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Impact of Livestock Farming on Human Onchocerciasis in Adamawa and North Regions Cameroon

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Abstract

Background: Onchocerciasis or "river blindness" is currently a major cause of blindness in the world. The use of Ivermectin by the high risk population showed its limits. In these regions, cattle raising is the main occupation and human onchocerciasis is reduced since the high density of cattle stocking protects by zooprophylaxis and cross immunization. This work was carried out in order to know if cattle protect humans against human onchocerciasis. To achieve this goal, two high densities of cattle stocking and onchocerciasis zones were selected: Wakwa and Touboro, two zones of Cameroon located on the Vina River.

Methods: Blackflies were collected in each zone and dissected thereafter. Skin biopsies were carried out on volunteers. Infested with Onchocerca blackflies were the subject of detailed accounts and biopsies for microfilariae.

Results: Dissections showed 83.87% of infected blackflies with *O. ochengi* in Wakwa and 82.5% in Touboro. Moreover, 17.5% of parasitized blackflies were parous of *O. volvulus* in Touboro against 16.13% in Wakwa. Biopsy results displayed that Wakwa recorded a rate of 2%, while Touboro 4% onchocerciasis microfilariae.

Conclusions: These results show that the cattle raising influence the transmission of onchocerciasis in humans.

Keywords: Onchocerciasis; Blackflies; Biopsy; Microfilariae

Introduction

Simulium damnosum is a complex group of over 40 sibling species of blackfly distributed throughout the Sub-Saharan Africa and the Arabian Peninsula [1]. Blackflies are responsible for more than 90% of onchocerciasis cases worldwide and 95% of cases in Africa [1]. About 54 species of *Simulium* have been reported to bite man, transmitting onchocerciasis [1]. In Cameroon, the cytospecies and cytotypes reliably recorded include *S. damnosum* s.s.; Nile form, *S. damnosum* s.s.; Volta form, *S. sirbanum* s.l., *S. squamosum* A , *S. squamosum* B, *S. squamosum* C, *S. squamosum* D and *S. mengens* [2]. Onchocerciasis is a parasitic disease caused by a worm of Onchocerca genus, parasitic nematode hoofed mammals [3,4] whose vectors are the blackflies. In humans, *Onchocerca volvulus* is primarily responsible for this disease [3]. Onchocerciasis is also called "river blindness" due to the fact that it is contacted in the vicinity of rapid streams [5]. This disease attacks not only the skin like nodule, hanging groin (6.5 million suffer from severe skin diseases), but also results in the permanent loss of sight. In a recent survey of Schwartz et al. [6] in the Central African Republic, onchocerciasis was found responsible for 73.1% cases of blindness in the population [7].

Onchocerciasis is currently a major cause of blindness in the world. The eradication of the disease is a great challenge for modern public health [7]. *O. volvulus* not only causes chorio-retinitis but also produces severe keratitis. Besides, it is developed as a result of an inflammatory response to dead microfilariae which are degenerated thereafter into the corneal stroma [7]. The infestation may clinically present palpable subcutaneous nodules. The female worms produce thousands of microfilariae that cross embryonic fibrous tissue and invade the epidermis [8]. *O. volvulus* embryonic forms migrate through the skin and causes severe itching, disfiguration, and ocular lesions [9]. Keratitis and blindness can be the result of heavy parasite loads in the human host over time. The parasite is encapsulated in fibrous tissue [9]. The female adult worm measures 30 cm to 80 cm long while the male measures 3 cm to 5 cm [8]. The female produces microfilariae whose length is between 250 µm to 300 µm.

The number of people infected by *O. volvulus* is estimated at 17.7 million through the 34 countries worldwide. Over 99% are found in Inter-Tropical Africa, Central and South America and the Middle East [4]. It is considered that 270,000-360,000 people are blind due to onchocerciasis [10]. In addition, about 500,000 other people suffer from severe visual disability associated with onchocerciasis [11]. We believe that over 860,000 people are currently having ocular involvement and are likely to become totally blind if no treatment has been initiated. According to Boussinesq [11], onchocerciasis is the fourth leading cause of blindness worldwide after cataract (16 million people), trachoma (5.9 million), and various forms of glaucoma (5.1 million) [11]. People affected by the disease lose 15% of their annual revenues for treatment. In addition, 30% of uncultivated land, school non-attendance of children and exacerbation of rural poverty are linked to onchocerciasis [11].

In 1983, 1.2 million Cameroonians' blind, have been identified by United Nation demographic report, 5,000,000 infected and 30,000 blinds [2]. Currently there are 32,000 blinds due to onchocerciasis. Facing these increasing and alarming victims over years, appropriates' measures shall have taken to predict the risk abandonment land which was the case in West Africa before independence [7].

Different drugs have been administered but showed their limits. For example, Microfilaricid (Diethylcarbamazin, Ivermectin and Modexin) cannot kill adult worm, Macrofilaricid (Suramin, Doxycyclin, Rifampicin, Azithromycin, Fosfomycin) cannot kill microfilaria. In addition, elders, pregnant women and children cannot use them because of their side effects. Beside this, *Simulium damnosum* is the same vector transmitting *Onchocerca volvulus*, *O. ochengi*, *O. gutturosa*, *O. armilata*, *O. dukei* and *O. bovis* cattle parasites in Cameroon

This work investigated if cattle raising is limiting factor for spreading human onchocerciasis. To assess the relevance of our research, blackflies and bloodless biopsies were submitted for parasite evaluation.

Methods and Material

Description of the study site

In the Adamawa Region: The Adamawa Region of Cameroon is located in Central Africa between the 6th and 8th degree of north latitude and the 10th-16th degree east longitude [12]. It covers an area of about 62,000 km^2, with altitudes of 900 m to 1,500 m [12]. It is characterized by two seasons: a rainy season and a dry season. The average annually rainfall, temperature and humidity are about 1,500 mm, 22℃ and 70% respectively.

The location of IRAD-Wackwa is as follows: 7°12.035'N of longitude, 13°34.933'E of latitude and 1032 m of altitude. That of Mandjiri as follows: 7°13.280'N of longitude, 13°35.637'E of latitude and 1065 m of altitude. Adamawa with its temperate climate has a permanent pasture [13]. The main activity is cattle raising with reportedly more than 5,600,000 heads estimated in 2005 [14] (Figure 1). Cattle production in the region represents about 86.6% of domestic livestock population and more than 28% of the national cattle head estimated at about 10 million [12]. The flocks do not go on transhumance in the dry season. Watering knows no major obstacles. The existence of grasslands is another parameter that promotes the raising of cattle.

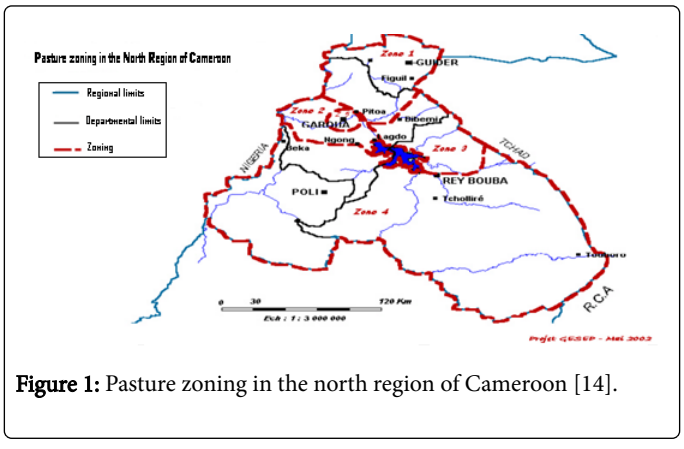

Figure 1: Pasture zoning in the north region of Cameroon [14].

In the North Region: The North region extends between 8° and 10°N latitude 12° and 16°E. The climate is of Sudanese type with six months of rainy season and six months of dry season, and an average temperature of 35°C. Agriculture and livestock account for 90% of the population, north Province, with 4 million of bovine in 2005 [14]. Touboro belongs to the Sudano-Sahelian zone whose vegetation is of shrubs and herbs. Geographical features are: longitude: 7°46'60"N, latitude: 15°22'0"E. It is located at an altitude of 531 meters above the sea. Cattle increasing are an important for livestock in this area. Lagaye is a village located 10 km from the city of Touboro on the road to Mbaïboum.

Skin snip collection

Biopsy as part of this study was a skin snip collected for search of microfilariae responsible of onchocerciasis. 50 people in each area were subjected for biopsy and biopsies were performed at the iliac crest, scapula and calf of each volunteer person (Table 1).

Localities	Number of volunteers	% of blindness	% of onchocerciasis
Mandjiri	50	2%	2%
Touboro-Lagaye	50	4%	4%
Wakwa-IRAD	50	0%	0%

Table 1: Rate of blindness and onchocerciasis in different localities.

Samples were collected with sterilized razor blade per person. Small pieces of skin about 2 mm to 3 mm of diameter were collected. The sampling site was previously disinfected with alcohol swab. The skin fragment was left for 15 minutes in 5% dextrose for microfilariae to leave the skin. Thereafter, 2 ml of 10% formalin was added and we obtain 4% of solution (w/v). Bottles were hand-shaken for proper mixture of the contents. Then, the bottles were closed with their screw caps and brought back to the laboratory for examination. The contents of each vial was labelled per volunteer then transferred in dry tubes. Tubes were grouped in lots of 10 and placed in the cooler, which had a capacity of 70 tubes. Tubes were centrifuged for 3 minutes at 10,000 rpm. The supernatant from each conical tube was put back in the bottle according to the serial number of samples. The pellet of each tube was removed and spread on object slide without thin slide. All slides were observed at 100X under microscope. Dead microfilariae were well observed by staining with an angular curved tail that is their characteristic and accounted (Figure 2).

Capture and storage of blackflies

Blackflies were aspired during their meal (Figure 2) and were collected in a plastic tube EDTA K3 for proper conservation and to avoid they get dried.

Identification of the blackfly

Blackfly was deposited on a slide which carried a drop of saline solution. The observation showed a foreleg wearing thin bristles combs, while the hind leg has a white spot band occupying three quarters of basi-tarsus. The abdomen bears silvery and shiny scales. This identification allowed us to avoid the confusion with other blackflies. Then, the dissection was performed by using two

entomological needles and dissecting forceps. The forceps were used to pick up a blackfly from the samples and deposit it on the slide. A needle kept the blackfly while another one was used to remove the first abdominal segments as well as the various internal organs (stomach and gut, malpighian tubes, ovaries and fat) as described by [15,16]. Abdomen, thorax and head were separately dissected and examined. Larvae of *Onchocerca* were counted.

Figure 2: Capture of blackflies near the Vina River Touboro (Vokna).

Internal organs showed the differences between nulliparous blackfly and parous blackfly, under a binocular microscope by highlighting the follicular relics in the ovaries, the presence of residual eggs indicating a previous nesting female, the presence of abundant abdominal fat, and presence of residual blood in the stomach indicating a previous blood meal. Overall malpighian tubes in nulliparous were opaque, thick and swollen [16].

The nulliparous ovaries were clear, slightly larger and more spherical, stretched very slowly before breaking abruptly. However, the parous had clear Malpighian tube under transmitted light, and ovaries stretched along their full length and tore slowly. The best examination was done in saline, 45% NaCl under a stereo microscope with 50X magnification.

The thorax muscles and the head were separately lacerated where the L1 and L2 and L3 larvae were located, respectively. The difference between *O. volvulus* and *O. ochengi* was observed in the size of L3 larvae; L3 for *O. volvulus* measured 440 microns to 700 microns and that of *O. ochengi*, 750 microns to 850 microns. This method was scrutinized for search of *O. volvulus* and *O. ochengi* larvae. The number and the developmental stages were established to determine the infestation rate and the parasitic loads of female *Simulium damnosum* complex.

Ethical approval: After sensitizing the community, many volunteers accepted to know their dermal microfilaria.

Results

Captured and dissected blackflies in Mandjiri

During three months, 594 blackflies were captured and 525 dissected out of which 346 were parous (blackflies those have laid eggs at least one time), 31 were infested rating 8.95% of infestation. 26 blackflies were infested by *O. ochengi* and five blackflies were infected by *O. volvulus* (Tables 2 and 3).

	August	October	November	Total
Blackflies captured	262	116	216	594
Blackflies dissected	193	116	216	525
Parous	88	99	159	346
Nulliparous females	105	17	57	179
Parous infectious (L1 / L2 / L3)	7	5	19	31
Number of parasites (*O. volvulus* + *O. ochengui*)	**20**	**7**	**53**	**80**
Parous infectious (with L3)	4	3	12	19
Rate Parous infectious	7,95%	5,05%	11,94%	8,95%
Rate Infected parous	4,54%	3,03%	7,75%	5,49%
Parous infectious (Number of *O. volvulus*)*	4 (11)	0(0)	1(4)	5(15)
Parous infectious(Number of *O. ochengi*)**	3(9)	5(7)	18(49)	26(65)
*Parous infectious (Number of *O. volvulus*): 4 (11): 4 means parous infectious and 11 *O. volvulus*				
**Parous infectious (Number of *O. ochengi*): 3 (9) means 3 parous infectious and 9 *O. ochengi.*				

Table 2: Results of the dissection of blackfly Mandjiri.

	Captured blackflies	Dissected blackflies	Parous infectious	Nulliparous Non infectious	Infectious parous	Rate of infectious parous
August	262	193	88	105	7	7.95
October	116	116	99	17	5	5.05
November	216	216	159	57	19	11.94
Total	594	525	346	179	31	8.95

Table 3: Results of the dissection of blackfly Mandjiri.

Captured and dissected blackflies in Touboro

During five months, 544 blackflies captured, 544 dissected, 399 blackflies were parous, and 40 were infested rating 10, 02% infestation. 33 blackflies infested by *O. ochengi* rating 8, 27%, seven blackflies were infected by *O. volvulus* giving a rate of 1.75% (Tables 4, 5 and 6).

	August	Septembe r	Octobe r	Novem ber	Decemb er	Total
Blackflies (females) captured	57	191	111	72	113	544
Females dissected	57	191	111	72	113	544
Parous	**44**	**157**	**90**	**35**	**73**	**399**
nulliparous	13	34	21	37	40	145
Infected parous (L1 / L2 / L3)	**3**	**11**	**9**	**6**	**11**	**40**
Nber parasites (*O. volvulus* + *O. ochengui*)	5	20	10	9	23	65
Parous infectious (with L3)	2	5	7	4	6	24
Rates of infectious parous	6.81%	7,00%	10.00%	17.14%	15.06%	10.0%
Rate Infected parous	5.54%	3.18%	7.77%	11.42%	8.21%	6.01%
Parous infectious (Number of *O. volvulus*)*	0(0)	3(6)	1(2)	1(2)	2(3)	7(13)
Parous infectious (Number of *O. ochengi*)**	3(5)	8(14)	8(8)	5(7)	9(20)	33(5)
*Parous infectious (Number of *O. volvulus*.): 4 (11) 4 means parous infectious and 11 *O. volvulus*						
**Parous infectious (Number of *O ochengi*.): 3 (9) means 3 parous infectious and 9 *O. ochengi*						

Table 4: Results of the dissection blackflly Touboro.

	Parasites	*Parous non infectious	Parous infected	Total parous
August	(0;5)=5	**41 (44-3)**	3	44
September	(6;14)=20	**146 (157-11)**	11	157
October	(2;8)=10	**81 (90-9)**	9	90
November	(2;7)=9	**29 (35-6)**	6	35
December	(3;20)=32	**62 (73-11)**	11	77
Total	(13;54)=67	**359 (399-40)**	40	399
Parous non-infectious comes from the difference between parous and parous infected.				

Table 5: Results of the dissection of blackfly Touboro.

	Captured blackflies	Dissected blackflies	Parous infected	Nulliparous non infected	Infectious Parous	Rate of infectious parous
August	57	57	3	13	2	14.29
September	191	191	11	34	5	3.18
October	111	111	9	21	7	7.78
November	72	72	6	37	4	11.43
December	113	133	11	40	6	14.29
Total	544	544	40	145	24	3.18

Table 6: Results of the dissection blackfly Touboro.

NB: Variation of dissected blackflies and *Onchocerca volvulus* for Wakwa and Touboro are according the dissected blackflies, not *O.volvulus* and *O. onchengi* founded same time in the blackfies collected.

Discussion

Many studies on onchocerciasis were conducted in Cameroon. This work is the first to be realized. We arbitrarily selected a quota of 200 blackflies dissecting per month. The parous blackflies and infested increasing in the end of the rainy season demonstrated by the Figures 3 and 5.

This analysis showed that November and December are dangerous. Exposed persons should protect themselves against nuisance and biting blackflies. The evolution of parous has been increasing in the two places (Figures 5 and 6).

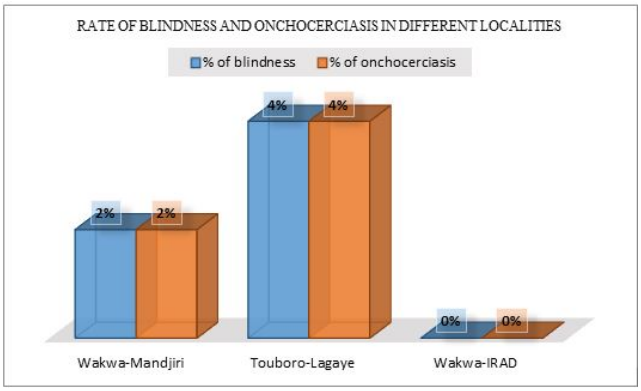

Figure 3: Results of biopsies.

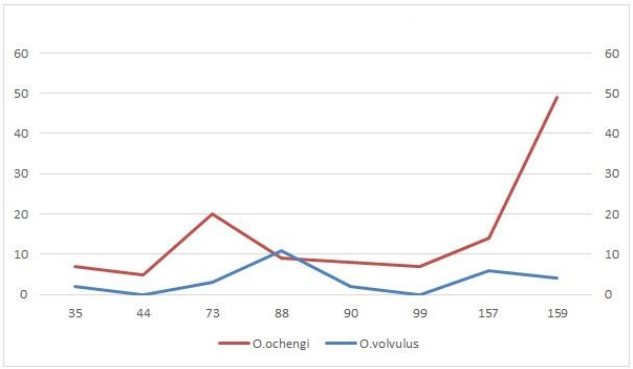

Figure 4: Trendline between *O. volvulus* and *O. ochengi*.

We noted that there's more to parous Wakwa that Touboro. This abundance of parous can be explained by the abundance of livestock (females are blood-sucking blackflies) which corroborate with Martinez who conducte his study about influencing the abundance of *Culicoides* in avian nets. Infestation of *O. volvulus* to Touboro began in November.

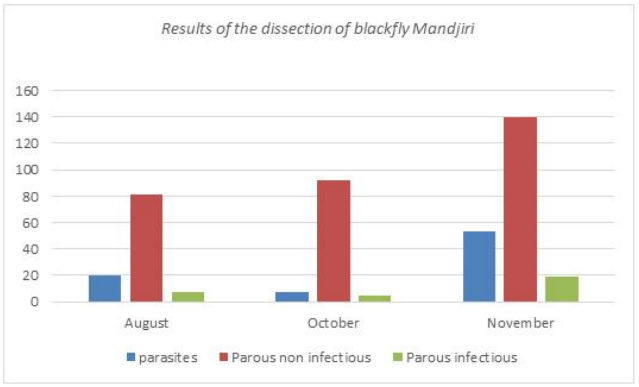

Figure 5: Distribution of parasites in the capture period.

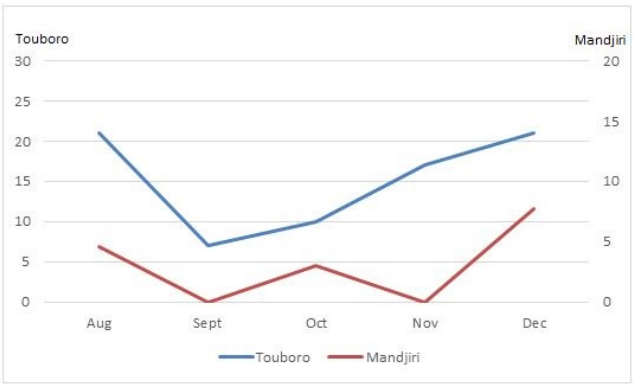

Figure 6: Evolution of the rate of parous infected in the period.

Wakwa site for a total of 346 parous dissected. 31 were parasitized with parous 8.95% infestation *Onchocerca*. 26 were *O. ochengi* representative 26/31=83.87%. For all pares dissected 26/346=7.51% represented the infestation rate of *O.ochengi*. For df=3. The value of x^2 5% was 7.81%. The value found was less than 7.51% to 7.81%. We can conclude that this result is significant. The IRAD capture point Wakwa recorded a percentage of 100% *O. ochengi*. Mandjiri the capture point for a total of 258 dissected. 5 females were parasitized giving a rate of 1.94% *O. volvulus* and 98.06% representative O.ochengi. Of the 31 parous parasitized, 5/31=16.13% were parasitized by *O. volvulus* compared to all parous dissected 5/346=1.45% of infestation *O. volvulus*. With df=3. 5% was 7.81%. The value found (1.45%) was very lower than this threshold value. This result is significant. WHO recommends 01 parous for 2000 blackflies dissected to expect disposal in an area [17]. The onchocerciasis transmission is active Mandjiri.

Skin snips were taken on 50 volunteers in Mandjiri. Only one microfilaria was recorded on one slide representing 2% of collected samples. In Lagaye (Touboro), two slides revealed one microfilaria each, rating 4%. In Wakwa-Mandjiri, one case of blindness was registered whereas Lagaye recorded two cases of blindness and three cases of onchocerciasis nodules among the 50 volunteers.

The results of the biopsies revealed microfilaria one *O. volvulus* found in a child of 6 years. One blindness case was found in a man of 70 years native of Mandjiri.

We can say that this point of capture is the most dangerous because it is the location of release of *O. volvulus*. This study was conducted one month after the distribution of Mectizan (ivermectin) by the program against onchocerciasis of the Regional Delegation of Adamawa. Probably this distribution had an impact on the lethality of microfilariae because most skin biopsies were negative.

Lagaye-Touboro for a total of 399 parous dissected. 40 were parasitized with parous 10.03% infestation by *Onchocerca* (Table7).

	Touboro	Mandjiri
August	21	4.54
September	7	No capture
October	10	3.03
November	17	No capture
December	21	7.75
Total	7	

Table 7: Rate of infected parous (%).

These pares parasitized; 33 were infested with *O. ochengi* representative33/40=82.5%. For all pares dissected 33/399=8.27% represented the infestation rate of *O.ochengi*. With df=5. the x^2 5% was 11.07% the value found was less than 8.27% at 5%. This result was significant. Zoophilia blackfly threatening more livestock than humans. The number of cattle dilutes the aggressiveness of blackfkies. Of the 399 black flies captured (7/399=1.75%) of dissected blackflies were infested by *O. volvulus*. The x^2 5%, df=5% was 11.07%.

The infestation rate of *O. volvulus* (1.45%) in Mandjiri-Adamawa was less than 1.75% in Lagaye-Touboro. This showed that the blackflies in Touboro were more infested than those of Wakwa thus livestock Touboro had not diluted enough aggressiveness of blackflies [18].

7 blackflies were parasitized by *O. volvulus* i.e. 7/40=17.5% of the 40 parous parasitized. These results are significant. In addition, 3 persons with cataracts caused by microfilariae and 4 people bore onchocerciasis nodules on 50 examinees. *O. volvulus* rate of 1.45% was observed in Mandjiri-Adamawa which was less than 1.75% in Lagaye-Touboro (Figures 7, 8, 9 and 10).

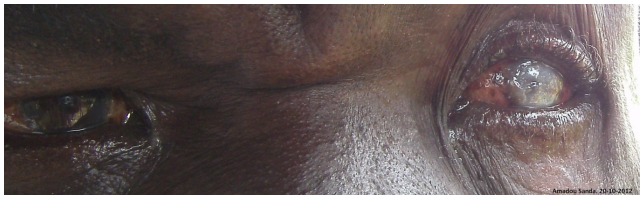

Figure 7: Keratitis ossifying of male 59 years old (Lagaye Touboro).

Figure 8: Nodules of male 45 years old (Lagaye -Touboro).

Figure 9: Blindness of the female 29 years old (LagayeTouboro).

This showed that the blackflies were more infested in Touboro than those of Wakwa. Thus the breeding of cattle in Touboro does not dilute enough aggressiveness of blackflies against human [18]. The study of graphics (Tables 8 and 9, Figures 11 and 12) increase through August to December (e.g.: 44 parous to 159; 5 parasites to 3).

Figure 10: Hanging groin of the female 59 year old (Lagaye Touboro).

Disected	Parous	Parasites
57	44	5
72	35	9
111	90	10
113	73	23
116	99	7
191	157	20
193	88	20
216	159	53

Table 8: Link between dissected, parous and parasites.

Parous	*O.volvulus*	*O.ochengi*
35	2	7
44	0	5
73	3	20
88	11	9
90	2	8
99	0	7
157	6	14
159	4	49

Table 9: Link between *O.volvulus* and *O.ochengi*.

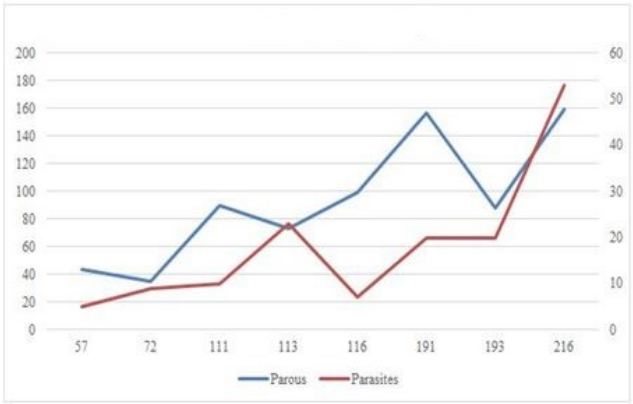

Figure 11: Trendline between dissected, parous and parasites.

The climate of Adamawa provides a framework for the cattle breeding of 1.9 million heads having been registered in connection with vaccination against 5.6 million cattle that account Cameroon. The North Region of the country has vaccinated 779.000 heads over all the cattle in the country [19,20]. Adamawa represents 74.51% of the cattle produced by the two Regions while the North Region has 25.50%. Adamawa cattle represent 3 times the North Region's production. In the Department of Vina, there is 2.55 million heads of cattle. This is evidence that would explain the differences of infestation in the two regions of Cameroon [21].

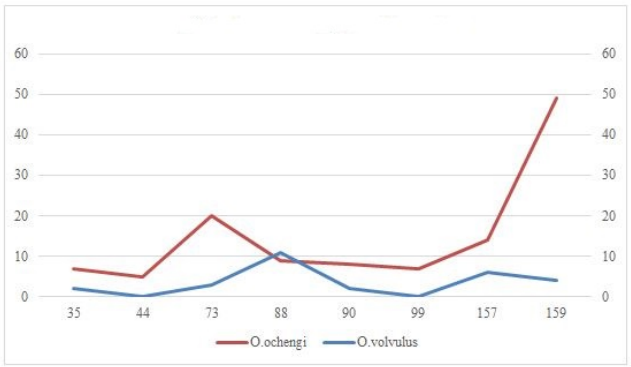

Figure 12: Trendline between *O. volvulus* and *O. ochengi*.

The climate of Adamawa provides a framework for the cattle breeding of 1.9 million heads having been registered in connection with vaccination against 5.6 million cattle that account Cameroon. The North Region of the country has vaccinated 779.000 heads over all the cattle in the country [19,20]. Adamawa represents 74.51% of the cattle produced by the two Regions while the North Region has 25.50%. Adamawa cattle represent 3 times the North Region's production. In the Department of Vina, there is 2.55 million heads of cattle. This is evidence that would explain the differences of infestation in the two regions of Cameroon [21].

We can state that the cattle are a limiting factor of blackfly infestation by *O. volvulus* responsible for human onchocerciasis. That is why in the Northern Region. Ocular and dermal manifestations are more pronounced than in Adamawa. Livestock farming therefore play a very important role in the control of human onchocerciasis. This corroborates with Fréderic Paris [22] who stated that "the incidence of the disease is almost zero among the Bororo pastoralists while the areas of transhumance herds have a protective effect due to the bovine herd. But beyond a ratio of 4 oxen per man there is cancellation of the anthropophile preference and even dilution of bites in herds".

Conclusion

This study showed that the rates of infected blackflies by *Onchocerca* varied between the two zones and *O. volvulus* is more common in Touboro (Nord) than in Wakwa (Adamawa). Thus livestock also influence among other factors the prevalence of human onchocerciasis in both regions. Longitudinal studies of anthropophagic blackflies activity should be done in combination with entomological and parasitological study in 10 Regions of Cameroon.

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