DEVELOPMENT BY INJECTION IN *SIMULIUM DAMNOSUM* S.L. OF TWO *ONCHOCERCA* SPECIES FROM THE WART HOG TO INFECTIVE LARVAE RESEMBLING TYPE D LARVAE (DUKE, 1967)

WAHL G.* & BAIN O.**

Summary :

Four wart hogs (*Phacochoerus aethiopicus*) examined in the Sudan savanna of North-Cameroon were all found infected with two types of skin microfilariæ. One was *O. ramachandri* Bain, Wahl and Renz, 1993, the adult worms of which live in the subcutaneous tissues of the feet. The other, smaller type belongs to a new *Onchocerca* species, the adult worms of which were not yet found. *O. ramachandri*-microfilariae were evenly distributed across the whole body surface, those of *Onchocerca* sp. were concentrated on the back. The two species of microfilariæ were isolated from an infected hide, separated under the dissecting microscope and injected into the thorax of pupae-hatched *S. squamosum* and *S. damnosum* s.str. females. Both filariæ developed in both flies at high rates (33 - 47% of injected microfilariae) and without pathological forms to infective larvae (L3). Both L3-species had a caudal tip, were long, slender and very motile and had a conspicuous glandular oesophagus. L3 from *O. ramachandri*-microfilariae had a long glandular oesophagus (55 % of total L3 length), a round head and measured an average of 955 µm long and 19.2 µm wide. L3 from the other microfilaria-species were shorter (845 µm, $P < 0.001$) and thinner (16.7 µm, $P < 0.001$) and had a shorter glandular oesophagus (36%, $P < 0.001$), a shorter tail ($P < 0.01$) and a conical head.

Both L3-species, by their caudal tip, their long and slender silhouette, their great motility and their conspicuous glandular oesophagus resemble non-*O. volvulus* filarial L3 known, since many years, to occur in "wild" *S. damnosum* s.l. in Cameroon (Type D larvae, Duke, 1967) and in Liberia (Agamofilaria Type VI, Voelker and Garms, 1972). During our study, 13 such larvae were found in 12 wild *S. damnosum* s.l. from two geographically different areas of North-Cameroon and all identified as *O. ramachandri*. The excellent development of the two *Onchocerca* species from the wart hog in *S. damnosum* s.l. after artificial infection, and the identification of all recently examined wild Type D larvae as *O. ramachandri* suggest that *S. damnosum* s.l. is a natural vector of *O. ramachandri* and that most (if not all) of the Type D larvae in onchocerciasis vectors in North-Cameroon originate from wart hogs.

KEY WORDS : onchocerciasis. *O. ramachandri*. *Onchocerca* sp. *Simulium damnosum* s.l. Type D larvae. Agamofilaria Type VI. suids. Cameroon. intra-thoracical injection.

Résumé : OBTENTION DE LARVES INFECTANTES SEMBLABLES AUX LARVES DE TYPE D PAR INJECTION CHEZ *SIMULIUM DAMNOSUM* S. L. DES MICROFILAIRES DES DEUX ESPÈCES D'*ONCHOCERQUES* DÉCOUVERTES CHEZ LES PHACOCÈRES

Quatre Phacochères (*Phacochoerus aethiopicus*) de la savane soudanaise au Cameroun sont tous quatre infestés par deux types de microfilaires dermiques. L'une correspond à *O. ramachandri* Bain, Wahl et Renz, 1993, dont les adultes se trouvent dans les tissus sous-cutanés des pieds. L'autre, plus petite, appartient à une nouvelle espèce d'*Onchocerca* dont l'adulte n'a pas été découvert. Les microfilaires d'*O. ramachandri* sont uniformément réparties, celles d'*Onchocerca* sp. sont concentrées dans la région dorsale. Des microfilaires prélevées dans une peau infestée ont été séparées sous la loupe binoculaire selon leur appartenance spécifique et injectées en intra-thoracique à des femelles de *S. squamosum* et *S. damnosum* s. str. prélevées à leur sortie de la pupa. Les deux espèces de Filaires se développent en larves infectantes (L3) dans les deux espèces de *Simulies* avec un rendement élevé (33 - 47 % des microfilaires injectées), et sans anomalies morphologiques. Les deux espèces de L3 sont longues, grêles et très mobiles; elles ont une pointe caudale et un oesophage glandulaire très développé. Les L3 d'*O. ramachandri* ont un oesophage atteignant 55 % de la longueur du corps et une tête arrondie; elles mesurent en moyenne 955 µm de long et 19,2 µm de large. Les L3 de l'autre espèce sont plus courtes (845 µm, $P < 0,001$) et plus minces (16,7 µm, $P < 0,001$). L'oesophage est plus court (36 %, $P < 0,001$), la queue plus courte ($P < 0,01$) et la tête est conique. Les caractères communs aux deux espèces de L3 ressemblent à ceux des L3 connues depuis de nombreuses années chez les *S. damnosum* s. l. sauvages et désignées comme « Larves de Type D » de Duke, 1967 au Cameroun, ou comme « Agamofilaria Type VI » de Voelker et Garms, 1972, au Liberia. Au cours de notre étude, 13 larves de ce type ont été trouvées chez 12 *S. damnosum* s. l. sauvages dans deux régions différentes du Nord-Cameroun et ont été identifiées comme *O. ramachandri*. Le parfait développement des deux espèces d'*Onchocerca* parasites des Phacochères, après inoculation intra-thoracique de *S. damnosum* s. l. et la présence du « Type D » dans les régions où se trouvent les Phacochères suggèrent que cette *Simulie* est le vecteur naturel de *O. ramachandri* et que la plupart, sinon toutes les L3 de « Type D » chez les vecteurs de l'onchocercose humaine au Nord-Cameroun proviennent des parasites des Phacochères.

MOTS CLÉS : onchocercose. *O. ramachandri*. *Onchocerca* sp. *Simulium damnosum* s. l. larve de Type D. Agamofilaria Type VI. phacochère. Cameroun. injection intrathoracique.

* Institut für Tropenmedizin, Eberhard-Karls-Universität, Wilhelmstrasse 27, D-72074 Tübingen, Germany.

** Laboratoire de Biologie Parasitaire, Protistologie, Helminthologie CNRS URA 114, Muséum national d'Histoire naturelle, 61 rue de Buffon, 75231 Paris Cédex 05, France.

INTRODUCTION

In some areas of Africa the *Simulium* vectors of *Onchocerca volvulus*, the causative agent of human onchocerciasis (riverblindness), also frequently transmit filariae of animals (Nelson and Pester, 1962; Duke, 1967; Garms and Voelker, 1969; Voelker and Garms, 1972; Renz *et al.*, 1989). These "animal filariae" in the onchocerciasis vectors are important for the epidemiology of the human disease, because they are indicators of the degree of zoophily of the vector populations, because they can hamper the correct assessment of *O. volvulus*-transmission and because they could have an immunological impact in man (Wahl *et al.*, 1994). Almost all of the animal filariae so far found in onchocerciasis vectors are unidentified and only known by their infective larvae (L3), which morphologically differ from *O. volvulus* ("non-*O. volvulus* larvae"). However, onchocerciasis vectors may also carry animal filariae, which closely resemble *O. volvulus*, as has been discovered by natural transmission experiments of the cattle-filaria *O. ochengi* to *S. damnosum* s.l. (Denke and Bain, 1978). One common type of non-*O. volvulus* larva in *S. damnosum* s.l. is longer, thinner and more motile than *O. volvulus* and has a caudal tip and a conspicuous glandular oesophagus. It was first described in Cameroon as Type D larva (Duke, 1967). A similar larva was later described in Liberia as *Agamofilaria* VI (Voelker and Garms, 1972) and thought by the authors to be the same species as Type D. The geographical and seasonal pattern of Type D fly-infections in Cameroon suggested that these larvae stemmed from game animals (Renz *et al.*, 1989).

In search for the host and adult stages of Type D larvae, we screened some common wild mammals of North-Cameroon for filariae and their possible development in *S. damnosum* s.l. A first paper described a new *Onchocerca* species, *O. ramachandrini* Bain, Wahl and Renz, 1993, discovered in the subcutaneous tissues of the feet of wart hogs (*Phacochoerus aethiopicus*). This paper reports the attempt to experimentally infect *S. damnosum* s.l. with *O. ramachandrini* and with a second *Onchocerca* microfilaria, coincidentally found in the same wart hogs. The possible relation of their L3 to Type D larvae is examined.

MATERIAL AND METHODS

Study site

The game animals were examined at the hunting lodge "Campement de Vogzoum" on the river Vina du Nord, 160 km to the North-East of Ngaoundéré, North-Cameroon.

Isolation of microfilariae

Large numbers of microfilariae for the experimental infection of flies were obtained by incubating superficial skin strips from the neck and the feet of an infected wart hog in 25 ml cone-bottomed plastic tubes with RPMI-1640 medium, penicillin (200 U/ml) and streptomycin (200 ug/ml) for several hours at ambient temperature (28 ± 3 °C).

Collection and identification of *S. damnosum* s.l.

S. damnosum s.l. pupae were collected in the nearby rapids of the Vina du Nord and left to emerge in cages of black cloth. Eight larvae were cytotaxonomically identified as *S. squamosum* (6 larvae) and *S. damnosum* s.str. (2). Adult flies were accordingly identified by the colour of the basal segments of the antennae as *S. squamosum* (≤ 2.5 pale segments) and *S. damnosum* s.str. (≥ 3 pale segments).

Experimental infection of flies

Microfilariae which had emerged from the skin were transferred to fresh RPMI-1640 medium, with penicillin and streptomycin (see above), amphotericin B (0.25 ug/ml) and fetal calf serum (10%) and injected in portions of 15 microfilariae/fly through fine glass capillaries into the thorax of pupae-hatched female flies. The flies were then kept individually in 2 ml gauze-covered plastic tubes in a humid chamber in total darkness and at ambient temperature (28 ± 3 °C), feeding on a 30% sugar solution with penicillin, streptomycin and amphotericin (same concentrations as above).

Control of identification of injected microfilaria

In each trial some of the injection capillaries with the microfilariae separated under the dissecting microscope (50x) were blown out into a drop of glycerol on a slide, covered with a slip and sealed with nail polish for later identification under the compound microscope (100 - 400x magnification).

Morphology of infective larvae

Mature third stage larvae from the head, thorax or abdomen of flies having reached infective age, were considered infective larvae = L3. Their length was measured through an ocular scale on a dissecting microscope at 50x magnification. The other main parameters (maximal diameter, muscular oesophagus + buccal cavity, glandular oesophagus, intestine and tail) were measured through a scale on a compound microscope at 400x magnification. Arithmetic mean measurements were compared by the t-test.

Hide mapping

The hide (without head and tail) of one wart hog

Fig. 1. – Distribution of *O. ramachandrinii* and *Onchocerca* sp. microfilariae in the hide of a *Phacochoerus aethiopicus*. Number of *O. ramachandrinii* / *Onchocerca* sp. microfilariae emerged per 100 mg skin biopsy.

infected with both microfilaria-species was sampled on four equidistant sites on the back, the sides and the belly and on each leg by taking a square of approximately 2 cm×2 cm superficial skin with a scalpel (Fig. 1). The biopsies were weighed, finely cut with scissors and incubated in medium (see above) for 4 hours. The microfilariae which emerged from each skin sample were quantitatively transferred to a slide with glycerol for later identification under the compound microscope (see above).

RESULTS

COMMON OCCURRENCE OF TWO DERMAL MICROFILARIAE, *O. RAMACHANDRINI* AND *ONCHOCERCA* SP., IN *PHACOCHOERUS AETHIOPICUS*

Two types of dermal microfilariae were found in the wart hog : a larger one with a cylindrical head and lashing movements and a smaller one with an attenuated anterior end and sluggish, coiling movements (Fig. 2, Table 1). The bimodal distribution of their lengths (Fig. 3), their differential distribution in the hide (Fig. 1) and their development into different types of infective larvae show that they are two distinct species. The larger one was identified as *O. ramachandrinii* Bain, Wahl and Renz, 1993. The small one, the adult stages of which were not yet found, is a new species. By the morphology of its L3 (see below), it was assigned to the genus *Onchocerca*. The two species were associated in all four wart hogs examined. Whereas *O. ramachandrinii*-microfilariae were more or less evenly distributed across the whole hide, with a local maximum at the flanks, those of

Onchocerca sp. were concentrated at the back and neck and were rare in the feet (Fig. 1).

DEVELOPMENT TO THE INFECTIVE STAGE IN *S. squamosum* AND *S. DAMNOSUM* S. STR., FOLLOWING INTRA-THORACICAL INJECTION

Experimental set-up

Microfilariae were isolated from the skin of an infected wart hog and injected into nulliparous (pupae-hatched) female flies. The development of the microfilariae was monitored in flies dying until day 6 post infection (p.i.). All surviving flies were killed and checked for filarial larvae on day 7 p.i. Three inoculation trials were conducted (Table 2). In Trial 1 and 2 microfilariae isolated from the neck were separated under the dissecting microscope into *O. ramachandrinii* and *Onchocerca* sp. prior to injection. In Trial 3 undifferentiated microfilariae from the feet were inoculated. This site was known, from hide-mapping, to contain almost exclusively *O. ramachandrinii*. In all trials the “purity” of the inoculum was checked by randomly leaving aside some of the injection capillaries and identifying the microfilariae in them under the compound microscope (Table 2).

Filarial development in the three trials

In all trials the microfilariae developed to infective larvae at a high rate (33 - 47%) and practically without pathological stages (Table 2). The time span of development was slightly faster than for *O. volvulus* and *O. ochengi* under the same conditions (Wahl unpubl. data), with first sausage stages (L1) appearing already on day 1 p.i., second stage larvae (L2) on day 3 p.i., preinfective larvae on day 4 p.i. and mature third stage larvae (L3) in the head on day 5 p.i. In the three trials six *S. squamosum* and two *S. damnosum* s. str. survived to infective age. Development in both fly species was equally good for both microfilaria species (Table 2). The total number of L3 (both species) recovered from the head, the thorax and abdomen was 24, 17, and 6 respectively.

Description of *O. ramachandrinii* and *Onchocerca* sp. infective larvae

Among the experimentally obtained infective larvae, two morphological types were found (Fig. 4, Tables 1 and 3) : a large one had a round head, a long tail and a conspicuously long glandular oesophagus, which made up about 1/2 of the total L3-length. This type was found for all L3 in Trial 3 (injection of almost pure samples of “big microfilariae”) and was identified as *O. ramachandrinii*. The other type was shorter ($P<0.001$) and thinner ($P<0.001$) and had a conical

Fig. 2. – The two species of dermal microfilariae found in *Phacochoerus aethiopicus*: *O. ramachandrani* and *Onchocerca* sp. A-C : *O. ramachandrani*; A : silhouette, cephalic hook in lateral view; B : cephalic region of another specimen, hook in front view; C : same specimen, posterior region. D-F : *Onchocerca* sp.; D : silhouette; E : attenuated anterior region of another specimen, hook in front view (on the left) or lateral view (on the right); F : posterior region of two specimens. (Only the first cephalic nuclei, R1 cell and last caudal nuclei were drawn; A-F : scale = 30 µm).

Fig. 3. – Bimodal length distribution of dermal microfilariae from a *Phacochoerus aethiopicus* infected with both species, *O. ramachandrani* with long microfilariae (ra) and *Onchocerca* sp. with shorter microfilariae (sp). Abscisse : classes of length : 220 to 229 µm, 230 to 239 µm, etc.

1. MICROFILARIAE (n)	35	29
LENGTH (μm) $\pm$ SD (RANGE)	301 $\pm$ 12 (272-333)	251 $\pm$ 10 (227-272)
DIAMETER (μm) $\pm$ SD (RANGE)	7.4 $\pm$ 0.3 (6.7-8.4)	6.9 $\pm$ 0.5 (5.7-7.4)
ANTERIOR END	cylindrical	attenuated
BEHAVIOUR	lashing front end	sluggish coiling
LOCALISATION IN THE HIDE	evenly distributed	neck and back
2. INFECTIVE LARVAE (n)	17	12
LENGTH (μm) $\pm$ SD (RANGE)	955 $\pm$ 55 (850-1040)	845 $\pm$ 65 (750-1000)
DIAMETER (μm) $\pm$ SD (RANGE)	19.2 $\pm$ 0.8 (17.8-21.2)	16.7 $\pm$ 0.8 (16.0-18.5)
GLAND. OES (% L3-length) $\pm$ SD (RANGE)	55.5 $\pm$ 2.3 (52.3-61.5)	36.3 $\pm$ 1.9 (32.6-39.2)
TAIL (% of L3-length) $\pm$ SD (RANGE)	5.7 $\pm$ 0.4 (5.1-6.3)	5.2 $\pm$ 0.4 (4.7-6.0)
HEAD	rounded	conical

Table 1. – Differential characters of *O. ramachandrini* and *Onchocerca* sp. (respectively left and right columns)

TRIAL NUMBER + SPECIFICATION	1 (selected OR)	2 (selected OSP)	3 (OR from foot)
CONTROL OF INJECTED MICROFILARIAE			
number of capillaries tested	1	2	4
number of <i>O. ramachandrini</i> identified	12	0	56
number of <i>Onchocerca</i> sp. identified	0	25	7
DEVELOPMENT IN THE FLIES			
number of flies injected (15 mff/fly)	14	18	22
number of SQUAM\DAM surviving	1/0	2/1	3/1
number of SQUAM\DAM with L3	1/-	2/1	3/1
total number of L3 in SQUAM\DAM	6/-	10/5	19/7
% development in SQUAM\DAM	40/-	33/33	42/47

Table 2. – Development of *O. ramachandrini* (or) and *Onchocerca* sp. (osp) in *S. squamosum* and *S. damnosum* s.str., following intrathoracal injection

head, a shorter tail ($P < 0.01$) and a shorter glandular oesophagus ($P < 0.001$), which made up only about 1/3 of the total L3-length. All L3 from Trial 2 (injection of selected "small microfilariae") corresponded to this second type and were identified as *Onchocerca* sp., with the exception of one *O. ramachandrini*-L3, which apparently developed from an accidentally injected "big microfilariae" (Table 3). Both L3-species were long and slender in general appearance and very motile, when recovered from fresh flies, with quick, curling movements. They all had a conspicuous, clearly visible glandular oesophagus (Fig. 4 A,F), a laterally flattened buccal cavity and a caudal tip, which was most commonly bent ventrally or dorsally and only rarely straight (Fig. 4 D, E, H, I).

DISCUSSION

TWO *ONCHOCERCA* SPECIES FROM THE WART HOG IN CAMEROON

This paper describes the microfilaria and the infective larvae (L3) of two filarial species from the wart hog, *Phacochoerus aethiopicus*, in Cameroon. One of them is *Onchocerca ramachandrini*, the L3 of which were unknown so far. The other is a new species, the adult worms of which were not yet found. Due to the morphology of its infective larvae the new species can be assigned to the genus *Onchocerca* (in Bain and Chabaud, 1986). In view of the high infection rate of *P. aethiopicus* (4 of 4 examined animals infected with both species), the wart hog seems to be a major host of the two filariae.

Fig. 4. – The infective larvae of *O. ramachandrini* and *Onchocerca* sp.

A-E : *O. ramachandrini*; A : silhouette, lateral view; B : anterior region, left lateral view; C : head, median view; D, E : tail, ventral and left lateral view respectively.

F-I : *Onchocerca* sp.; F : silhouette, lateral view; G : head, lateral view; H, I : tail, ventral and right lateral view respectively;

(A, F : scale = 200 µm; B,D,H,I : scale = 30 µm; C, G : scale = 10 µm).

FLY/LOC	LENGTH (µm)	DIAMETER (µm)	M. OES (% of length)	G. OES (% of length)	INTEST (% of length)	TAIL (% of length)	L3 IDENT
TRIAL N° 2: SELECTED ONCHOCERCA SP. (OSP)							
F1H1	840	16.0	17.1	37.8	40.0	5.0	OSP
F1H2	800	16.0	18.8	35.4	40.3	5.5	OSP
F1T1	820	17.0	17.4	36.7	41.2	4.7	OSP
F1T2	800	/	15.9	33.7	45.1	5.3	OSP
F2H1	860	16.0	17.3	39.2	38.3	5.0	OSP
F2H2	800	16.5	19.3	37.0	38.8	5.0	OSP
F2H3	750	17.3	15.4	35.9	43.6	5.1	OSP
F2H4	900	17.3	16.1	32.6	46.3	4.9	OSP
F2H5	850	16.1	13.2	38.0	42.7	6.0	OSP
F2H6	900	18.5	18.2	36.5	39.6	5.6	OSP
F2H7	1000	16.1	/	/	42.0	4.9	OSP
F2T1	820	16.8	16.1	36.7	41.9	5.3	OSP
F3T1	850	19.8	16.0	57.5	21.4	5.1	OR
TRIAL N° 3 : O. RAMACHANDRINI (OR) FROM THE FOOT							
F1H1	1010	19.5	14.8	56.8	22.2	6.2	OR
F1H2	1000	21.2	16.5	54.5	23.6	5.3	OR
F1H3	940	18.5	16.4	57.5	20.9	5.2	OR
F1H4	960	20.0	14.5	53.8	26.2	5.5	OR
F2T1	940	19.0	16.4	57.0	20.7	5.8	OR
F2T2	950	18.8	16.0	52.8	25.3	5.9	OR
F3H1	990	19.8	16.8	61.5	15.4	6.3	OR
F3A1	1040	18.5	16.6	53.9	24.0	5.5	OR
F3A2	990	18.5	17.1	55.0	22.2	5.8	OR
F3A3	880	18.5	16.2	53.3	24.8	5.7	OR
F3A4	990	18.5	15.4	56.6	22.6	5.4	OR
F4H1	1040	19.5	16.8	55.7	21.5	6.0	OR
F4H2	940	19.8	15.6	52.9	25.2	6.3	OR
F4H3	900	17.8	14.7	55.2	24.2	5.9	OR
F4H4	900	18.5	16.5	52.3	25.8	5.4	OR
F4T1	920	19.8	16.0	57.2	20.9	5.9	OR

Table 3. Morphology of infective larvae from two injection trials

FLY/LOC = number of fly and localisation where L3 were found (F=fly number, H=head, T=thorax, A=abdomen); M. OES = muscular oesophagus + buccal cavity; G. OES=glandular oesophagus.

DEVELOPMENT IN *SIMULIUM* VECTORS

Complete extrinsic development of the two species was obtained by intrathoracical injection of microfilariae into pupae-hatched *S. damnosum* s.l. females. Development was exceptionally good and without pathological forms, so that, despite the artificial route of infection used, it can be concluded with near certainty that both species are naturally transmitted by *Simulium* species. This is the first account of life cycles of *Onchocerca* species of suids.

RELATION OF THE TWO *ONCHOCERCA* SPECIES FROM THE WART HOG WITH NON-*O. VOLVULUS* LARVAE IN WILD ONCHOCERCIASIS VECTORS

The infective larvae of both species, by their long and slender silhouette, their caudal tip, their great motility and their conspicuous glandular oesophagus, resemble a certain form of "wild" filarial larva commonly found in onchocerciasis vectors in Africa : it was first described in Cameroon and named Type D (Duke, 1967). This form of larva has since been frequently found by other authors in Cameroon, as well

in *S. damnosum* s.l. (Franz and Renz, 1980; Renz *et al.*, 1989; Schibel and Renz, 1990; Wahl *et al.*, 1994) as in *S. bovis* (Renz *et al.*, 1989; Wahl and Renz, 1991). A similar type of larva was found in *S. damnosum* s.l. in Liberia and named *Agamofilaria* Type VI (Voelker and Garms, 1972). The authors thought it to be "[...] most probably identical with Type D in Cameroon [...]". Long and slender non-*O. volvulus* L3 with a caudal tip were also found in other parts of the Onchocerciasis Control Programme area (Baker and Bain, unpubl. data).

The (retrospective) synonymization of our two L3-species from the wart hog with the historical accounts of Type D- and/or *Agamofilaria* Type VI larvae seems, however, impossible: although most of the morphological characters given by Voelker and Garms (1972) for *Agamofilaria* Type VI larvae correspond to *O. ramachandrini* (length : 915-985 µm, glandular oesophagus : 49% of total length, tail : 5.8%), its inferior diameter (mean : 17.5 µm) precludes a formal synonymization. Many of the characters of Type D larvae given by Duke (1967) show a wide range (length : 750 - 925 µm, diameter : 15 - 20

µm, glandular oesophagus 270 - 475 µm) and thus cover the morphology of both *O. ramachandri* and *Onchocerca* sp. Moreover, the abundance of *Onchocerca* species in game animals in Africa (Bain *et al.*, 1978; Denke and Bain, 1981), all of them with unknown life cycles, cautions to consider that other L3-species with similar morphology may occur.

However, since the description of the two *Onchocerca* L3 from wart hogs, we were able to examine 13 Type D-like larvae from 11 wild *S. damnosum* s.str. and one *S. squamosum* in two geographically different regions in North-Cameroon. Significantly, they were all morphologically indistinguishable from *O. ramachandri*.

ONCHOCERCA SPECIES FROM WART HOGS IN THE EPIDEMIOLOGY OF HUMAN ONCHOCERCIASIS

In view of the excellent development of *O. ramachandri* injected into *S. damnosum* s.str. and *S. squamosum* and its L3 being indistinguishable from "wild" Type D-like larvae found in these fly species, it seems almost certain that *S. damnosum* s.str. and *S. squamosum* are natural vectors of *O. ramachandri*. We cannot speculate at this stage of knowledge, whether *Onchocerca* sp. is also naturally transmitted by *S. damnosum* s.l. or is insusceptible, nor whether *S. damnosum* s.l. is the only natural vector of *O. ramachandri* (and *Onchocerca* sp. ?), nor can we exclude that there are other unknown infective larvae with Type D morphology. However, the coincidence of all recently examined Type D larvae in North-Cameroon being identified as *O. ramachandri* and the abundance of *P. aethiopicus* in this area, strongly suggests that many (if not all) of the Type D larvae in *S. damnosum* s.l. in Cameroon (and other parts of Africa ?) stem from wart hogs. The annual proportion of Type D larvae in the Sudan savanna of Cameroon is as high as one fifth of all filarial infections (Wahl *et al.*, 1994). This underlines the high degree of zoophily of the vector populations and raises questions as to a possible immunological impact of these animal filariae in man.

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