy season) and 4·5 times (dry season) higher than that on man (Table 2 b), and thus roughly conforms to the factor of boophily (2·6–3·2) assessed by simultaneous catching on both blood hosts (Wahl *et al.* 1998).

Parous rates. The proportion of parous flies among all flies caught throughout the study was higher in Galim than in Karna (Table 2 e, χ^2: $P < 0{\cdot}0001$). There was some evidence that at both sites it is generally higher in the dry than in the rainy season (man-caught flies in Karna and cattle-caught flies in Galim: $P < 0{\cdot}0001$, but not man-caught flies in Galim: $P > 0{\cdot}05$).

Infection rate of the flies with different Onchocerca *species.* Of about 23 000 parous flies dissected at the 2 study sites, 8 % carried Lx-*Onchocerca* larvae (L1, L2 and/or L3) and 2·5 % carried L3 (Table 2 f and h). When the 2 villages are compared by considering separately seasons of high and seasons of low infection rates, no significant difference in Lx-infection rate was seen (Table 2 f, χ^2: $P > 0{\cdot}02$). At both sites, morphological identification of the L3 revealed 3 *Onchocerca* species in the *S. damnosum* vectors: *O. volvulus*, *O. ochengi* and *O. ramachandrini*. This proves that the fly populations at the 2 study sites feed on humans, cattle and wart hogs. In both sites, the proportion of 'animal' *Onchocerca* spp. infections in the flies was amazingly high: whereas in Galim the overwhelming majority of the filarial infections throughout the year were *O. ochengi* (89 %), in Karna, *O. ochengi* infections were predominant in the dry season (54 %), while *O. ramachandrini* infections prevailed in the rainy season (52 %, Table 2 h). 160, 88 and 15 morpho-

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Table 1. Relative abundance and vector-rôle of *Simulium damnosum* species at the 2 study sites

Village	Karna								Galim							
Season	Dry				Rain†				Dry				Rain			
Fly species*	*squa*			*dam*	*squa*			*dam*	*squa*			*dam*	*squa*			*dam*
Cytotypes	Stand	IIL/18	IIL-18		Stand	IIL/18	IIL-18		Stand	IIL/18	IIL-18		Stand	IIL/18	IIL-18	
No. larvae identified	24	5	0	15	12	5	0	8	34	7	6	0	13	0	2	1
% among all identified larvae		66 %		34 %		65 %†		31 %†		100 %		0 %		94 %		6 %
No. adult flies‡ identified		934		4629		911		822		1987		53		5424		24
% among all identified flies		17 %		83 %		53 %		47 %		97 %		3 %		99 %		1 %
No. flies dissected		934		4629		911		822		852		39		5424		24
% parous		49 %		66 %		27 %		33 %		75 %		95 %		58 %		83 %
No. flies with L3 (*Ov*/*Oo*/*Or*)§		4/7/0		29/46/10		2/0/3		8/0/8		9/74/0		1/1/0		12/53/0		0/1/0
% of parous with L3 (*Ov*/*Oo*/*Or*)		0·9/1·6/0		0·9/1·5/0·3		0·8/0/1·2		3·0/0/3·0		1·4/11·5/0		2·7/2·7/0		0·4/1·7/0		0/5·0/0

* *squa*, *S. squamosum*; *dam*, *S. damnosum* s.st.
† 1 *S. sirbanum* larva (4 % of all identified larvae) identified.
‡ Flies caught on man or on cattle.
§ *Ov*, *O. volvulus*; *Oo*, *O. ochengi*; *Or*, *O. ramachandrini*.

Table 2. *Simulium damnosum* s.l. biting – and parous rates and *Onchocerca* spp. transmission potentials at the 2 study sites

Village	Karna			Galim			
Caught on	Man		Cattle	Man		Cattle	
Season	Dry	Rain	Dry	Dry	Rain	Dry	Rain
a Catching days	145	30	2	21	22	15	15
b DBR	41·6	39·2	392·0*	320·7	315·8	1456·6*	591·8*
c ABR	14750			116161		373833	
d Flies dissected	7874	1733	102	2868	4057	2414	3915
e Flies parous (%)	4649 (59%)	509 (29%)	59 (58%)	2067 (72%)	2842 (70%)	1554 (64%)	1963 (50%)
f Flies with Lx§ (% of par.)	439 (9%)	103 (20%)	12 (20%)	434 (21%)	272 (10%)	383 (25%)	204 (10%)
g Flies with L3 (*Ov*/*Oo*/*Or*)†	44/66/11	10/0/11	2/1/1	18/137/0	15/49/0	9/141/1	8/42/1
h % L3-inf. flies of parous	0·95/1·42/0·24	1·96/0/2·16	3·4/1·7/1·7	0·87/6·63/0	0·53/1·72/0	0·58/9·07/0·06	0·20/1·07/0·05
i Total L3 *Ov*/*Oo*/*Or*	84/137/18	23/0/20	2/14/1	37/591/0	32/226/0	13/652/1	21/145/1
j amL3/infected fly‡	1·91/2·08/1·64	2·30/–/1·82	–	2·06/4·31/–	2·13/4·61/–	1·44/4·62/1·00	2·63/3·45/1·00
k DTP *Ov*/*Oo*/*Or*	0·44/0·73/0·09	0·52/0/0·45	3·9/27·0/1·9	4·22/65·9/0	2·46/17·31/0	7·83/393·1/0·56	1·56/10·94/0·15
l ATP *Ov*/*Oo*/*Or*	175/133/99			1219/15186/0		1714/73733/130	

* By doubling the catch of 1 fly collector.
‡ am, arithmetic mean.
† *Ov*, *O. volvulus*; *Oo*, *O. ochengi*; *Or*, *O. ramachandrini*.
§ Lx, L1, L2 and/or L3.

Table 3. Verification of morphologically identified L3 from Karna and Galim by DNA probes

Village	Karna		Galim			
Season	Rainy		Dry		Rainy	
L3 species*	*Ov*	*Or*	*Ov*	*Oo*	*Ov*	*Oo*
Morphologically identified	20	15	15	30	53	130
Verification by DNA probes:						
p*Ov*12+/p*Oo*5/1− (= *Ov*)	17	0	13	4	43	17
p*Ov*12−/p*Oo*5/1+ (= *Oo*)	1	0	1	23	5	103
p*Ov*12−/p*Oo*5/1−	2†	15‡	1†	3†	5†	10†

* *Ov*, *O. volvulus*; *Oo*, *O. ochengi*; *Or*, *O. ramachandrini*.
† Non-reaction of these dots due to loss of larvae (Wahl & Schibel 1998).
‡ Non-reaction of these dots partly due to loss of larvae (8%), but mainly due to the larvae belonging to an *Onchocerca* species that does not react with p*Ov*12 and p*Oo*5/1.

logically identified L3 of *O. ochengi*, *O. volvulus* and *O. ramachandrini* respectively were sequentially hybridized with an *O. volvulus*-specific DNA probe (pOv12) and a DNA probe (pOo5/1) which reacts with DNA of *O. volvulus* and *O. ochengi*, but not with other *Onchocerca* species (Wahl & Schibel, 1998). This experiment showed that 86, 91 and 100% of the morphological identifications respectively had been correct (Table 3).

Mean numbers of L3/infective fly. For each of the 3 species, the distribution of the numbers of L3/infective fly roughly conformed to the negative binomial distribution (data not shown). There exists no general consensus how to express the mean of such parasite distributions (Fulford, 1994). In this and the preceeding articles, we chose the arithmetic mean in order to compare our results with most other published data. Whereas in Karna, the arithmetic mean of L3/infective fly was roughly the same for all 3 *Onchocerca* species (1·6–2·3), in Galim the mean load of *O. ochengi*-L3 (3·5–4·6) was considerably higher than that of the 2 other species (1·4–2·6 and 1·0 respectively, Table 2j). In Galim, the proportion of *O. ochengi*-infected flies carrying more than 1, 2, 3 and 4 L3 was 69, 55, 42 and 32% respectively, in comparison to 46, 24, 12 and 8% respectively for *O. volvulus* (difference between all proportions: χ^2: $P < 0{\cdot}001$).

Onchocerca *spp. annual transmission potentials on man*. Due to the high biting rate and the large number of *O. ochengi*-L3 in the flies in Galim, the total annual transmission potential (without differentiating the 3 *Onchocerca* species) was very high (16405 L3/man/year), and about 40 times higher than the total ATP on man in Karna (Table 2l). However, in Galim, throughout the year only 10% of the fly infections and 7% of the total ATP were due to *O. volvulus* (Fig. 3). Nevertheless, owing to the high biting rate in Galim, the *O. volvulus*-ATP was 7 times higher than in Karna (1219 and 175 L3/man/year respectively).

Seasonal variations of infection rates and transmission potentials. The Lx-infection rate showed an inverse seasonality at the 2 study sites: whereas in Karna it was high in the rainy (20%) and low in the dry season (9%), in Galim it was high in the dry (23%) and low (10%) in the rainy season (Table 2f, difference of infection rates between dry and rainy season in both sites: χ^2: $P < 0{\cdot}0001$). In Galim, the most obvious seasonal variation was the strikingly high L3-infection rate in the dry season (Table 2h). This was primarily due to an increased infection rate with *O. ochengi*, and secondly due to the fact that almost all infections in this season were L3, while earlier stages were rare (Table 2f and h, difference in the ratio of L3- and Lx-infections between dry and rainy season: χ^2: $P < 0{\cdot}0001$). Since, in this season, the biting rate on cattle was also increased, the daily transmission potential (DTP) of *O. ochengi* on cattle was strikingly high in the dry season (393 L3/animal/day), and 36 times higher than in the rainy season (Table 2k). In Karna, the most obvious seasonal changes were (a) the interruption of *O. ochengi* transmission in the rainy season, when the nomadic cattle move away, (b) the low infection rate in the dry season, when the flies apparently frequently feed on animal species which are not infective to them (see Discussion section) and (c) the 9-fold increased transmission of *O. ramchandrini* in the rainy season (Table 2f, h and k).

Differences between flies caught on man and on cattle. Only in 1 case was there evidence that fly populations attacking cattle might differ from those attacking man: in the dry season in Galim, flies caught on cattle had a lower parous rate, a higher Lx-infection rate (of parous), a higher *O. ochengi*-L3-infection

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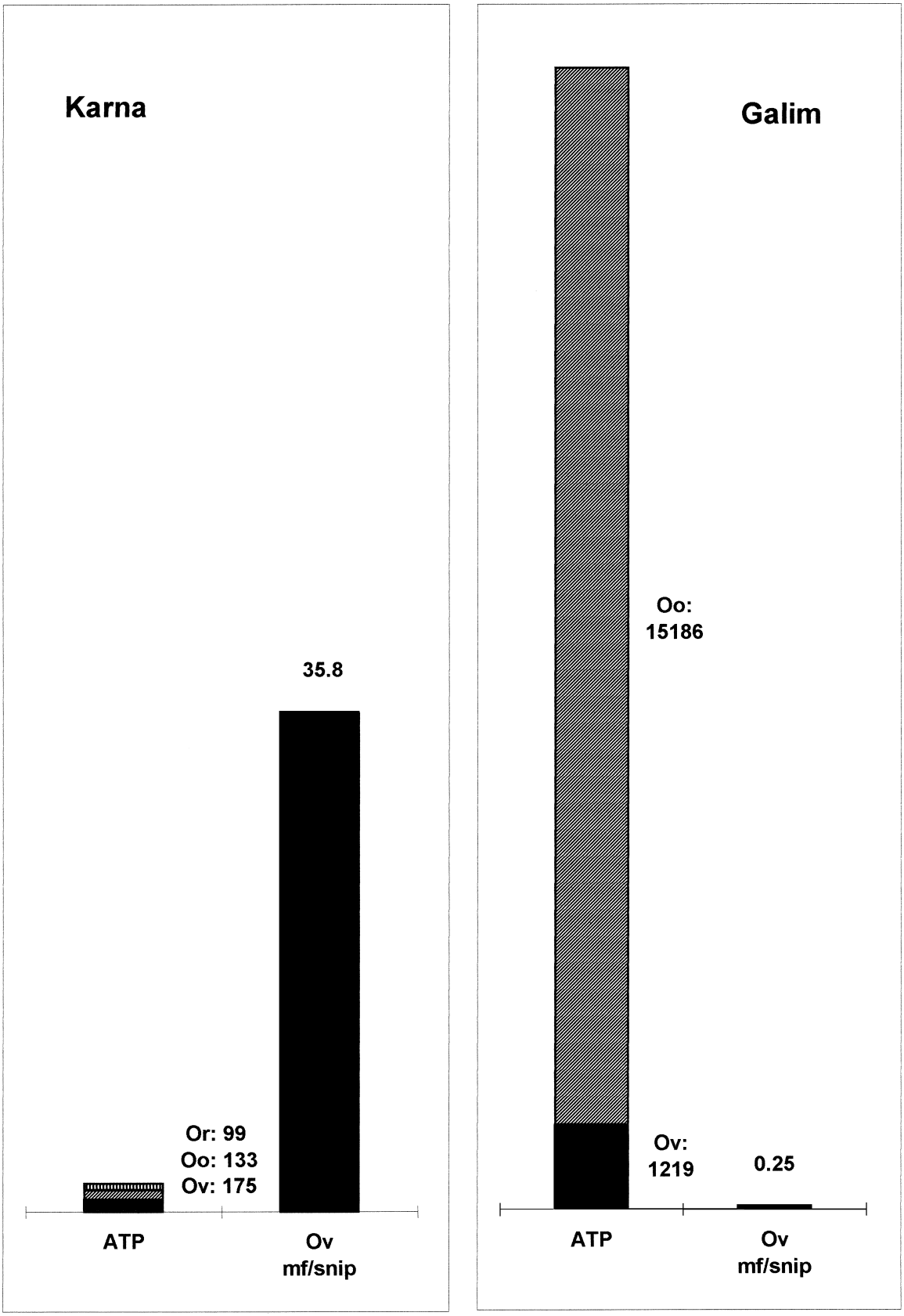

Ov = *O. volvulus*, Oo = *O. ochengi*; Or = *O. ramachandrini*

Fig. 3. *Onchocerca* spp. transmission potentials and endemicity of human onchocerciasis in the 2 study sites.

rate (Table 2e, f and h, χ^2 for all differences: $P < 0{\cdot}05$) and a smaller proportion of *S. damnosum* s.str. (Wahl *et al*. 1998). However, one must keep in mind that in Galim fly-catching on cattle was done at some distance from where flies were collected on human bait.

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Table 4. Human onchocerciasis in the 2 study villages

(mf, Microfilariae; nod, nodules; oconch, ocular onchocerciasis; m/f ex, males/females examined; % snip+, % of examined with 1 or 2 positive skin snips; mf/snip, arithmetic mean number of mf/1 positive snip; % nod+, % of examined with palpable nodules; nod/pat, arithmetic mean number of nod/patient.)

		Parasitology					Ophthalmology	
Village	Age group	m/f ex	% snip+	mf/snip	% nod+	nod/pat	m/f ex	% oconch
Karna	5–19	112/125	49	17·8	10	1·3	28/13	15
	(0–19)*	(110/97)*					(46/41)*	
	20–39	27/35	81	53·9	29	1·8	26/26	37
		(50/63)*					(21/26)*	
	40–59	31/25	82	46·5	46	2·5	27/18	69
		(33/39)*					(14/16)*	
	>59	33/22	91	82·0	51	2·6	22/13	83
		(8/7)*					(3/3)*	
	Total	203/207	64	56·0	23	2·1	103/70	49
			65*					33*
Galim	5–19	44/37	10	1·7	4	1·0	10/5	0
	(0–19)*	(27/24)*					(9/8)*	
	20–39	1/5	33	1·8	0		1/5	0
		(12/15)*					(4/5)*	
	40–59	6/1	14	5·0	17	1·0	5/1	17
		(8/10)*					(3/3)*	
	>59	4/2	33	1·5	0		3/2	0
		(2/2)*					(1/1)*	
	Total	55/45	13	1·9	4	1·0	19/13	3
			18*					3*

* Corrected for OCP standard population (Moreau *et al*. 1978).

Human onchocerciasis

In Karna, 410 autochthonous persons were examined parasitologically (2 skin snips and palpation of nodules) and 173 were examined ophtalmologically with a slit lamp. In Galim the respective numbers were 100 and 32 (Table 4). There was a striking difference in prevalence and severity of onchoncerciasis between the 2 villages: whereas in Karna 64 % of the subjects had microfilariae (56 mf/positive snip), 23 % had palpable nodules (2·1/patient) and 49 % had ocular onchocerciasis, in Galim only 13 % were microfilariae-positive (2 mf/positive snip), 4 % had palpable nodules (1·0/patient) and 3 % had ocular onchocerciasis (χ^2: $P = 5{\cdot}4 \times 10^{-20}$, $P = 1{\cdot}7 \times 10^{-5}$ and $P = 1{\cdot}3 \times 10^{-6}$ repectively). In Karna, almost half of the 5 to 19-year-olds already had skin microfilariae, whereas in Galim only 10 % of this age group were positive ($P = 4{\cdot}9 \times 10^{-10}$).

Animal onchocercosis

In Galim 123 cattle $\geqslant$ 3 years were cast with ropes and skin-snipped on the ventral midline between the umbilicus and the udder/scrotum. A total of 84 % was found infected with *O. ochengi*. In Karna, no such large-scale survey could be done, neither for cattle (the nomadic Fulani cattle were too wild to be cast with ropes), nor for wart hogs (rareness of hunted animals). However, 2 adult cattle were slaughtered and 4 adult wart hogs hunted during our study; all had microfilariae of *O. ochengi* and *O. ramachandrini* respectively.

DISCUSSION

Zoophily of the S. damnosum *s.l. populations in North Cameroon*

The annual proportion of flies infected with 'not *O. volvulus* filariae', identified by morphology and verified by DNA probes, was amazingly high in the 2 study sites (57–90 % of all infections). This shows that the flies in the study area predominantly feed on animals. High infection rates with animal filariae were also found in other study sites in North Cameroon (Duke, 1967; Renz, 1987; Renz *et al*. 1989, Wahl, unpublished observations). Provocatively, one could say that in North Cameroon, *S. damnosum* s.l. is principally a vector of animal onchocercosis and in some areas (e.g. Galim) only occasionally transmits the human parasite, *O. volvulus*. *O. ochengi* is the prevailing filarial species in *S. damnosum* s.l. both in the Guinea- and Sudan savanna. This is primarily due to the abundance of cattle, mainly in the Adamawa highlands (about 5 times more cattle than humans), but also due to the flies' preference of cattle as a blood-host (Wahl *et al*. 1998). High infection rates of *S. damnosum* s.l. with

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not-*O. volvulus* filariae have, so far, been published only from few areas in Africa (Voelker & Garms, 1972, Garms, 1987), but are now beginning to be discovered in more and more regions (Toe, Merriweather & Unnasch, 1994).

Impact of zoophily of S. damnosum *s.l. on the epidemiology of* O. volvulus

The high degree of zoophily of *S. damnosum* s.l. in North Cameroon considerably lowers the vectorial capacity *sensu* Garrett-Jones (1964) of the vector populations for *O. volvulus* (Renz, Bathelmess & Eisenbeiß, 1987*b*, Renz, Enyong & Wahl, 1994). This effect has been called 'zooprophylaxis'. In this study, a striking additional effect of zoophily became evident when the mean microfilarial load in our 2 study villages was related to the *O. volvulus*-ATP: even though the *O. volvulus*-ATP (on man) was 7 times higher in Galim than in Karna, the microfilarial load was 143 (!) times lower in Galim than in Karna.

The situation in Karna, where a moderate *O.volvulus*-ATP was associated with hyperendemic onchocerciasis and frequent ocular implications, corresponds well to other Sudan savanna villages in Cameroon examined in an earlier study. In this group of villages the mean microfilarial load in the population was linearly related to the logarithm of the ATP, fitting a regression line of the formula: $y = 37{\cdot}6\ (\log \mathrm{ATP}) - 33{\cdot}8$ ($r = 0{\cdot}87$).

The situation in Galim, on the other hand, did not fit this regression at all, in that the microfilarial load in the population was far lower than could be expected from the high *O. volvulus*-ATP. Even if one calculates the ATP (as some authors do) only from L3 found in the *head* of the flies, the endemicity in Galim is still far lower than could be expected from this '$\mathrm{ATP}^{\mathrm{head}}$'. The relatively low $\mathrm{ATP}^{\mathrm{head}}$ in Galim is due to an unexplained, significantly lower proportion of head-L3 (32 % of all L3) than in Karna (56 %, χ^2: $P < 0{\cdot}002$). Several other villages in the cattle-raising areas of the Adamawa highlands in Cameroon show a similar epidemiological situation as in Galim, i.e. high *S. damnosum* s.l. biting rates on man (and cattle), high infection rate of the flies with *O. ochengi* and low endemicity of human onchocerciasis (Wahl & Renz, unpublished observations).

The unproportionally low endemicity of human onchocerciasis in Galim (and other cattle-farming villages in the Adamawa), in conjunction with a very high *O. ochengi*-ATP in these places, leads us to hypothesize that in these areas anthropo-boophilic *S. damnosum* s.l. permanently inoculate *O. ochengi*-L3 into man, which then cause a cross-reactive immunization against *O. volvulus*. This hypothesis of 'natural heterologous vaccination' by *O. ochengi* was strengthened by immunological studies, which showed a high degree of homology between *O. volvulus* and *O. ochengi* in protein profile and serological recognition, and demonstrated significant differences in the serological reactivity between patients from Galim and Karna (Hoch *et al.* 1992, 1993).

The low prevalence of palpable nodules in the population at Galim indicates that the putative cross-reactive immunity against *O. volvulus* is *protective* (i.e. directed against pre-adult stages of the parasite) and not merely anti-microfilarial.

For the hypothesis of cross-protection by *O. ochengi* to be valid, *O. ochengi*-L3 must actually leave the vectors when these bite the 'inappropriate' host (i.e. man). Our data from the dry season at Galim indicate that this is the case: of 188 parous *S. damnosum* s.l. which had accidentally taken a partial bloodmeal on the fly collectors, only 5 (2·7 %) carried *O. ochengi*-L3, as compared to 132 (7 %) of 1879 parous flies which didn't have any traces of blood in the gut (χ^2: $P < 0{\cdot}0002$). From the total number of L3 found in these 2 groups of flies (29 and 562 L3 respectively), it was calculated that the flies shed 48 % of their *O. ochengi*-L3 load during their (partial) bloodmeal on man.

In Karna, where the *O. volvulus* transmission roughly equalled the transmission of animal filariae (*O. ochengi* and *O. ramachandrini*), the relation between *O. volvulus*-ATP and onchocerciasis endemicity was not significantly different from West African savanna sites where no transmission of animal filariae was reported (e.g. Thylefors, Philippon & Prost, 1978). Assuming cross-protection exists, this would indicate that it is only adequately provoked when the ATP of animal *Onchocerca* species is considerably higher than that of *O. volvulus*. Therefore a possible immunizing effect of *O. ramachandrini* could not be assessed in this study: in both study sites the ATP of this filaria was lower than the one of *O. volvulus*. It seems improbable that very high transmission potentials of *O. ramachandrini* exist in any onchocerciasis area, because this would require extremely high population densities of wart hogs near human habitations.

Epidemiology of O. ochengi

Despite high *S. damnosum* s.l. biting rates along the big rivers in the Adamawa highlands, the overall prevalence of *O. ochengi* in cattle is lower than in the nomadic cattle from the Sudan savanna (Wahl *et al.* 1994). This was explained by the more stationary maintenance of cattle in the Adamawa, many of which are therefore never close to *S. damnosum* s.l. breeding sites, whereas the nomadic cattle in the Sudan savanna will always seek the big rivers in the dry season, when smaller tributaries stop flowing. However, when cattle farms in the Adamawa are near *S. damnosum* s.l. breeding sites, the annual

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transmission of *O. ochengi* is very high, e.g. 74000 L3/animal/year in Galim. In view of this high transmission pressure it is astonishing that 16% of the examined adult cattle in Galim were skin snip-negative. In an earlier study we have shown that this is probably due to acquired immunity against skin microfilariae in older animals (Trees *et al*. 1992).

The most striking feature in the epidemiology of *O. ochengi* at Galim was the seasonal variation of transmission. In the dry season, there was an increase in the flies' biting rate on cattle (2·5-fold), the parous rate (1·3-fold), the L3-infection-rate (8·5-fold) and the mean L3-number per infected fly (1·3-fold), resulting in a 36-fold increased daily transmission potential of *O. ochengi* on cattle as compared to the rainy season. This is probably due to the flies taking many more bloodmeals on cattle in the dry season, because in this season the cattle are brought daily to the river. Alternatively, it could be due to the cattle having a higher microfilarial density in the dry season and thus being more 'infective' for the flies (no longuitudinal survey of individual microfilarial densities has been done yet).

Epidemiology of O. ramachandrini

In view of the high prevalence of *O. ramachandrini* in a small, random subsample of wart hogs, this animal species seems to be the main host of *O. ramachandrini*. As yet we do not know whether other natural vectors besides *S. damnosum* s.l. exist, but in view of the vector specifity of all other known *Onchocerca* species, this seems improbable. A common feature of the epidemiology of *O. ramachandrini* in the Sudan savanna of North Cameroon seems to be a strong seasonal variation of the infection rate of fly populations near human habitations, which is low in the dry season, but high in the rainy season (Renz *et al*. 1989). This is probably mainly due to the presence of nomadic cattle during the dry season in many riverine villages in the Sudan savanna. The cattle scare the wart hogs away and are possibly also more attractive to the flies. Moreover, in the rainy season the flies disperse farther away from the human habitations and thus more frequently encounter wart hogs.

The role of other blood hosts in the epidemiology of onchocerciasis

In the savanna areas, about 40% of *S. damnosum* s.l. females feeding on humans infected with *O. volvulus* (Philippon, 1977) and 17% of flies feeding on cattle with *O. ochengi* (Wahl *et al*. 1998) become infected. It is therefore surprising that the overall infection rate of the flies in Karna and Galim was sometimes remarkably low: only 348 of 4708 parous flies dissected in the dry season in Karna and 380 of 4805 parous flies dissected in the rainy season in Galim carried sausage stage (L1) or 2nd-stage (L2) larvae. This means that only 7·4% and 7·9% of the flies respectively had acquired infections during their last bloodmeal (L3 are not considered, since they stem from earlier bloodmeals). In Galim, the low infection rate in the rainy season can be explained by the cattle staying away from the river (see above) and the flies therefore feeding predominantly on the human population, the majority of which is not infected in Galim. In Karna, however, where most of the humans carry *O. volvulus* microfilariae, the low infection rate in the dry season is most probably due to the flies feeding on other, non-infective animal species. In this season, 36% of the L3 infections in the flies in Karna were *O. volvulus*, 54% *O. ochengi* and 10% *O. ramachandrini*. Since L1 and L2 cannot be identified to the species by morphology, their infection rate can only be calculated indirectly. Thus, the putative species-specific infection rate with L1 or L2 of *O. volvulus* in the dry season in Karna was 7·4 (total L1/L2 infection rate) × 0·36 (proportion of *O. volvulus* among L3 infections) = 2·7%. If all flies had taken their last bloodmeal on the village population, 100% × 0·64 (= prevalence of *O. volvulus*) × 0·4 (chance of *S. damnosum* s.l. becoming infected) = 25·6% of the parous flies should have carried L1 or L2 of *O. volvulus*. From the observed infection rate (2·7%) and the expected infection rate (25·6%) it can be calculated that apparently only 11% of the bloodmeals were taken on man. In the same way we calculated that 28% of the bloodmeals were taken on cattle and 5% were taken on wart hogs. This would mean that in the dry season in Karna as many as 100 − 11 − 28 − 5 = 56% of all bloodmeals were taken on other, non-infective blood hosts. Such non-infective blood hosts could be other game animals (e.g. antelopes), which are abundant in the Sudan savanna of North-Cameroon. As yet we do not know why these blood hosts apparently play a less important role in the rainy season.

Conclusions

Our study provides epidemiological evidence that live infective larvae of *O. ochengi*, inoculated into man in high numbers by anthropo-boophilic *S. damnosum* s.l., can cause partial protection from infection with *O. volvulus*. However, since detailed data were gathered only in 1 village sofar (Galim) and since, in this village, the *O. volvulus*-ATP was different from that in the 'control village' (Karna), our hypothesis needs consolidation. Full understanding of the interlinkage of human and animal onchocercosis essentially depends on the exact knowledge, at each study site, of the size, the infection rates and the diurnal and seasonal movements of all implicated populations, i.e. fly popu-

https://doi.org/10.1017/S003118209700228X Published online by Cambridge University Press

lations, humans, cattle, wart hogs and other wild animals (microepidemiology). In this study these parameters could be assessed only in a very preliminary and approximate way, and it seems justified that more detailed micro-epidemiological studies in selected sites be carried out.

We would like to thank Drs D. Mbah, M. D. Achu-Kwi, V. Tania (Centre de Recherches Zootechniques et Vétérinaires, Ngaoundéré, Cameroon) and Dr O. Dawa (Ministère d'Elevage, des Pêches et des Industries Animales, Ngaoundéré, Cameroon) for their kind support and helpful advice. G. Wahl received scholarships from the Graduiertenförderung des Landes Baden-Württemberg, the Deutsche Akademische Austauschdienst (DAAD) and the Studienstiftung des Deutschen Volkes. This study was supported by the Commission of European Communities (TS2-0184 and TS3 CT92-006).

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