ELSEVIER

Veterinary Parasitology 52 (1994) 297–311

veterinary parasitology

Bovine onchocercosis in North Cameroon

G. Wahl*[,a], M.D. Achu-Kwi[b], D. Mbah[b], O. Dawa[c], A. Renz[a]

[a]*Institut für Tropenmedizin, Wilhelmstr. 27, D-7400 Tübingen, Germany*
[b]*Centre de Recherches Zootechniques Wakwa, BP 65, Ngaoundere, Cameroon*
[c]*Ministère d'Elevage, des Pêches et des Industries Animales, BP 64, Ngaoundere, Cameroon*

(Accepted 22 June 1993)

Abstract

Ventral skin biopsies from 204 Gudali cattle of the Vina division in the Adamawa highlands revealed microfilariae of *Onchocerca gutturosa, O. ochengi* and *O. dukei* in 85%, 51% and 8% of the animals, respectively. In 60 Fulani cattle from the Tcholliré division in the Sudan savanna, the same microfilaria species were detected in 92%, 83% and 47% of the animals. *Onchocerca armillata* adult worms were found in 67% of the Gudalis and in 100% of the Fulanis. In areas of high transmission the prevalences declined in old animals, possibly indicating acquired resistance. For all species no significant difference in prevalence was found between male and female cattle. The microfilariae of *O. ochengi* and *O. dukei* were concentrated in the skin of the posterior and anterior belly, respectively. *Onchocerca gutturosa* microfilariae had highest densities on the hump and near the umbilicus, whereas those of *O. armillata* were distributed more evenly across the body surface. In infected hides the mean microfilarial densities of *O. gutturosa, O. ochengi, O. dukei* and *O. armillata* were respectively 3.1 microfilariae (mff) mg^{-1}, 0.6 mff mg^{-1}, 0.7 mff mg^{-1} and 0.092 mff mg^{-1} for the whole body surface and 9.3 mff mg^{-1}, 3.8 mff mg^{-1} and 1.9 mff mg^{-1} for the sites of highest density (*O. armillata* had no predilection site). Ninety-five per cent of the microfilariae were located in the uppermost skin layer of 2 mm depth, 5% were in the corium and none were found in the subcutis. Two cattle had skin microfilariae of a hitherto unknown *Onchocerca* species.

Key words: Onchocerca spp.; Cattle-Nematoda; Epidemiology-Nematoda

*Corresponding author at: CIRMF, BP 769, Franceville, Gabon.

SSDI 0304-4017(93)00561-C

1. Introduction

Four *Onchocerca* species are common parasites of cattle in Africa: *O. gutturosa* Neumann, 1910, the adult worms of which live in the loose connective tissues of the nuchal ligament and other tendons, *O. armillata* Raillet and Henri, 1909, which inhabits the intima of the aorta, and *O. ochengi* Bwangamoi, 1969, and *O. dukei* Bain et al., 1974, which form nodules in the ventral skin and on the fasciae of the thoracic muscles (Fig. 1).

Onchocerca ochengi is transmitted by *Simulium damnosum* s.l. (Denke and Bain, 1978; Omar et al., 1979; Séchan, 1984) and *O. dukei* by *S. bovis* (Wahl and Renz, 1991). *Onchocerca gutturosa* is transmitted by *Culicoides* species in the Sudan and in Sierra Leone (ElSinnary and Hussein, 1980; Davies et al., 1989), whereas in Tanazania it was reported to develop in *Simulium vorax* (Mwaiko, 1981). The vector of *O. armillata* is as yet unknown, *Onchocerca dukei*, *O. gutturosa* and *O. armillata* do not develop in *S. damnosum* s.l. (Wahl, 1991).

The four *Onchocerca* species of African cattle are generally believed to have a low pathogenicity and are thus of minor veterinary interest. *Onchocerca ochengi*, however, has recently received increasing attention because of its co-transmission by the vector of human onchocercosis (Wahl et al., 1994) and because of its potential as a model for *O. volvulus* (Trees et al., 1992; Trees, 1992).

As part of an investigation on the implication of bovine onchocercosis for the epidemiology of human onchocercosis (Wahl et al., 1994), we assessed the prevalence of *Onchocerca* species in cattle from the Adamawa highlands and from the Sudan savanna of North Cameroon.

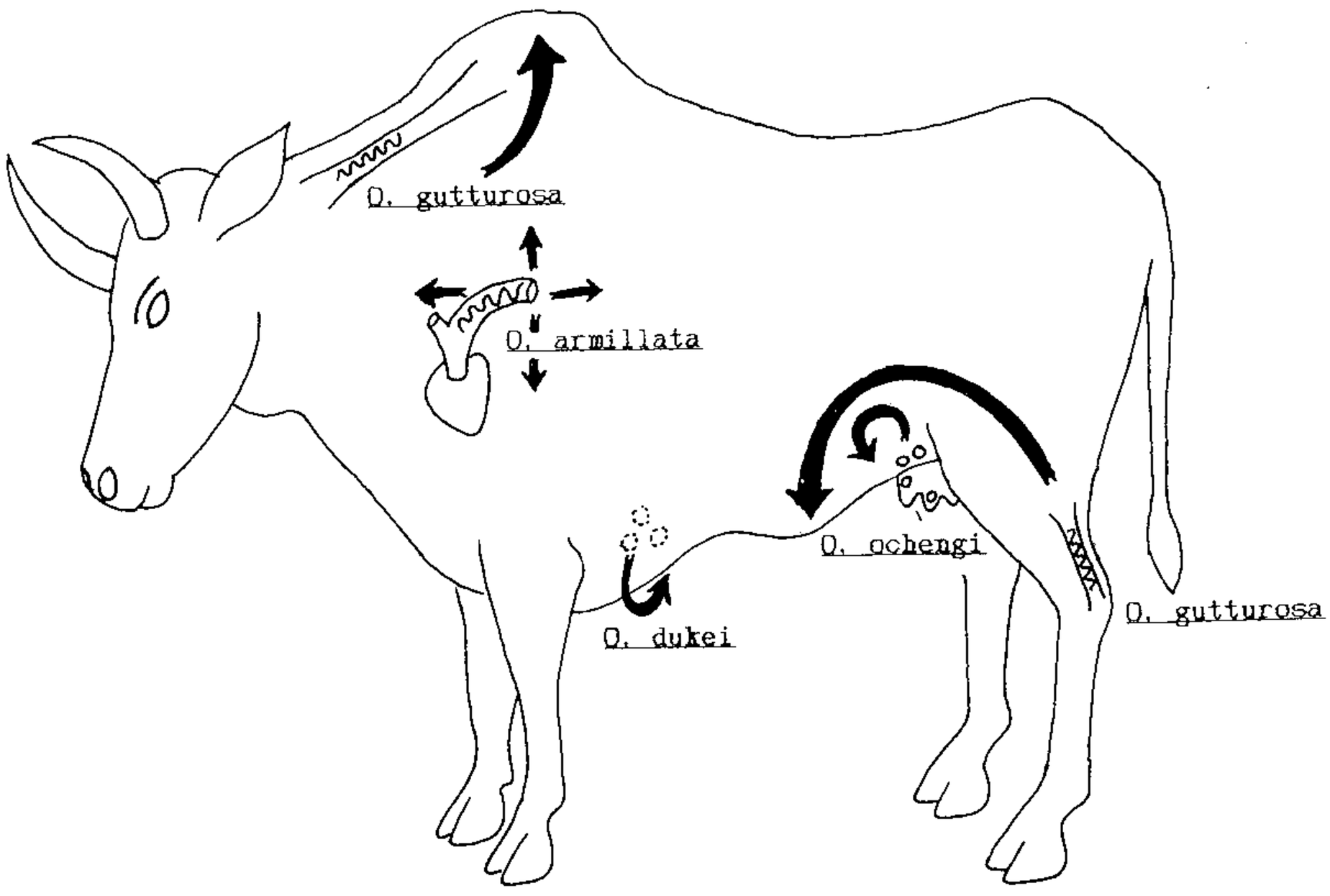


Fig. 1. Predilection sites of *Onchocerca* adult worms and microfilariae in zebu cattle in North Cameroon. Arrows indicate concentrations of microfilariae.

2. Material and methods

2.1. Study areas and cattle breeds

Cattle were examined in the North Province and Adamawa Province of Cameroon. The cattle in this region are almost exclusively *Bos indicus*. Those in the North Province (Sudan savanna) are almost exclusively Fulani (mainly Aku with some Djafun), which are reared by the nomadic Mbororo tribe. In the Adamawa highlands (Guinea savanna) the cattle population comprises Ngaoundere-Gudalis (35%), Akus (30%), Djafuns (20%) and Banyo-Gudalis (15%). They are mainly reared by semi-sedentary farmers and Mbororos, migrating only during the driest months to nearby better grazing areas. The prevalence of bovine onchocercosis was assessed in Fulanis from the Tchollire division at the abattoir of Touboro (7°45′N, 15°21′E) and in Ngaoundere-Gudalis from the Vina division at the abattoir of Ngaoundere (7°13′N, 13°34′E).

2.2. Assessment of prevalence

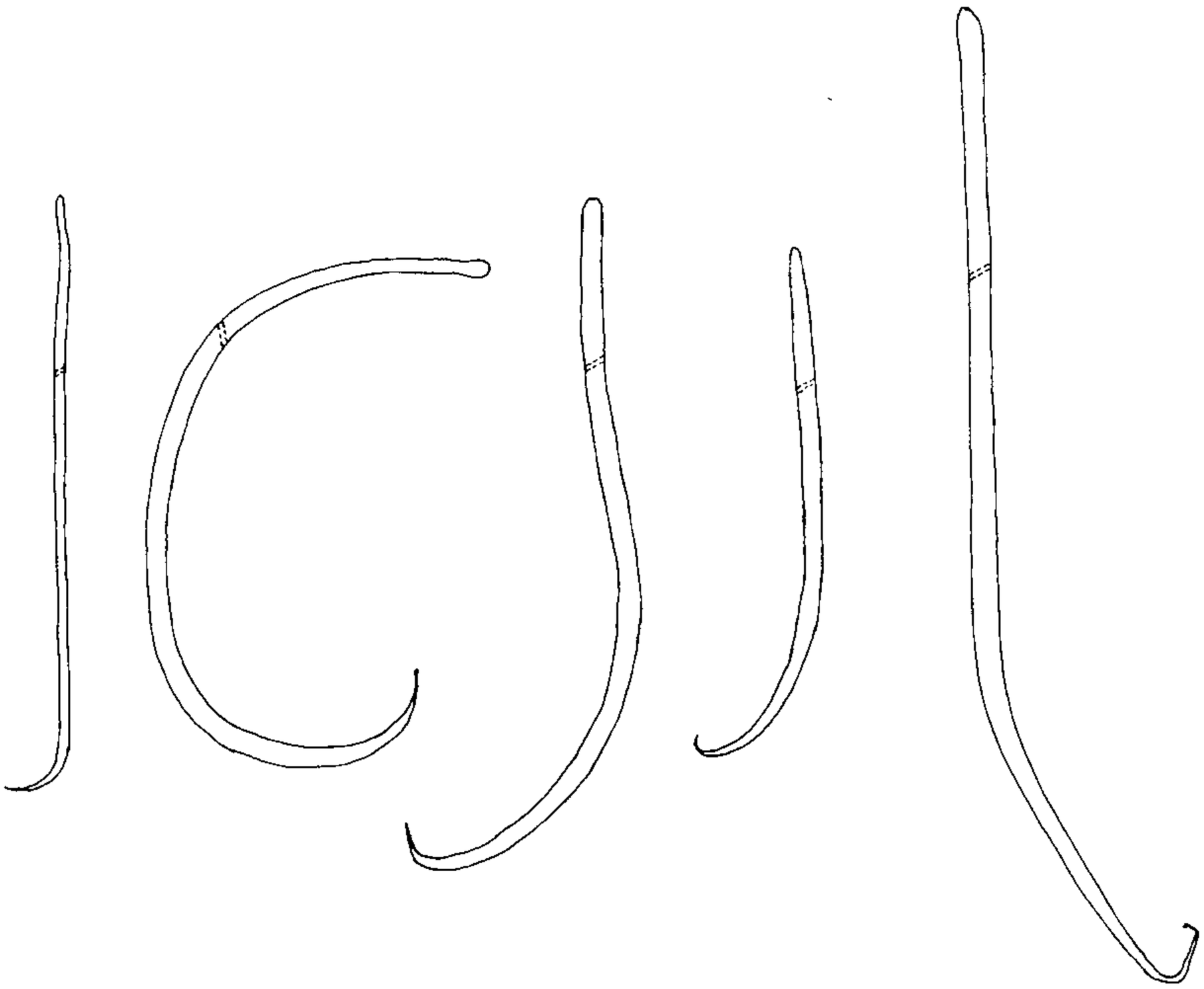
Slaughter cattle were examined for *Onchocerca* adult worms at their predilection sites (Fig. 1) and for microfilariae by taking one superficial skin sliver (mean surface 15×5 mm (i.e. 75 mm^2), mean wet weight 75 mg) with a scalpel (after shaving the skin) on the ventral midline between the umbilicus and the udder/scrotum. Sub-samples of adult female worms were isolated from each predilection site and identified by the structure of the cuticle, the form of the posterior end and (when present) the microfilariae in the uterus (Bain, 1981). Skin biopsies were incubated for 4 h at ambient temperature in RPMI 1640 medium and the emerged microfilariae identified under a dissecting microscope at 50× magnification (Fig. 2).

2.3. Distribution of microfilariae across the body surface

Eleven hides of slaughter cattle were skin-sampled at six equidistant sites on the back, on the sides and on each side of the ventral cut (Fig. 3). As a result of slaughter procedures, most of the hides could only be examined without the head, the legs, the tail and the udder/scrotum. The chosen sites were shaved and superficially perforated with a punch of 20 mm diameter. The upper skin layers of approximately 2 mm (epidermis and upper corium) were removed with a scalpel within the perforated area, yielding skin discs of 314 mm^2 surface and 574 ± 122 mg wet weight (63 punches weighed), and incubated as mentioned above.

2.4. Vertical distribution of the microfilariae

Four ventral skin strips, of approximately 2 mm width and 8 mm depth, were sampled in vertical sequence with a corneoscleral trepane of 2 mm diameter (Zahner and Schulz-Key, 1990). Five columns of three cores were taken on each



	O. gutturosa	O. armillata	O. ochengi	O. dukei	Onchocerca spec.
Measurements (in µm in demineralized water/after hemalum staining):					
Length	263 / 249	382 / 346	298 / 277	240 / 217	- / 374
Diameter	4.4 / 3.4	6.8 / 5.5	7.9 / 5.9	6.7 / 4.8	- / 7.3
n	26 / 13	15 / 13	22 / 13	22 / 8	0 / 8
Movement in physiological medium:	quick, compressed-sinuous undulation, often with trembling	sluggish, wide-spaced, snake-like undulation	lashing front end, contorting back end, often as if into a knot	very active, vigourously lashing front end, back end oscillating	

Fig. 2. *Onchocerca* microfilariae from the skin of zebu cattle in North Cameroon.

skin, with one horizontal cylinder in the uppermost skin layer (epidermis and upper corium), one in the lower corium and one in the subcutis. The skin cylinders were digested with 0.5% collagenase at 35°C (Schulz-Key et al., 1977).

2.5 Emergence of microfilariae from the skin in relation to the biopsy method and the time and temperature of incubation

Four slaughter hides were each sampled on the ventral midline between the udder and the umbilicus by taking two slivers with a scalpel, two 20 mm punches and two vertical 2 mm cores with a trepane. The sequence of slivers, punches and cores was different for every skin, to compensate for possible directional distri-

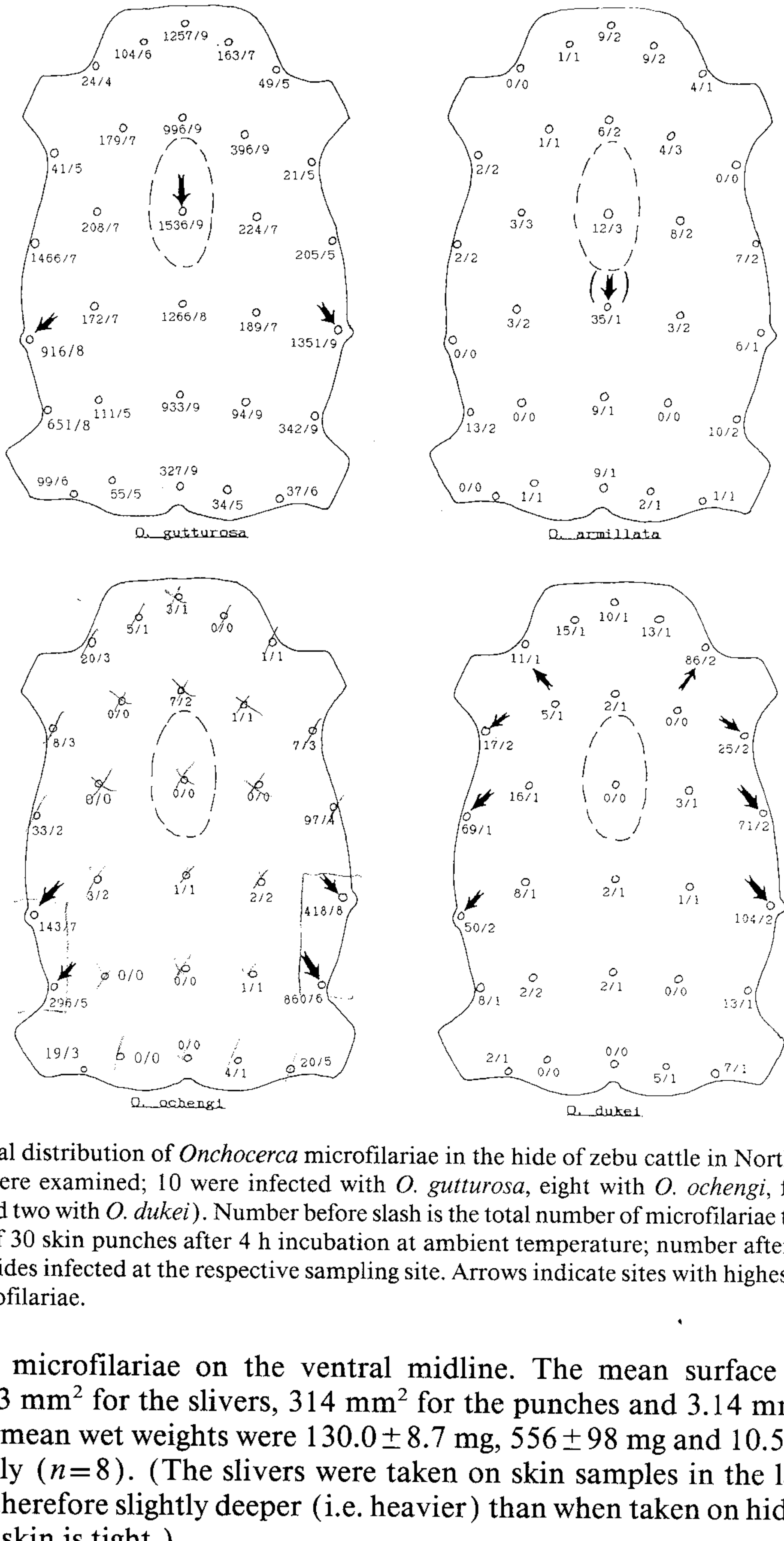


Fig. 3. Spatial distribution of *Onchocerca* microfilariae in the hide of zebu cattle in North Cameroon (11 hides were examined; 10 were infected with *O. gutturosa*, eight with *O. ochengi*, four with *O. armillata* and two with *O. dukei*). Number before slash is the total number of microfilariae that emerged from each of 30 skin punches after 4 h incubation at ambient temperature; number after slash is the number of hides infected at the respective sampling site. Arrows indicate sites with highest concentration of microfilariae.

bution of microfilariae on the ventral midline. The mean surface area was 75.3 ± 10.3 mm^2 for the slivers, 314 mm^2 for the punches and 3.14 mm^2 for the cores; the mean wet weights were 130.0 ± 8.7 mg, 556 ± 98 mg and 10.5 ± 3.3 mg, respectively ($n=8$). (The slivers were taken on skin samples in the laboratory and were therefore slightly deeper (i.e. heavier) than when taken on hides in situ, where the skin is tight.)

The biopsies were incubated in 2 ml plastic tubes with RPMI 1640 medium and 200 units ml^{-1} penicillin and 200 μg ml^{-1} streptomycin for up to 48 h. Microfilariae were counted and the medium was changed after 4, 24 and 48 h incubation. For each skin and each method one biopsy was incubated at ambient temperature ($22 \pm 4°C$) and the other at 37°C. After 48 h the biopsies were digested with 0.5% collagenase at 35°C. The microfilariae remaining in the skin were counted after 24 h digestion, when the collagenase was changed, and after 48 h, when digestion was complete. Because of the high numbers of microfilariae and the large amount of debris, the digestion medium for slivers and punches was diluted to 20 ml and the microfilariae were counted in a 1 ml sample.

2.6. Statistical analysis

Prevalences in the two study areas and in the different age groups were compared by χ^2 tests when applicable.

3. Results

3.1. Endemicity of bovine onchocercosis in North Cameroon

All of the four known *Onchocerca* species of African cattle are endemic in North Cameroon. The overall prevalences of *O. gutturosa*, *O. ochengi*, *O. dukei* (assessed by the detection of microfilariae in the skin) and *O. armillata* (assessed by detection of adult worms) were 85%, 51%, 8% and 67%, respectively, in 204 Gudali cattle from the Adamawa highlands and 92%, 83%, 47% and 100%, respectively, in 60 Fulani cattle examined in the Sudan savanna (Table 1). A critical assessment of the methods used showed that the 'true' prevalences are approximately 10% higher. *Onchocerca armillata*, *O. ochengi* and *O. dukei* were significantly ($P<0.01$) more frequent in cattle from the Sudan savanna than in highland cattle. Two Gudali cattle had skin microfilariae of a hitherto unknown *Onchocerca* species (Fig. 2).

3.2. Prevalence in relation to age and sex of cattle

In Gudali cattle from the highlands the prevalence of microfilariae of *O. gutturosa* peaked in 3–4-year-old cattle and declined significantly in cattle older than 7 years ($P<0.05$). *Onchocerca ochengi* adults and microfilariae, on the other hand, were significantly less frequent in the younger age groups ($P<0.01$). Prevalences of *O. armillata* and *O. dukei* were not significantly different between the age groups (Table 1). In Fulani cattle from the Sudan savanna the prevalences of all species reached their maxima earlier than in the highland cattle and all declined in cattle older than 7 years (Table 1). However, too few cattle were examined to show whether the differences were statistically significant. No significant sex-related

Table 1
Prevalence of *Onchocerca* species in zebu cattle in North Cameroon

Age group (years)	Prevalence method	*O. gutturosa*		*O. armillata*		*O. ochengi*		*O. dukei*	
		n	% Positive	*n*	% Positive	*n*	% Positive	*n*	% Positive
Adamawa highlands (Gudali)									
1–2	AW	–		1	0	5	40	0	–
	MF	4	75	4	0	4	25	4	0
3–4	AW	13	62	9	56	29	38	0	–
	MF	40	95	40	15	40	28	40	5
5–7	AW	10	100	16	82	37	70	0	–
	MF	45	84	45	13	45	53	45	7
>7	AW	20	90	13	62	32	100	0	–
	MF	41	76	41	22	41	71	41	10
Total[a]	AW	89	88	100	67	154	60	0	–
	MF	204[b]	85	204[b]	16	146	51	146	8
Sudan savanna (Fulani)									
1–2	AW	1	100	1	100	1	0	0	–
	MF	1	100	1	100	1	0	1	0
3–4	AW	3	100	3	100	11	82	0	–
	MF	13	100	13	23	13	92	13	69
5–7	AW	3	100	3	100	18	89	0	–
	MF	19	95	19	32	19	89	19	47
>7	AW	2	100	2	100	15	80	0	–
	MF	15	87	15	13	15	73	15	27
Total[a]	AW	30	100	38	100	79	87	0	–
	MF	60	92	60	27	60	83	60	47

[a]Animals which could not be aged are included.
n, Number of animals examined (animals examined for adult worms and for microfilariae are not the same); AW, prevalence as assessed by detection of adult worms (animals could often not be examined simultaneously for all four adult worm species); MF, prevalence as assessed by presence of microfilariae in ventral skin snip (all animals were examined for all four microfilariae species, except: fifty-eight Gudali slaughter cattle which had been preselected for intradermal nodules; in these, only *O. gutturosa* and *O. armillata* microfilariae were considered.

differences in prevalence of adults or microfilariae were found for any parasite–cattle breed combination (Table 2).

3.3. Adult worm versus microfilarial prevalence

Microfilariae of *O. armillata* always occurred in low densities (Fig. 3, Table 4) and were therefore detected in only 14% of animals positive for adult worms ($(b+d)/b+c)\times 100$, Table 3). The prevalence of this species was therefore based only on the presence of adult worms. As a result of the slaughter procedures, a routine search for *O. dukei* adult worms could not be made. Its prevalence was therefore calculated only from the occurrence of skin microfilariae. The prevalence of *O. gutturosa* and *O. ochengi* was assessed by both methods: groups of cattle which were independently examined either for adult worms or for micro-

Table 2
Prevalence of bovine *Onchocerca* species in relation to sex

Breed	Sex	Method	Mean age (years)	*O. gutturosa*		*O. armillata*		*O. ochengi*		*O. dukei*	
				n	% Positive	*n*	% Positive	*n*	% Positive	*n*	% Positive
Gudali	f	AW	6.6	36	81	31	68	83	71	0	–
		MF	5.8	159	80	159	16	159	58	159	4
	m	AW	5.4	9	89	8	73	28	57	0	–
		MF	4.8	66	82	66	12	66	64	66	8
Fulani	f	AW	7.1	6	100	6	100	30	87	0	–
		MF	6.6	21	95	21	24	21	81	21	38
	m	AW	5.7	3	100	3	100	16	75	0	–
		MF	5.0	17	100	17	41	17	76	17	47

n, Number of animals examined; f, female; m, male; AW, prevalence as assessed by presence of adult worms (animals could often not be simultaneously examined for all four species); MF, prevalence as assessed by presence of microfilariae in one ventral skin snip (the same animals were examined for all four microfilariae species).

Table 3
Macro-, microfilarial and true prevalence of bovine onchocercosis

	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*
Onchocerca species	Ex. (*n*)	AW+/MF+ (*n*)	AW+/MF− (*n*)	AW−/MF+ (*n*)	AW−/MF− (*n*)	AW-Prev. (*b*+*c*) / *a* (%)	MF-Prev. (*b*+*d*) / *a* (%)	MT-Prev. (*b*+*c*+*d*) / *a* (%)	T-Prev. (%)
O. gutturosa	67	47	6	13	1	79.1	89.6	98.5	98.5
O. ochengi	168	109	12	12	35	72.0	72.0	79.2	79.3
O. armillata	54	5	30	0*	19	64.8	9.3	–[a]	–[a]

[a]Proportion of false adult worm negatives not known as a result of inefficiency of skin snip method for *O. armillata*.
Ex, Number of animals examined; AW+ (MF+), adult worms (microfilariae) detected, AW− (MF−), no adult worms (microfilariae) detected; AW-Prev. (MF-Prev.), prevalence as assessed by the detection of adult worms (microfilariae); MT-Prev., 'minimal true prevalence'; T-Prev, true prevalence ($=h+((c/a)\times(d/a)\times(e/a)\times 100)$).

filariae had more or less equal infection rates ($P>0.05$, Table 1). When cattle were examined for both adults and microfilariae (Table 3), macro- and microfilarial prevalences were also roughly the same ($P>0.05$), but it was revealed that 7–19% of the infections were missed when only one of the methods was applied. The per cent positives and false negatives of each method gives the 'minimal true prevalences' (Table 3). To this must be added a small proportion of animals which are false negative both for macro- and microfilariae.

3.4. Location of adult worms

Female worms from the nuchal ligament and the hind tarsal joints were always *O. gutturosa*, and those from the aorta were always *O. armillata*. Intradermal

Table 4
Emergence of microfilariae from biopsies in relation to time and temperature

Species	Method	Temperature (°C)	Mff emerged after			Mff after digestion	Mff mg^{-1} after digestion	Mff mm^{-2} after digestion
			4 h	24 h	48 h			
O. gutturosa[a]	Sliver	Ambient	28	61	536	5806	11.17	19.28
		37	29	3836	5015	5905	11.36	19.60
	Punch	Ambient	94	245	556	5076	2.28	4.04
		37	264	2187	2508	4438	2.00	3.53
	Core	Ambient	3	9	28	187	4.45	14.89
		37	0	146	251	287	6.83	22.85
Mean density							6.35	14.03
O. ochengi[a]	Sliver	Ambient	17	41	528	4448	8.55	14.78
		37	36	832	904	2344	4.51	7.78
	Punch	Ambient	314	429	780	9790	4.40	7.79
		37	317	792	889	3889	1.75	3.10
	Core	Ambient	15	61	250	308	7.33	24.52
		37	2	172	190	199	4.74	15.84
Mean density							5.22	12.30
O. armillata[a]	Silver	Ambient	0	2	2	2	0.005	0.009
		37	1	6	7	27	0.069	0.120
	Punch	Ambient	0	6	7	77	0.046	0.082
		37	3	4	5	65	0.039	0.069
	Core	Ambient	0	0	0	0	0	0
		37	0	0	0	0	0	0
Mean density							0.040[b]	0.070[b]

[a]Four skins examined for *O. gutturosa* and *O. ochengi*; three skins for *O. armillata*; i.e. four (or three) biopsies per method and temperature. Ambient temperature was 22 ± 4 °C.
[b]Microfilariae densities in cores were not considered, owing to high possible sampling error.

nodules at the belly and in the udder/scrotum were exclusively *O. ochengi*, whereas nodules on the fasciae of the thoracic muscles were mainly *O. dukei*. In animals with numerous nodules of *O. ochengi*, some were also found subcutaneously and on the fasciae of the thoracic muscles. A systematic search in whole carcasses for other habitats of the four adult worm species (outside their predilection sites) was not carried out.

3.5. Distribution and relative abundance of microfilariae in the hide

In hides without head, legs, tail and udder/scrotum, the highest densities of microfilariae of *O. gutturosa* were found in the anterior portion of the back and at the belly (Fig. 3). However, considerable numbers were also found at all other sampling sites. In contrast, the microfilariae of *O. ochengi* were located almost exclusively just before the udder/scrotum and at the umbilicus. *Onchocerca dukei* microfilariae were concentrated in the anterior portion of the belly and along the breast. The microfilariae of *O. armillata* were concentrated on the mid-back in one animal, but were more evenly distributed across the whole body surface in

the others. One whole hide infected with *O. gutturosa* and *O. ochengi* was additionally skin-punched at five sites on the head and on the inside of both ears, the lower front and hind legs, the upper and lower tail and the tip of one teat. Other than in their predilection sites (see above), significant numbers of *O. gutturosa* microfilariae were found between the horns (0.26 microfilariae (mff) mg^{-1}). Additional *O. ochengi* microfilariae were recovered from the chin (0.014 mff mg^{-1}) and the teat (0.017 mff mg^{-1}).

The mean number of *O. gutturosa* microfilariae that emerged after 4 h incubation from all skin punches was 0.078 mg^{-1} (or 0.143 mm^{-2}, Fig. 3). This was five times higher than the number for *O. ochengi* (0.014 mff mg^{-1}) and *O. dukei* (0.017 mff mg^{-1}), and 24 times higher than that for *O. armillata* (0.0023 mff mg^{-1}). At the sites of highest concentration (arrows in Fig. 3), the same microfilaria species emerged at 0.221 mff mg^{-1}, 0.093 mff mg^{-1} and 0.047 mff mg^{-1}, respectively (*O. armillata* showed no predilection site).

3.6. Vertical distribution of microfilariae

Four ventral skin strips from cattle infected with *O. gutturosa* (two skins), *O. ochengi* (four skins) and *O. armillata* (three of the cattle with adult worms) were sampled for microfilariae in three different skin layers. It was found that 97.7% of all *O. ochengi* and 92.4% of all *O. gutturosa* microfilariae digested from 60 skin cores (882 and 262 total counts, respectively) were located in the uppermost 2 mm of the skin (epidermis and upper corium); only 2.3% and 7.6%, respectively, were recovered from the lower corium (3–5 mm depth). No microfilariae were found in the subcutis (6–8 mm). No microfilariae of *O. armillata* were detected in any of the skin layers.

3.7. Emergence of microfilariae from skin biopsies

Biopsies from four ventral skins infected with microfilariae of *O. gutturosa* (four skins), *O. ochengi* (four skins) and *O. armillata* (three skins) were incubated for up to 48 h at 37°C and ambient temperature and subsequently digested with collagenase. After 4 h of incubation, only 0–8.2% (mean 2.5%) of the total number of microfilariae recovered after digestion had emerged from the biopsies, irrespective of the microfilaria species, the biopsy method and the temperature of incubation (Table 4). A high emergence rate (88–95% of all microfilariae) was only observed in 2 mm cores incubated at 37°C for 48 h. In slivers and punches incubated for 2 days, 27.3% of the *O. gutturosa* and 71.3% of the *O. ochengi* microfilariae remained in the skin at 37°C, whereas as many as 90.0% and 90.8%, respectively, remained in the skin at ambient temperature. One out of four slivers infected with *O. gutturosa* and *O. ochengi* and all three slivers infected with *O. armillata* released no microfilariae after 4 h when incubated at ambient temperature. They were thus 'false microfilariae negative' according to our routine skin snip method. The mean microfilarial density (after digestion of biopsies) of *O.*

armillata was more than 100-fold lower than that of the two other species (Table 4).

4. Discussion

More than 50% of the cattle examined were concomitantly infected with three (Adamawa) or even four (Sudan savanna) *Onchocerca* species. This prevalence of bovine onchocercosis is high as compared with that found in the Sahel savanna of North Cameroon (Graber et al., 1966) and at other study sites in Africa (Amegee, 1974; Ottley et al., 1985).

For the unknown *Onchocerca* microfilaria species, which was found in only two of 264 animals and in small numbers, cattle are probably not the main hosts. Instead, this species might be, as has been proposed for *O. denkei* (Bain et al., 1982), a parasite of wild ungulates.

Cattle from the Sudan savanna were more frequently infected with *O. ochengi* than those from the Adamawa highlands, despite high biting rates of its vector, *S. damnosum* s.l., in the highlands (Wahl, 1991; Wahl et al., 1994). This may be due to the seasonal disappearance of small tributaries in the Sudan savanna and the nomadic life of the Mbororo herdsmen, which brings the Fulani cattle more frequently in contact with large rivers, the preferred breeding sites of *S. damnosum* s.l. Cattle in the Adamawa highlands, where small tributaries flow throughout the year, are generally kept more stationary, and some of them may never be exposed to *O. ochengi* transmission. Although the overall infection rate is thus lower, in some highland sites the prevalence in adult cattle exceeds 90% (Trees et al., 1992). *Onchocerca dukei* is rare in the highlands, because its vector, *Simulium bovis*, is scarce in this area (Wahl and Renz, 1991). The uniformly high prevalence of *O. gutturosa* in both study areas may indicate that the vectors, as in Sierra Leone and in the Sudan, are ceratopogonid flies, which breed in a wide range of landscapes.

The high infection rates of Gudalis in some areas make it unlikely that the observed differences in prevalence between the study areas are due to differences in the susceptibility of the two cattle breeds.

The age-related differences in prevalence between the species and between the study areas indicate that wherever cattle are exposed to a high rate of *Onchocerca* transmission, maximal prevalences are reached in middle-aged animals and then decline in older ones. A similar pattern has previously been reported for the microfilarial prevalence of *O. gutturosa* in Togo (Denke, 1986) and of *O. armillata* in the Sudan (Atta El Mannan et al., 1984). Such a decline in prevalence with age in hyperendemic areas might indicate that cattle acquire a certain degree of immunity, as has already been shown to occur against microfilariae of *O. ochengi* (Trees et al., 1992).

The prevalence of the four bovine *Onchocerca* species was apparently equal in male and female cattle. This is in agreement with most other studies on bovine onchocercosis (e.g. Schillhorn van Veen and Robl, 1975; Denke, 1986). In the

Sudan, however, a higher prevalence and density of *O. armillata* microfilariae was found in bulls than in cows (Atta El Mannan et al., 1984). *O. lienalis* also seems to be more frequent in male cattle (Beveridge et al., 1979; Ferenc et al., 1986).

Infections of *O. gutturosa*, *O. ochengi* and (most probably) *O. dukei* can be detected by the identification of microfilariae from a single ventral skin sliver with equally high confidence as by the (laborious) search for adult worms (Tables 1 and 3). However, when cattle were examined by both methods, it was revealed that 7–19% of the infections would have been missed if only one method had been used (Table 3). The true prevalences are therefore roughly 10% higher than the ones determined by the skin snip or the adult worm method alone. However, the sensitivity of the skin snip method can probably be increased considerably by mincing the biopsies and incubating them at 37°C for at least 24 h (see below). In our study area, the prevalence of *O. armillata* must be assessed by examination of aortae, as microfilariae occur in very low densities in the skin (Fig. 3, Table 4) and are therefore often not detected by the skin snip method (Tables 1 and 3).

The microfilariae of *O. ochengi* and *O. dukei* were located almost exclusively ventrally. Bain et al. (1977) found high densities also in the legs, which we could not confirm in one animal examined. The accumulation of these species in the lower body parts relates well to the preferred feeding sites of their vectors (Wahl and Renz, 1991; Wahl, 1991). *Onchocerca gutturosa* microfilariae in this study were found in high densities on the back, between the horns and at the belly. The concentration at predilection sites was, however, much less marked than with *O. ochengi* and *O. dukei* (Fig. 3), and with *O. gutturosa* in Sudanese cattle (ElBihari and Hussein, 1978). In Sierra Leone, *O. gutturosa* microfilariae were found in high densities also around the dewlap, the brisket and upper forlegs (Trees et al., 1989). As in Togo and Sierra Leone (Bain et al., 1977; Trees et al., 1989), *O. armillata* microfilariae in this study were more evenly distributed across the whole body surface and always occurred in low numbers in the skin. In other studies, however, they were found concentrated around the hump (ElBihari and Hussein, 1976) and in higher densities (ElBihari and Hussein, 1976; Atta El Mannan et al., 1984; Ogbogu et al., 1990). A differing distribution of skin microfilariae of the same species in different geographical regions might be due to an adaptation to the biting habits of the local vectors or to different predilection sites of the adult worms (Muller, 1979; Trees et al., 1989). Our experiments show that the low density of *O. armillata* microfilariae in this study as compared with the high densities reported from the Sudan and Nigeria was not due to different biopsy methods, but may be a geographical variation of the parasite.

Reports on the microfilarial density of bovine *Onchocerca* species in the skin of their hosts are few and vary greatly. Densities of *O. gutturosa*, for example, range from approximately 0.17 mff mg^{-1} skin (Trees et al., 1989; Ogbogu et al., 1990) to 22 mff mg^{-1} (Mwaiko, 1981; Zahner and Schulz-Key, 1990). The low numbers of microfilariae that emerged from skin biopsies after 4 h in this study, especially of *O. armillata*, prompted us to investigate whether a high proportion

of microfilariae might remain in the biopsies or be found in deeper skin layers. Our experiments showed that almost all microfilariae of *O. gutturosa* and *O. ochengi* were located in the uppermost skin layer of 2 mm depth and that *O. armillata* microfilariae were not 'hidden' in deeper skin layers. However, the three biopsy methods tested released microfilariae inefficiently; a mean of 97.5% of all microfilariae remained in the biopsies after 4 h and 53.7% after 2 days of incubation. When the microfilariae were left to emerge at ambient temperature, 90% remained in the biopsies, even after a 2 day incubation. The low emergence rates might be due to the large size of the biopsies in combination with the tight texture of cattle skin; possibly, only peripheral microfilariae received outer stimuli or were capable (at elevated temperatures) of migrating to the surface of the biopsies. The actual mean microfilarial densities of the four species can therefore roughly be calculated by multiplying the relative abundances found during hide-mapping by a factor of at least 40. Thus *O. gutturosa*, *O. ochengi*, *O. dukei* and *O. armillata* would have mean densities of 3.1 mff mg^{-1}, 0.6 mff mg^{-1}, 0.7 mff mg^{-1}, and 0.092 mff mg^{-1}, respectively, for the whole body surface and 9.3 mff mg^{-1}, 3.8 mff mg^{-1} and 1.9 mff mg^{-1}, respectively, at the sites of highest concentration (*O. armillata* had no predilection site). The densities per surface area are 1.83 times higher (574 mg punch, i.e. 314 mm^2). The microfilarial density of *O. armillata* is probably even lower, as it was calculated mainly from one very heavily infected animal (compare Fig. 3 and Table 4). If these microfilarial densities can be considered representative for the African bovine *Onchocerca* species in hyperendemic areas, they would be much lower than that of *O. volvulus* in hyperendemic areas, which is in the order of 50–100 mff mg^{-1} (or mm^{-2}) skin snip at the predilection sites of the microfilariae (e.g. Renz et al., 1987).

Acknowledgements

The unknown microfilaria species was identified by Dr. O. Bain, Muséum National d'Histoire Naturelle, Paris. The skilful and efficient technical assistance of Mr. D. Ekale is gratefully acknowledged. We are indebted to Dr. Schulz-Key and Professor P. Wenk, Institute for Tropical Medicine, Tübingen, for advice and stimulation. We thank the Ministry of Higher Education and Research of Cameroon for granting research permits to two of us (G.W. and A.R.). This investigation received financial support from the Land Baden Württemberg, the Deutscher AkademischeAustauschdienst, the Studienstiftung des Deutschen Volkes (all FRG) and the Commission of European Communities (TS2/0184-D(AM)).

References

Amegee, Y.E., 1974. Les onchocerques bovines en Afrique. Etude de deux formes nodulaires à *Onchocerca dukei* et *O. dermata* au Togo, Thèse de Doctorat Vétérinaire, Faculté de Médecine et de Pharmacie, Université de Dakar.

Atta El Mannan, A.M., Hussein, H.S., El-Sinnary, K. and Magzoub, M., 1984. *Onchocerca armillata* prevalence and pathology in Sudanese cattle. Ann. Trop. Med. Parasitol., 78: 619–625.

Bain, O., 1981. Le genre *Onchocerca*: hypothèses sur son évolution et clé dichotomique des espèces. Ann. Parasitol. Humaine Comparée, 56: 503–562.

Bain, O., Denke, A.M. and Amegee, Y., 1977. Les onchocerques des bovins au Togo: les microfilaires et leurs distributions. Ann. Univ. Bénin, 3: 117–123.

Bain, O., Vassiliades, G. and Delbove, P., 1982. Une nouvelle onchocerque, parasite de bovin domestique, au Sénégal. Ann. Parasitol. Hum. Comp., 57: 587–591.

Beveridge, I., Kummerow, E. and Wilkinson, P., 1979. The prevalence of *Onchocerca lienalis* in the gastro-spleenic ligament of cattle in North Queensland. Aust. Vet. J., 55: 204–205.

Davies, J.B., Trees, A.J., McCall, P.J., Bockarie, M.J., Thomson, M.C. and McKellar, S.B., 1989. On the possibility of bovine *Onchocerca* species infecting *Simulium damnosum* s.l. in the forest zone of Sierra Leone. II. Biting densities and filarial infections in *Simulium* spp. and *Culicoides* spp., Ann. Trop. Med. Parasitol., 83: 603–614.

Denke, A.M., 1986. The prevalence of *Onchocerca* spp. in cattle in Northern Togo in 1979. Trop. Med. Parasitol., 37: 46–48.

Denke, A.M. and Bain, O., 1978. Données sur le cycle d'*Onchocerca ochengi* chez *Simulium damnosum* s.l. au Togo. Ann. Parasitol. Hum. Comp., 53: 757–760.

ElBihari, S. and Hussein, H.S., 1976. The distribution and redescription of the microfilariae of *Onchocerca armillata* Raillet and Henry, 1909. Sudan J. Vet. Sci. Anim. Husb., 17: 77–85.

ElBihari, S. and Hussein, H.S., 1978. *Onchocerca gutturosa* (Neumann, 1910) in Sudanese cattle. I. The microfilariae. Rév. Elev. Méd. Vét. Pays Trop., 31: 179–182.

ElSinnary, K. and Hussein, H.S., 1980. *Culicoides kingi*, Austen: a vector of *Onchocerca gutturosa* (Neumann, 1910) in the Sudan. Ann. Trop. Med. Parasitol., 74: 655–656.

Ferenc, S.A., Copeman, D.B., Turk, S.R. and Courtney, C.H., 1986. *Onchocerca gutturosa* and *Onchocerca lienalis* in cattle: effect of age, sex and origin on prevalence of onchocerciasis in subtropical and temperate regions of Florida and Georgia. Am. J. Vet. Res., 47: 2266–2268.

Graber, M., Fernagut, R. and Oumate, O., 1966. Helminthes des zébus adultes de la région de Maroua (Nord-Cameroun). Rev. Elev. Méd. Vét. Pays Trop., 19: 149–162.

Muller, R., 1979. Identification of *Onchocerca*. In: A.E.R. Taylor and R. Muller (Editors), Problems in the identification of parasites and their vectors. Symposium of the British Society of Parasitology, 27 October 1978, London. Blackwell Scientific, Oxford, UK, Vol. 17, pp. 175–206.

Mwaiko, G.L., 1981. The development of *Onchocerca gutturosa* Neuman to infective stage in *Simulium vorax* Pomeroy. Trop. Med. Parasitol., 32: 276–277.

Ogbogu, V.C., Bablis, J.M. and Ajanusi, O.J., 1990. Prevalence of microfilariae in cattle at slaughter in Zaria, Nigeria. Vet. Parasitol., 36: 171–175.

Omar, M.S., Denke, A.M. and Raybould, J.N., 1979. The development of *Onchocerca ochengi* (Nematoda: Filarioidea) to the infective stage in *Simulium damnosum* s.l. with a note on the histochemical staining of the parasite. Trop. Med. Parasitol., 30: 157–162.

Ottley, M.L., Moorhouse, D.E. and Holdsworth, P.A., 1985. Equine and bovine onchocerciasis. Vet. Bull., 55: 571–588.

Renz, A., Wenk, P., Anderson, J. and Fuglsang, H., 1987. Studies on the dynamics of transmission of onchocerciasis in a Sudan-savanna area of North Cameroon V. What is a tolerable level of Annual Transmission Potential? Ann. Trop. Med. Parasitol., 81: 263–274.

Schillhorn van Veen, T. and Robl, M.G., 1975. Aortic onchocerciasis in cattle in Zaria (Nigeria). Rev. Elev. Méd. Vét. Pays Trop., 28: 305–310.

Schulz-Key, H., Albiez, E.J. and Büttner, D.W., 1977. Isolation of living adult *Onchocerca volvulus* from nodules. Trop. Med. Parasitol., 28: 428–430.

Séchan, Y., 1984. Développement d'onchocerques animales chez le veceur de l'onchocercose humaine *Simulium sirbanum* Vajime et Dunbar, 1975 (Diptera: Simuliidae) en zone subsahélienne du Mali, Afrique de l'Ouest, Cahiers O.R.S.T.O.M. (Editors), Travaux et Documents de l'O.R.S.T.O.M., No. 178, 234 pp.

Trees, A.J., 1994. *Onchocerca ochengi: mimic, model or modulator of O. volvulus*? Parasitol. Today, 8: 337–339.

Trees, A.J., McCall, P.J. and Davies, J.B., 1989. On the possibility of bovine *Onchocerca* species in-

fecting *Simulium damnosum* s.l. in the forest zone of Sierra Leone. I. Parasitological aspects. Ann. Trop. Med. Parasitol., 83: 595–601.

Trees, A.J., Wahl, G., Kläger, S. and Renz, A., 1992. Age related differences in parasitosis may indicate acquired immunity against microfilariae in cattle naturally infected with *Onchocerca ochengi*. Parasitology, 104: 247–252.

Wahl, G., 1991. Die *Onchocerca* Arten der Rinder in Nord-Kamerun und ihre Bedeutung für die Epidemiologie der menschlichen Onchozerkose. Thesis, University of Tübingen, 103 pp.

Wahl, G. and Renz, A., 1991. Transmission of *Onchocerca dukei* by *Simulium bovis* in North-Cameroon. Trop. Med. Parasitol., 42: 368–370.

Wahl, G., Enyong, P., Schibel, J.M., Ngosso, A., Tubbesing, H., Moyoo, R. and Renz, A., 1994. Transmission of *Onchocerca volvulus, O. ochengi* and Type D filariae by *Simulium damnosum* s.l. in North-Cameroon and its influence on the epidemiology of human onchocerciasis. Parasite (Previously: Ann. Parasitol. Hum. Comp.), Vol. 1 (Suppl.), March 1994, in press.

Zahner, S. and Schulz-Key, H., 1990. Rinderonchozerkose in Süddeutschland: Verteilung der Mikrofilarien in ihrem Wirtshabitat. Mitt. Österr. Ges. Tropenmed. Parasitol., 12: 87–94.