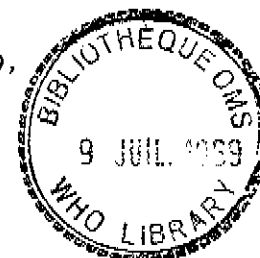




TRIAL OF TWO IMMUNOLOGICAL TESTS
(INTRADERMAL TEST AND COMPLEMENT FIXATION TEST)
FOR THE DETECTION OF FILARIASIS IN POPULATION GROUPS
IN UPPER VOLTA WHERE WUCHERERIA BANCROFTI (COBBOLD, 1877),
ONCHOCERCA VOLVULUS (LEUCKART, 1893) AND
DIPETALONEMA PERSTANS (MANSON, 1891) CO-EXIST

by

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1. INTRODUCTION

1.1 Aim of the study

The present investigation was made at the request of the World Health Organization (Parasitic Diseases unit, Division of Communicable Diseases) in accordance with the recommendation of the Expert Committee on Onchocerciasis, on Filariasis (Wuchereria and Brugia infections) and on Immunology and Parasitic Diseases (WHO, 1962, 1965, 1966, 1967).

A detailed review of the immunological methods used for the diagnosis of filariasis has been published by Kagan (1963). It emerges in particular from that study that the results obtained by the different authors are difficult to compare in view of the lack of standardization, as regards both the antigens used and the methods employed.

The aim of our study was to test two purified filarial antigens in areas where several forms of filariasis co-exist. One was the FST antigen (also called FSC-D1) used for the intradermal test and the other the FP 111 antigen used for the complement fixation test. Both were prepared by Professor Sawada (School of Medicine, Gunma University, Maebashi, Japan) and were sent us from Geneva by Professor Ansari and Dr Kent.

So as to facilitate comparison of our results with those of other authors using the same antigens, we followed the methods advocated by WHO for the intradermal test and by Professor Sawada for complement deviation.

1.2 Description of the villages surveyed

1.2.1 Geography, climate and vegetation

The three villages selected for the survey are situated in the Bobo-Dioulasso region between 10° and 12° North latitude and 4° and 5° West longitude (see Maps 1, 2 and 3).

The climate is of the Sudanese-Guinea type (Aubreville, 1950), characterized by a dry season (October-April) and a rainy season (May-September). The average annual rainfall lies between 1100 and 1200 mm.

In regard to vegetation, this is a wooded savannah region with high grass, resulting from degradation of the original dense forest which survives in the form of residual forests. The permanent water courses are bordered by forest galleries.

1.2.2 Situation of the three villages surveyed (see Map 3)

The first of these villages, Samandéni (4° 28' West longitude and 11° 28' North latitude) is situated 45 km north-west of Bobo-Dioulasso, on the banks of the Black Volta. Onchocerciasis is predominant there.

The second is Tingréla (4° 49' West long. and 10° 39' North lat.) situated close to Banfora on the shores of a lake in a marshy region, 90 km south of Bobo-Dioulasso. This is a Bancroftian filariasis zone.

The third village is Banankélédaga (4° 19' West longitude and 11° 19' North latitude) situated some 15 km north-west of Bobo-Dioulasso. It was chosen as control. The village is not free from filariasis (such a village seems very difficult to find) but nevertheless a certain percentage of subjects are encountered in whom neither Onchocerca volvulus, nor Wuchereria bancrofti nor Dipetalonema perstans can be detected by the methods usually employed.

2. METHODS OF WORK

2.1 Description of methods

2.1.1 Organization of field surveys

The survey covered a total of 369 subjects. The field work was carried out at night, between 10 p.m. and 2 a.m., during the second half of June and the first half of July 1967. Each subject underwent a certain number of tests: intradermal test; sampling of capillary and venous blood with and without anticoagulant; clinical examinations and skin biopsy; finally, reading of the intradermal test.

2.1.2 Parasitological methods

(a) Search for microfilariae in the blood

Calibrated thick drop

20 mm³ of capillary blood were taken from the middle finger using a calibrated pipette and placed on a grease-free slide measuring 7.5 x 2.5 cm. The drop was immediately defibrinated and spread out a little so as to obtain an average diameter of about 17 mm. The following day, in the laboratory, the drops were dehaemoglobinized by dipping the slides for a few minutes (five to seven) in a dish containing distilled water. After drying the slides, the drops were fixed for 10 minutes with a methyl alcohol and then stained with Giemsa,¹ using three drops of Giemsa rapide in 2 ml of neutral distilled water.

Search after concentration

This was carried out by the technique suggested by Sang & Petithory (1963), the basic principles of which are as follows: 5 ml of blood collected over sodium citrate are transferred to a conical centrifuge tube. 10 ml of physiological saline are added as well as 5-10 drops (generally eight) of a two per cent. saponin solution in physiological saline (8.5 g NaCl, 1000 ml distilled water). The tube is then shaken until complete haemolysis of the blood. Next it is centrifuged for 10 minutes at 2500 rpm. After centrifugation the supernatant is discarded and the walls of the tube dried with a clean cotton-wool plug, without touching the pellet. The latter is then taken up in a few drops of physiological saline with the aid of a Pasteur pipette. Finally, a few drops of this liquid are examined between slide and coverslip under the microscope.

(b) Search for microfilariae in the skin

Skin biopsies were performed using a special instrument normally employed in ophthalmology but which proved to be extremely useful for skin snips, since it made that operation instantaneous and painless.²

The instrument used has the further advantage of furnishing specimens with the same surface area and volume. A double biopsy was carried out on each subject, in the trochanter region. Three successive readings were made under the microscope using a 40X objective: immediately after the biopsy, 10 minutes and then 20 minutes later.

2.1.3 Clinical methods

Each subject underwent a rapid clinical examination so as to detect on the one hand, the blind and those with nodules, and on the other elephantiasis cases.

¹ Giemsa rapide RAL, Kuhlmann Laboratories, Paris.

² Scleral punch ("Sclerastanze") manufactured by Leanhard Klein, Heidelberg, Federal Republic of Germany.

2.1.4 Immunological methods

(a) Intradermal test

The intradermal test was effected with the FST filarial antigen (also termed FSC-D1) developed by Professor Sawada (Sawada et al., 1965). This highly purified antigen is prepared from Dirofilaria immitis. It is essentially of a protein nature with a small glucidic component (2.5 per cent.). The antigen is freeze-dried and is reconstituted at the time of use by adding physiological solution so that 1 ml of the solution contains 2.5 micrograms of purified protein. Each subject received 0.02 ml of the antigen (corresponding to 0.05 micrograms of protein) in the skin of the anterior side of the left forearm. A control injection of the same volume was administered to the skin of the other forearm, using physiological solution.

Intradermal injection of 0.02 ml usually results in a papule 0.2 cm² in area (5 mm in diameter). The reading is made 15 minutes after injection. The perimeter of the papule is traced on the skin with a ballpoint pen and its area is measured with a transparent stencil, calibrated from 0.2 to 14 cm². When the reaction is negative there is no increase in the area of the papule and in general it even decreases, or sometimes completely disappears. We adopted as criterion of positivity the one given by Desowitz et al., (1966), so that our results could be compared to the ones obtained by those authors. The test is regarded as positive if the area of the papule has doubled, i.e. if it reaches 0.4 cm² (7 mm in diameter).

(b) Complement fixation

This test was performed using the technique described to us by Professor Sawada, which is of the rapid Kolmer type. The antigen used was FP III prepared by Sawada from Dirofilaria immitis and freeze-dried. It is reconstituted at the time of use with physiological solution containing magnesium ions (NaCl: 8.5 g; MgSO₄·7H₂O: 0.1 g; distilled water; 1000 ml), so that 1 ml of the solution contains 100 micrograms of purified protein. The test is carried out after titrating the complement in the presence of 0.2 ml of antigen in solution. For the test 0.2 ml of complement-free serum is used, diluted 1:5 with physiological solution containing magnesium ions, to which is added two complement units in a volume of 0.2 ml, in the dilution indicated by the titration, and 0.2 ml of antigen. After shaking and incubation for three hours at +4°C in the refrigerator, the tubes are left for 15 minutes in the water bath at 37°C. 0.2 ml of sensitized sheep red cells are added (two per cent. sheep red cells mixed with an equal volume of haemolytic serum 30 minutes beforehand). The reading is made after 30 minutes in the water bath at 37°C and the results are noted as ± to +++, according to the degree of haemolysis (+++ complete absence of haemolysis, ++ less than 25 per cent. haemolysis, + from 25 to 50 per cent. haemolysis, ± from 50 to 75 per cent. haemolysis. When haemolysis was over 75 per cent. the test was recorded as negative).

2.2 Discussion of the methods

2.2.1 Parasitological methods

Comparison of the results obtained with the thick drop and concentration

The results obtained are collected together in Table 1. Study of this table shows that the technique using concentration resulted in the detection of 53 persons who gave a negative thick drop test (i.e. 14.36 per cent. of the 369 examined), but that on the other hand 25 persons (i.e. 6.78 per cent. of the 369 examined) gave a positive thick drop but negative concentration test. The two methods should therefore be regarded as complementary.

2.2.2 Clinical methods

(a) Results of skin biopsies taken from persons with nodules and cases of blindness

All blind cases and all those with nodules, apart from one subject at Samandéni, gave a positive skin biopsy.

These subjects were distributed between the three localities surveyed in accordance with the details shown in Table 2.

(b) Results of blood tests and skin biopsies of elephantiasis cases in the three localities surveyed

The results of these different examinations are given in Table 3.

It is clear from the table that in almost half the subjects with lesions of the elephantiasis type neither the microfilariae of W. bancrofti nor of O. volvulus could be detected. On the other hand, 12 of these cases were carriers of D. perstans of microfilariae, only one being completely without microfilariae in the blood or the skin.

3. DESCRIPTION AND INTERPRETATION OF THE RESULTS

3.1 Results of parasitological and clinical examination in the three localities surveyed

These results appear in Table 4 which also shows the distribution of positive subjects according to the filarial species involved.

It appears, on studying this table, that in the two localities where onchocerciasis predominates on the one hand (Samandéni: 85.2 per cent. onchocerciasis cases) and Bancroft's filariasis on the other (Tingréla: 73.5 per cent. Bancroft's filariasis cases) a higher proportion of subjects are also infected with D. perstans (Samandéni: 79 per cent.; Tingréla: 72.2 per cent.). In most cases the latter species is associated with O. volvulus at Samandéni and with W. bancrofti at Tingréla. Furthermore, in the village of Banankélédaga, chosen as a control, there are nevertheless 35.7 per cent. of positive individuals most of whom (29.9 per cent.) are carriers of D. perstans.

3.2 Immunological results obtained with positive subjects

To simplify subsequent discussion, we shall term subjects found to be infected by parasitological or clinical tests "positive" subjects.

3.2.1 Subjects carrying only one filarial species

Subjects carrying only one filarial species are generally few in number. This arises from the fact that the following associations are frequently observed: O. volvulus and D. perstans; W. bancrofti and D. perstans; W. bancrofti, O. volvulus and D. perstans (Table 4).

(a) O. volvulus

The results appear in Table 5, which indicates that only 20 subjects are carriers of O. volvulus alone.

The immunological results show that 15 subjects gave a positive intradermal test and 12 a positive complement fixation test. Two subjects were negative in both tests.

(b) W. bancrofti

The results in Table 6 show that 29 subjects are carriers of W. bancrofti alone. Of these 75.9 per cent. gave a positive intradermal test and only 10.4 per cent. a positive complement fixation test; 17.2 per cent. of these individuals were negative in both tests.

(c) D. perstans

The results in Table 7 show that among subjects carrying D. perstans alone, 84.6 per cent. gave a positive intradermal test and only 26.9 per cent. a positive complement fixation test; 13.5 per cent. were negative in both tests.

(d) Discussion

In regard to the intradermal test, there is no significant difference between the results obtained with the three groups of filariasis cases studied above ($\chi^2 = 1.018$ for 2 degrees of freedom, $P > 0.50$).

On the other hand, in regard to the complement fixation test, there is a highly significant difference ($\chi^2 = 12.980$ for 2 degrees of freedom, $0.01 > P > 0.001$) between the results obtained for the same three groups, revealing a pronounced excess of positive tests among O. volvulus carriers and a marked deficit among W. bancrofti carriers.

3.2.2 Subjects carrying several filarial species(a) O. volvulus and W. bancrofti

The results for the four subjects concerned are given in Table 8. In view of the very small number of persons in this category, no conclusions can be drawn.

(b) W. bancrofti and D. perstans

The corresponding results are shown in Table 9. Of the 51 subjects concerned, 74.5 per cent. gave a positive intradermal test and only 17.7 per cent. a positive complement fixation test; 19.6 per cent. were negative in both tests.

(c) O. volvulus and D. perstans

Of the 69 subjects in Table 10, 55.0 per cent. gave a positive intradermal test and 56.6 per cent. a positive complement fixation test, while 18.8 per cent. were negative in both tests.

(d) O. volvulus, W. bancrofti and D. perstans

This association was found in 42 subjects (Table 11). Of these, 73.7 per cent. gave a positive intradermal test and 33.3 per cent. a positive complement fixation test, while 11.9 per cent. were negative in both tests.

(e) Discussion

In regard to the intradermal test a significant difference exists between the percentage of positive tests observed in the types of association (b), (c) and (d) ($\chi^2 = 6.508$ for 2 degrees of freedom, $0.05 > P > 0.02$). This difference is due to a pronounced deficit of positive tests with the association O. volvulus and D. perstans.

In regard to complement fixation, a significant difference can again be seen between the percentage of positive tests observed in the different types of association ($\chi^2 = 20.787$ for

2 degrees of freedom, $P < 0.001$). This difference is due to a large excess of positive tests with the association O. volvulus and D. perstans and a large deficit with the association W. bancrofti and D. perstans.

3.2.3 Subjects carrying one filarial species alone or in association with other species

(a) O. volvulus

Of the 135 subjects carrying O. volvulus alone or in association with D. perstans and/or W. bancrofti, we found that 63.7 per cent. gave a positive intradermal test and 48.9 per cent. a positive complement fixation test, 16.3 per cent. being negative in both tests (Table 12).

(b) W. bancrofti

126 subjects carried W. bancrofti alone or associated with O. volvulus and/or D. perstans (Table 13); 75.4 per cent. of them gave a positive intradermal test and 22.2 per cent. a positive complement fixation test, 15.9 per cent. being negative in both tests.

(c) D. perstans

Table 14, which gives results for carriers of D. perstans either alone or in association with O. volvulus and/or W. bancrofti, shows that of the 214 subjects concerned, 70.0 per cent. gave a positive intradermal test and 35.5 per cent. a positive complement fixation test, 16.4 per cent. being negative in both tests.

3.3 Immunological results obtained with negative subjects

102 subjects gave negative clinical and parasitological tests (Table 15). However, 54.9 per cent. of them were positive in the intradermal test and 38.2 per cent. positive in the complement fixation test. Only 31.4 per cent. were negative in both tests.

3.4 Comparison of the immunological results obtained with positive and with negative subjects (Tables 16 and 17)

(a) Intradermal test

On comparing the results given in Table 3, it can be seen that the positive tests are significantly more frequent in positive subjects than in negative ones ($\chi^2 = 8.792$ for 1 degree of freedom, $0.01 > P > 0.001$). This difference is due to a lower frequency of positive tests in negative subjects in the first two age-groups, as will be seen in paragraph 3.5.

(b) Complement fixation

Unlike the intradermal test, we found that negative subjects gave just as many positive tests as positive ones ($\chi^2 = 0.536$ for 1 degree of freedom, $P > 0.30$), whatever the age-group considered (paragraph 3.5).

3.5 Immunological results in relation to age

Because of a certain reluctance on the part of the population, we were only able to test a small number of young children and we were obliged to adopt the following age-groups, so as to have in each group a sufficient number of subjects to calculate statistically valid percentages.

- Group 1: subjects from 0 to 13 years of age.
- Group 2: subjects from 14 to 21 years of age.
- Group 3: subjects from 22 to 44 years of age.
- Group 4: subjects aged 45 years and above.

An examination of Table 18 shows the expected increase with age in the percentage of subjects who are parasitologically and clinically positive.

3.5.1 Immunological results in relation to age among positive subjects (Table 19)

(a) Intradermal test

The percentages of immunologically positive subjects vary from 61.5 per cent. (Group 4) to 82.0 per cent. (Group 2). Statistical analysis shows that there is no significant difference on comparing the four age-groups with one another ($\chi^2 = 7.422$ for 3 degrees of freedom, $P > 0.05$). On the other hand, if the first three age-groups (average percentage of subjects with positive intradermal tests = 76.7 per cent.) are compared with the fourth (61.5 per cent. of subjects with positive intradermal tests) it is found that these percentages are significantly different ($\chi^2 = 6.624$ for 1 degree of freedom, $0.02 > P > 0.01$).

(b) Complement fixation

The percentages of immunologically positive subjects vary from 30.7 per cent. (Group 4) to 43.6 per cent. (Group 2). On comparing the four age-groups with one another no significant difference is found between these percentages ($\chi^2 = 2.707$ for 3 degrees of freedom, $P > 0.30$).

Similarly, a comparative study of the first two age-groups, where the percentages are highest, and Groups 3 and 4 where the percentages are lowest, reveals no significant difference ($\chi^2 = 3.076$ for 1 degree of freedom, $P > 0.05$).

3.5.2 Immunological results in relation to age in negative subjects (Table 20)

(a) Intradermal test

A very clear increase is seen in the percentage of positive tests with age for the first three groups and, on the other hand, a slight decrease for the group of oldest subjects. The differences between the percentage observed are highly significant ($\chi^2 = 23.079$ for 3 degrees of freedom, $P < 0.001$).

The very clear increase in the percentage of positive intradermal tests found among the negative subjects in the first three age-groups is paralleled by that observed in the percentages of parasitologically or clinically positive subjects in the same three groups (Table 18). It may be mentioned, furthermore, that among positive subjects the percentages of positive intradermal tests do not differ significantly in the first three groups.

These findings seem to show that the intradermal test would make it possible to detect weakly infected subjects not detectable by the usual parasitological and clinical methods.

(b) Complement fixation

The percentages of negative subjects who are immunologically positive vary from 34.6 per cent. (Group 3) to 42.9 per cent. (Group 4). These percentages are not significantly different ($\chi^2 = 0.365$ for 3 degrees of freedom, $P > 0.90$).

3.6 Immunological results in relation to sex

3.6.1 Among positive subjects (Table 21)

(a) Intradermal test

No significant difference is seen between the results obtained with either sex ($\chi^2 = 0.295$ for 1 degree of freedom, $P > 0.50$).

(b) Complement fixation

Similarly, no difference is seen between the results obtained with either sex ($\chi^2 = 0.267$ for 1 degree of freedom, $P > 0.50$).

3.6.2 Among negative subjects (Table 22)

(a) Intradermal test

No significant difference can be seen between the results obtained with either sex ($\chi^2 = 0.990$ for 1 degree of freedom, $P > 0.30$).

(b) Complement fixation

No significant difference can be found between results obtained with either sex ($\chi^2 = 0.166$ for 1 degree of freedom, $P > 0.50$).

3.7 Immunological results in relation to locality

3.7.1 Among positive subjects (Table 23)

(a) Intradermal test

Statistical study of the results given in Table 23 shows that there is a significant difference between the results obtained in the three localities ($\chi^2 = 18.898$ for 2 degrees of freedom, $P < 0.001$). This difference is due to a pronounced excess of positive tests at Tigréla as compared with the other two localities.

(b) Complement fixation

The results differ in a highly significant manner from one locality to another ($\chi^2 = 64.372$ for 2 degrees of freedom, $P < 0.001$). A very pronounced excess of positive tests is observed at Samandéni and a high deficit at Tigréla.

3.7.2 Among negative subjects (Table 15)

The number of subjects in this category observed at Tigréla and Samandéni is too small for any conclusion to be drawn from these results.

3.8 Immunological results in relation to their intensity

3.8.1 Intradermal test

Tables 24 and 25 show the distribution of positive and negative subjects in relation to the area of the skin reaction.

These Tables seem to indicate that reactions equal to or greater than 1.0 cm^2 are more frequent among positive subjects. However, on comparing the means we obtained: $\Sigma = 1.89$, or $P \neq 0.06$. The results are therefore not significantly different.

3.8.2 Complement fixation

Tables 26 and 27 show the distribution of positive and negative subjects in relation to the intensity of their reaction.

Statistical study reveals that the results obtained in the four categories do not differ significantly for positive and negative subjects ($\chi^2 = 3.796$ for 3 degrees of freedom, $P > 0.20$).

It would seem, however, that among positive subjects there is a higher percentage of strongly positive reactions (C'++ and C'+++). However, with a probability equal to or less than 0.05, no significant difference is found between the percentages of highly positive (C'++ and C'+++) and weakly positive (C'+ and C'++) reactions observed among the positive as well as negative subjects ($\chi^2 = 3.077$ for 1 degree of freedom, $0.10 > P > 0.05$).

4. GENERAL CONCLUSIONS

From the parasitological viewpoint, it should be recalled that the two methods used in searching for sanguicolous microfilariae are complementary and that it would seem desirable to use them jointly whenever possible (see 2.2.1). If the concentration technique cannot be used, because of the impossibility of obtaining an adequate sample of venous blood (e.g. in the case of very young children), recourse should be had to the technique recommended by Edeson (1959), which consists in taking a 60 mm³ sample of capillary blood.

It should also be mentioned that like many authors, we found the microfilariae of W. bancrofti and of O. volvulus to be absent in nearly half the subjects presenting lesions of an elephantiasis type. On the other hand, skin biopsies were positive in all persons with nodules, apart from a single subject, illustrating the known reliability of the skin snip for the detection of onchocerciasis.

4.1 Intradermal test

(a) In positive subjects

The average percentage of positive tests was 71.5 per cent. (maximum 84.6 per cent. in carriers of D. perstans only, minimum 55.0 per cent. in carriers of O. volvulus and D. perstans).

The results for those carrying a single filarial species do not differ according to the nature of the latter (see 3.2.1 (d)). On the contrary, if carriers of a single or of several filarial species apart from O. volvulus and W. bancrofti carriers (numbers too small), are considered simultaneously then the results vary from one category to the other ($\chi^2 = 14.789$ for 5° of freedom, $0.02 > P > 0.01$). This is due essentially to a high deficit of positive tests with the association O. volvulus and D. perstans, although no satisfactory explanation can be given.

In regard to age, the percentage of positive tests is not different in the first three age groups, but a slight decrease in the number of positive reactions is found in Group 4 (see 3.5.1) which might be a sign of a fall in allergic response or the appearance of energy beyond a certain age. Some authors have noted an increase in positivity with age (Desowitz et al., 1966; Ciferri et al., 1965), there being a very low percentage of positive tests in the youngest subjects. The structure we have adopted for the age groups renders it difficult to compare our results with those of these authors, all the more so in that only four subjects out of 32 were eight years of age or less in our first age-group (Group 1)

The results when studied in relation to sex did not show any significant difference (see 3.6.1). This moreover has also been noted by numerous authors, in particular by Ciferri et al., (1965), Desowitz et al., (1966), Ata et al., (1967).

Finally, on studying the results in relation to locality, a distinct deficit of positive tests is noted in two localities (Banankélédaga and Samandéni) due essentially to the frequency of the association O. volvulus and D. perstans for which we found the highest percentage of negative tests (see above).

(b) In negative subjects

54.9 per cent. of these subjects gave a positive skin test. It may be mentioned in this connexion that Desowitz et al., (1966) noted, on the one hand, that in a filariasis-free zone all the subjects tested gave a negative result, apart from those who had spent one year or more in an endemic filariasis zone, and, on the other, that the antigen used seemed specific for filariae since, in particular, there was no reaction with subjects carrying intestinal helminths.

The high percentage of negative subjects giving a positive intradermal test seems to show that such individuals have in fact been infected, but that the filarial infection cannot be detected by the usual parasitological or clinical methods.

Unlike what was seen among positive subjects, a high increase in the percentage of positive tests in relation to age is found here (see 3.5.2 (a)) in the first three age-groups. On the other hand, as with the positive subjects, the percentage of positive tests decreases in the fourth group, probably for the same reasons.

This increase in the percentage of positive tests with age among negative subjects, which runs parallel to the increase in the percentage of subjects who are clinically or parasitologically positive with age, seems to confirm the fact that the intradermal test would make it possible to detect subjects not diagnosed by the usual examinations.

As with positive subjects, no significant difference was observed between the two sexes (see 3.6.2).

(c) Finally, we may point out that the percentage of subjects giving a positive intradermal test is appreciably the same among both positive and negative individuals over 22 years of age (Groups 3 and 4) and that, furthermore, the intensity of the reaction does not differ significantly as between positive and negative subjects, although Tables 24 and 25 show that tests with a surface area of over 1.3 cm² were observed only in positive subjects.

4.2 Complement fixation

While positive subjects gave only 34.5 per cent. of positive tests, 38.2 per cent. of positive tests were obtained among negative subjects. These two percentages are, moreover, not significantly different.

No difference was detected among either positive or negative subjects in relation to sex and age.

On the other hand, the results vary considerably according to the filarial species involved (10.4 per cent. positive tests among carriers of W. bancrofti only; 60 per cent. among carriers of O. volvulus only).

Filarial associations gave intermediate results depending on the presence or absence of O. volvulus or W. bancrofti (see 3.2.1 (d); 3.2.2 (e)).

For positive subjects the results vary according to locality and the predominance of O. volvulus or W. bancrofti. As with the intradermal test, the intensity of the reaction does not differ significantly whether the subjects are positive or negative, although a higher percentage of strongly positive tests (C⁺⁺ and ⁺⁺⁺) were observed in positive individuals.

It therefore appears that this test gives the best results with onchocerciasis. This is perhaps due to the fact that in the case of W. bancrofti and D. perstans a certain neutralization of circulating antibody by the numerous sanguicolous microfilariae takes place (WHO, 1965).

4.3 Comparison of the intradermal and complement fixation tests

On considering the positive subjects as a whole (Table 23), it can be seen that the intradermal test gives distinctly better results than the complement fixation one (71.5 per cent. positive as against 34.5 per cent. positive complement fixation). On the other hand, if the results obtained in each of the three localities are studied separately, it is found that the intradermal test gives the best results at Tingréla where W. bancrofti predominates, whereas complement fixation gives results better than those of the intradermal test at Samandéni where O. volvulus predominates.

4.4 Value of these two tests

In conclusion, the intradermal test, although giving a higher percentage of positive results (about 75 per cent.) among subjects detected by the usual parasitological and clinical methods, cannot be used alone for the diagnosis of filariasis because of its failure with about a quarter of confirmed filariasis sufferers. Its essential interest would seem to be that it would make it possible to detect the disease in suspect cases who are only weakly infected, when the diagnosis cannot be established by means of the classical methods. These conclusions would seem to hold for all three of the filarial species we have investigated.

In regard to the complement fixation test, it would appear that similar conclusions can be drawn if use of the method is restricted to the detection of onchocerciasis. However, the choice of another serum dilution or another antigen concentration might lead to appreciable improvements in the performance of this method and perhaps make it possible to extend its use to the case-finding of other types of filariasis. In particular, it should be noted that Rosseau-Baelde & Janssens (1961) obtained good results in the diagnosis of various forms of filariasis by using other antigens and applying a slightly different technique.

LEGENDS FOR TABLES

- Table 1: Comparative results obtained with the thick drop (20 mm³ sample of capillary blood) and with concentration (5 ml sample of venous blood).
p: D. perstans; b: W. bancrofti.
- Table 2: Distribution of blinded subjects and of subjects with nodules in the three localities surveyed.
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- Table 4: Number of subjects parasitologically or clinically positive or negative in the three localities surveyed, with distribution of the positive subjects according to the filarial species involved.
O.v.: O. volvulus; W.b.: W. bancrofti; D.p.: D. perstans.
- Table 5: Immunological results given by subjects infected with O. volvulus alone.
(k) This was a subject presenting a nodule with negative skin snip.
(1) ID = intradermal test; C' = complement fixation.
(2) Total of figures given in columns 4 and 6.
(3) Total of figures given in columns 5 and 6.
(4) Total of figures in columns 4, 5 and 6.
(5) The percentages are shown in quotes for they were calculated on less than 25 subjects.
- Table 6: Immunological results given by subjects infected with W. bancrofti alone.
(c) This refers to an elephantiasis case where the presence of W. bancrofti microfilariae could not be detected.
(1), (2), (3), (4): see Table 5.
- Table 7: Immunological results given by subjects infected with D. perstans alone.
(1), (2), (3), (4): see Table 5.
- Table 8: Immunological results given by subjects infected with O. volvulus and W. bancrofti.
(The percentages were not calculated because the number of cases was too low.)
(1), (2), (3), (4): see Table 5.
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(c) These are cases of elephantiasis where the presence of the microfilariae of W. bancrofti could not be detected.
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- Table 10: Immunological results given by subjects infected with O. volvulus and D. perstans
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(c) See Table 9.
(1), (2), (3), (4): see Table 5.

- Table 14: Immunological results given by subjects infected with D. perstans either alone or associated with W. bancrofti and/or O. volvulus.
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- Table 15: Immunological results given by subjects showing negative clinical and parasitological tests.
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- Table 16: Over-all immunological results given by positive subjects in relation to locality.
(1) ID = intradermal test.
(2) C' = complement fixation.
- Table 17: Over-all immunological results given by negative subjects in relation to locality.
(1) ID = intradermal test.
(2) C' = complement fixation.
- Table 18: Results of clinical and parasitological tests in relation to age.
- Table 19: Immunological results given by positive subjects in relation to age.
(1), (2), (3), (4): see Table 5.
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(1), (2), (3), (4): see Table 5.
(5): the percentages in quotes were calculated on less than 25 cases.
- Table 21: Immunological results given by positive subjects in relation to sex.
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- Table 23: Detailed immunological results given by positive subjects in relation to locality.
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- Table 24: Distribution of positive subjects giving a positive intradermal test according to the area of the reaction in cm² and in relation to locality.
- Table 25: Distribution of negative subjects giving a positive intradermal test according to the area of the reaction in cm² and in relation to locality.
- Table 26: Distribution of positive subjects giving a positive complement fixation test according to the intensity of the reaction expressed by the notation \pm (= feebly positive) to +++ (= strongly positive) and in relation to the locality.
(1) C' = complement fixation.
- Table 27: Distribution of negative subjects giving a positive complement fixation test according to the intensity of the reaction expressed by the notation \pm (= feebly positive) to +++ (= strongly positive) and in relation to locality.
(1) C' = complement fixation.

TABLE 1

Localities	Number of subjects				
	Examined	Negative thick drop and concentration	Positive thick drop and concentration	Positive thick drop and negative concentration	Positive concentration and negative thick drop
Banankélédega	137	96	13	4p	24p
Samandéni	81	16	44	13 including (12p (1b+p)	8 including (7p (1b
Tingréla	151	11	111	8 including (1p (5b (2b+p)	21 including (18p (3b
Totals	369	123	168	25 including (17p (5b (3b+p)	53 including (49p (4b

TABLE 2

Localities	Number of subjects			
	Examined	With nodules	Blind	With nodules and blind
Banankélédega	137	1	-	-
Samandéni	81	13	6	4
Tingréla	151	1	-	-

TABLE 3

Localities	Number of subjects with elephantiasis				
	Total	Without W.b. or O.v. mf	With W.b. and O.v. mf	With W.b. mf but without O.v. mf	With O.v. mf but without W.b. mf
Banankélédega	1	-	-	-	1
Samandéni	-	-	-	-	-
Tingréla	28	13 (46.4%)	5	4	6

TABLE 4

Localities	No. of subjects examined	No. of negative subjects	Number of positive subjects													
			Total	O.v.+	W.b.+	D.p.+	O.v.+	W.b.+	O.v.+	W.b.+	O.v.+	W.b.+	D.p.+	All subjects	All subjects	All subjects
														O.v.+	W.b.+	D.p.+
Banankélédaga	137	88 64.3%	49 35.7%	8	-	23	1	14	3	25	4	29.9%	41	29.9%		
Samandéni	81	4 4.9%	77 95.1%	12	-	7	1	47	9	69	11	13.6%	64	79.0%		
Tingréla	151	10 6.6%	141 93.4%	-	29	22	3	8	30	41	111	73.5%	109	72.2%		
Totals	369	102 27.6%	267 72.4%	20 7.5%	29 10.9%	52 19.5%	4 1.5%	69 25.8%	42 15.7%	135 36.6%	126 34.1%		214	58.0%		

TABLE 5

Localities	Number of subjects									
	Solely O.v.+	ID - C' - (1)	ID + C' - +1(k)	ID - C' +	ID + C' +	All subjects ID + (2)	All subjects C' + (3)	All subjects ID + or C' + (4)		
Banankélédaga	8	2	4	1	1	5	2	6		
Samandéni	11 +1(k)	-	1 +1(k)	2	8	9 +1(k)	10	11 +1(k)		
Tingréla	-	-	-	-	-	-	-	-		
Totals	20	2 ("10.0%")	6 ("30.0%")	3 ("15.0%")	9 ("45.0%")	15 ("75.0%")	12 ("60.0%")	18 ("90.0%")		

TABLE 6

Localities	Number of subjects							All subjects ID + or C' + (4)
	Solely W.b. +	ID - C' - (1)	ID + C' -	ID - C' +	ID + C' +	All subjects ID + (2)	All subjects C' + (3)	
Banankélédaga	-	-	-	-	-	-	-	-
Samandéni	-	-	-	-	-	-	-	-
Tingréla	28 +1(c)	5	20 +1(c)	2	1	21 +1(c)	3	23 +1(c)
Totals	29	5 (17.2%)	21 (72.4%)	2 (6.9%)	1 (3.5%)	22 (75.9%)	3 (10.4%)	24 (82.8%)

TABLE 7

Localities	Number of subjects							All subjects ID + or C' + (4)
	Solely D.p. +	ID - C' - (1)	ID + C' -	ID - C' +	ID + C' +	All subjects ID + (2)	All subjects C' + (3)	
Banankélédaga	23	5	12	1	5	17	6	18
Samandéni	7	1	1	-	5	6	5	6
Tingréla	22	1	18	-	3	21	3	21
Totals	52	7 (13.5%)	31 (59.6%)	1 (1.9%)	13 (25.0%)	44 (84.6%)	14 (26.9%)	45 (86.5%)

TABLE 8

Localities	Number of subjects							
	O.v. + W.b. +	ID - C' - (1)	ID + C' -	ID - C' +	ID + C' +	All subjects ID + (2)	All subjects C' + (3)	All subjects ID + or C' + (4)
Banankélétaga	-	-	-	-	-	-	-	-
Samandéni	1	-	-	-	1	1	1	1
Tingréla	3	1	2	-	-	2	-	2
Totals	4	1	2	-	1	3	1	3

TABLE 9

Localities	Number of subjects							
	W.b. + D.P. +	ID - C' - (1)	ID + C' - +10(c)	ID - C' +	ID + C' +	All subjects ID + (2)	All subjects C' + (3)	All subjects ID + or C' + (4)
Banankélétaga	1	1	-	-	-	-	-	-
Samandéni	1	1	-	-	-	-	-	-
Tingréla	37 +12(c)	7 +1(c)	22 +10(c)	3	5 +1(c)	27 +11(c)	8 +1(c)	30 +11(c)
Totals	51	10 (19.6%)	32 (62.7%)	3 (5.9%)	6 (11.8%)	38 (74.5%)	9 (17.7%)	41 (80.4%)

TABLE 10

Localities	Number of subjects									
	Solely O.v. + and D.p. +	ID - C' - (1)	ID + C' -	ID - C' +	ID + C' +	All subjects ID + (2)	All subjects C' + (3)	All subjects ID + and C' + (4)		
Banankélédaga	14	4	5	3	2	7	5	10		
Samandéni	47	9	5	15	18	23	33	38		
Tingréla	8	-	7	-	1	8	1	8		
Totals	69	13 (18.7%)	17 (24.6%)	18 (26.1%)	21 (30.4%)	38 (55.0%)	39 (56.5%)	56 (81.2%)		

TABLE 11

Localities	Number of subjects									
	O.v. + W.b. + and D.p. +	ID - C' - (1)	ID + C' -	ID - C' +	ID + C' +	All subjects ID + (2)	All subjects C' + (3)	All subjects ID + and C' + (4)		
Banankélédaga	2 +1(c)	- +1(c)	-	2	-	-	2	2		
Samandéni	9	2	2	2	3	5	5	7		
Tingréla	24 +6(c)	2	18 +3(c)	1 +1(c)	3 +2(c)	21 +5(c)	4 +3(c)	22 +6(c)		
Totals	42	5 (11.9%)	23 (54.7%)	6 (14.3%)	8 (19.0%)	31 (73.7%)	14 (33.3%)	37 (88.1%)		

TABLE 12

Localities	Number of subjects									
	Subjects with O.v. alone or associated with D.p. and/or W.b.	ID - C' - (1)	ID + C' - +1(k)	ID - C' + 6	ID + C' + 3	All subjects ID + (2)	All subjects C' + (3)	All subjects ID + or C' + (4)		
Banankélédaga	25	7	9	6	3	12	9	18		
Samandéni	68 +1(k)	12	7 +1(k)	19	30	37 +1(k)	49	56 +1(k)		
Tingréla	41	3	30	2	6	36	8	38		
Totals	135	22 (16.3%)	47 (34.8%)	27 (20.0%)	39 (28.9%)	86 (63.7%)	66 (48.9%)	113 (83.7%)		

TABLE 13

Localities	Number of subjects									
	Subjects with W.b. alone or associated with O.v. and/or D.p.	ID - C' - (1)	ID + C' - +1(c)	ID - C' + 2	ID + C' + 4	All subjects ID + (2)	All subjects C' + (3)	All subjects ID + or C' + (4)		
Banankélédaga	3 +1(c)	1	2 +1(c)	2	4	2 +1(c)	2	2 +1(c)		
Samandéni	11	3	2	2	4	6	6	8		
Tingréla	92 +19(c)	15 +1(c)	61 +14(c)	6 +1(c)	10 +3(c)	71 +17(c)	16 +4(c)	77 +18(c)		
Totals	126	20 (15.9%)	78 (61.9%)	11 (8.7%)	17 (13.5%)	95 (75.4%)	28 (22.2%)	106 (84.1%)		

TABLE 14

Localities	Number of subjects							
	Subjects with D.P. alone or associated with W.b. and/or O.v.	ID - C' - (1)	ID + C' -	ID - C' +	ID + C' +	All subjects ID + (2)	All subjects C' + (3)	All subjects ID + or C' + (4)
Banankélédaga	41	11	17	6	7	24	13	30
Samandéni	64	13	8	18	25	33	43	51
Tingréla	109	11	78	5	15	93	20	98
Totals	214	35 (16.4%)	103 (48.1%)	29 (13.6%)	47 (22.0%)	150 (70.0%)	76 (35.5%)	179 (83.6%)

TABLE 15

Localities	Number of negative subjects							
	Total	ID - C' - (1)	ID + C' -	ID - C' +	ID + C' +	All subjects ID + (2)	All subjects C' + (3)	All subjects ID + or C' + (4)
Banankélédaga	88	28 (31.8%)	26 (29.5%)	13 (14.8%)	21 (23.9%)	47 (53.4%)	34 (38.6%)	60 (68.2%)
Samandéni	4	1	1	-	2	3	2	3
Tingréla	10	3	4	1	2	6	3	7
Totals	102	32 (31.4%)	31 (30.4%)	14 (13.7%)	25 (24.5%)	56 (54.9%)	39 (38.2%)	70 (68.6%)

TABLE 16

Localities	Number of positive subjects				
	Total	With ID - (1)	With ID + (1)	With C' - (2)	With C' + (2)
Banankéledaga	49	20 (40.8%)	29 (59.2%)	34 (69.4%)	15 (30.6%)
Samandéni	77	32 (41.6%)	45 (58.4%)	23 (29.9%)	54 (70.1%)
Tingréla	141	24 (17.0%)	117 (83.0%)	118 (83.7%)	23 (16.3%)
Totals	267	76 (28.6%)	191 (71.4%)	175 (65.5%)	92 (34.5%)

TABLE 17

Localities	Number of negative subjects				
	Total	With ID - (1)	With ID + (1)	With C' - (2)	With C' + (2)
Banankéledaga	88	41 (46.6%)	47 (53.4%)	54 (61.4%)	34 (38.6%)
Samandéni	4	1	3	2	2
Tingréla	10	4	6	7	3
Totals	102	46 (45.1%)	56 (54.9%)	63 (61.8%)	39 (38.2%)

TABLE 18

Age-group	Total subjects examined	Positive subjects		Negative subjects	
		Number	%	Number	%
Group 1 0-13 years	63	32	50.8	31	49.2
Group 2 14-21 years	63	39	61.9	24	38.1
Group 3 22-44 years	131	105	80.2	26	19.8
Group 4 45 years and above	112	91	81.3	21	18.7

TABLE 19

Age-group	Number of positive subjects							
	Total	ID - C' - (1)	ID + C' -	ID - C' +	ID + C' +	All subjects ID + (2)	All subjects C' + (3)	All subjects ID + or C' + (4)
Group 1 0-13 years	32	6 (18.8%)	13 (40.6%)	1 (3.1%)	12 (37.5%)	25 (78.1%)	13 (40.6%)	26 (81.2%)
Group 2 14-21 years	39	3 (7.7%)	19 (48.7%)	4 (10.3%)	13 (33.3%)	32 (82.0%)	17 (43.6%)	36 (92.3%)
Group 3 22-44 years	105	18 (17.1%)	53 (50.5%)	9 (8.6%)	25 (23.8%)	78 (74.3%)	34 (32.4%)	87 (82.9%)
Group 4 44 years and over	91	16 (17.6%)	47 (51.7%)	19 (20.8%)	9 (9.9%)	56 (61.5%)	28 (30.7%)	75 (82.4%)

TABLE 20

Age-group	Number of negative subjects							
	Total	ID - C' - (1)	ID + C' -	ID - C' +	ID + C' +	All subjects ID + (2)	All subjects C' + (3)	All subjects ID+ or C'+ (4)
Group 1 0-13 years	31	17 (54.8%)	2 (6.4%)	8 (25.8%)	4 (12.9%)	6 (19.3%)	12 (38.7%)	14 (45.1%)
Group 2 14-21 years	24	8 ("33.3%")	7 ("29.2%")	2 ("8.3%")	7 ("29.2%")	14 ("58.3%")	9 ("37.5%")	16 ("66.6%")
Group 3 22-44 years	26	3 (11.5%)	14 (53.8%)	2 (7.7%)	7 (26.9%)	21 (80.8%)	9 (34.6%)	23 (88.5%)
Group 4 45 years and over	21	4 ("19.1%")	8 ("38.1%")	2 ("9.5%")	7 ("33.3%")	15 ("71.4%")	9 ("42.9%")	17 ("80.9%")

TABLE 21

Sex of subjects	Number of positive subjects							
	Total	ID - C' - (1)	ID + C' -	ID - C' +	ID + C' +	All subjects ID + (2)	All subjects C' + (3)	All subjects ID+ or C'+ (4)
Male	142	20 (14.1%)	71 (50%)	18 (12.7%)	33 (23.2%)	104 (73.2%)	51 (35.9%)	122 (85.9%)
Female	125	23 (18.4%)	61 (48.8%)	15 (12.0%)	26 (20.8%)	87 (69.6%)	41 (32.8%)	102 (81.6%)
Totals	267	43 (16.1%)	132 (49.4%)	33 (12.4%)	59 (22.1%)	191 (71.5%)	92 (34.5%)	224 (83.9%)

TABLE 22

Sex of subjects	Number of negative subjects							
	Total	ID - C' - (1)	ID + C' -	ID - C' +	ID + C' +	All subjects ID + (2)	All subjects C' + (3)	All subjects ID + or C'+ (4)
Male	52	14 (26.9%)	17 (32.7%)	7 (13.5%)	14 (26.9%)	31 (59.6%)	21 (40.4%)	38 (73.1%)
Female	50	18 (36.0%)	14 (28.0%)	7 (14.0%)	11 (22.0%)	25 (50.0%)	18 (36.0%)	32 (64.0%)
Totals	102	32 (31.4%)	31 (30.4%)	14 (13.7%)	25 (24.5%)	56 (54.9%)	39 (38.2%)	70 (68.6%)

TABLE 23

Localities	Number of positive subjects							
	Total	ID - C' - (1)	ID + C' -	ID - C' +	ID + C' +	All subjects ID + (2)	All subjects C' + (3)	All subjects ID + or C'+ (4)
Banankélédaga	49	13 (26.5%)	21 (42.9%)	7 (14.3%)	8 (16.3%)	29 (59.2%)	15 (30.6%)	36 (73.5%)
Samandéni	77	13 (16.9%)	10 (13.0%)	19 (24.7%)	35 (45.4%)	45 (58.4%)	54 (70.1%)	64 (83.1%)
Tingréla	141	17 (12.1%)	101 (71.6%)	7 (5.0%)	16 (11.3%)	117 (82.9%)	23 (16.3%)	124 (88.0%)
Totals	267	43 (16.1%)	132 (49.4%)	33 (12.4%)	59 (22.1%)	191 (71.5%)	92 (34.5%)	224 (83.9%)

TABLE 24

Localities	Number of positive subjects with ID +																
	Total	Distribution of these subjects according to area of reaction in cm ²															
		0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9
Banankélédaga	29	3	5	3	5	1	5	3	2	-	-	-	-	-	1	1	-
Samandéni	45	6	6	8	11	4	4	3	-	3	-	-	-	-	-	-	-
Tingréla	117	6	12	7	20	11	20	9	11	15	1	2	-	1	-	1	1
Totals	191	15	23	18	36	16	29	15	13	18	1	2	-	1	1	2	1

TABLE 25

Localities	Number of negative subjects with ID +																
	Total	Distribution of these subjects according to area of reaction in cm ²															
		0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9
Banankélédaga	47	5	4	10	5	6	9	2	3	2	1	-	-	-	-	-	-
Samandéni	3	1	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Tingréla	6	-	-	-	1	4	-	-	-	1	-	-	-	-	-	-	-
Totals	56	6	5	10	7	10	9	2	3	3	1	-	-	-	-	-	-

TABLE 26

Localities	Number of positive subjects with C' + (1)				
	Total	Distribution of these subjects according to intensity of the reaction			
		C'±	C'+	C'++	C'+++
Banankélédaga	15	4	6	4	1
Samandéni	54	19	18	7	10
Tingréla	23	11	7	1	4
Totals	92	34 (37.0%)	31 (33.7%)	12 (13.0%)	15 (16.3%)

TABLE 27

Localities	Number of negative subjects with C' + (1)				
	Total	Distribution of these subjects according to intensity of the reaction			
		C'±	C'+	C'++	C'+++
Banankélédaga	34	16	12	2	4
Samandéni	2	1	1	-	-
Tingréla	3	3	-	-	-
Totals	39	20 (51.3%)	13 (33.3%)	2 (5.1%)	4 (10.3%)

SUMMARY

At the request of WHO, two immunological tests (intradermal and complement fixation) were tried out in the south-west of Upper Volta for the case-finding of filariasis caused by W. bancrofti, O. volvulus and D. perstans.

The antigens used were developed by Professor Sawada from the adult worms of D. immitis.

Surveys (carried out by night) covered 369 subjects spread over three villages, one situated in an only slightly infested zone serving as a control and the two others in zones where W. bancrofti or O. volvulus predominate, respectively.

All the subjects were subjected to a clinical and parasitological examination (search for sanguicolous microfilariae by the calibrated thick drop and concentration techniques; search for dermal microfilariae by skin biopsy).

71.5 per cent. of subjects parasitologically or clinically positive and 54.9 per cent. of negative subjects gave a positive intradermal test. If the carriers of one filarial species only are considered, the results do not differ whatever the species involved. These results led to the conclusion that the intradermal test cannot be used alone for the case-finding of the three types of filariae studied, since some 25 per cent. of subjects with confirmed filariasis gave a negative skin test.

On the contrary, the high percentage of positive tests observed among parasitologically negative subjects is not thought to indicate a lack of specificity of the antigen but to signify rather that such subjects living in an endemic zone are actually infected but too weakly for this to be detected by the usual methods. Various observations supported this theory.

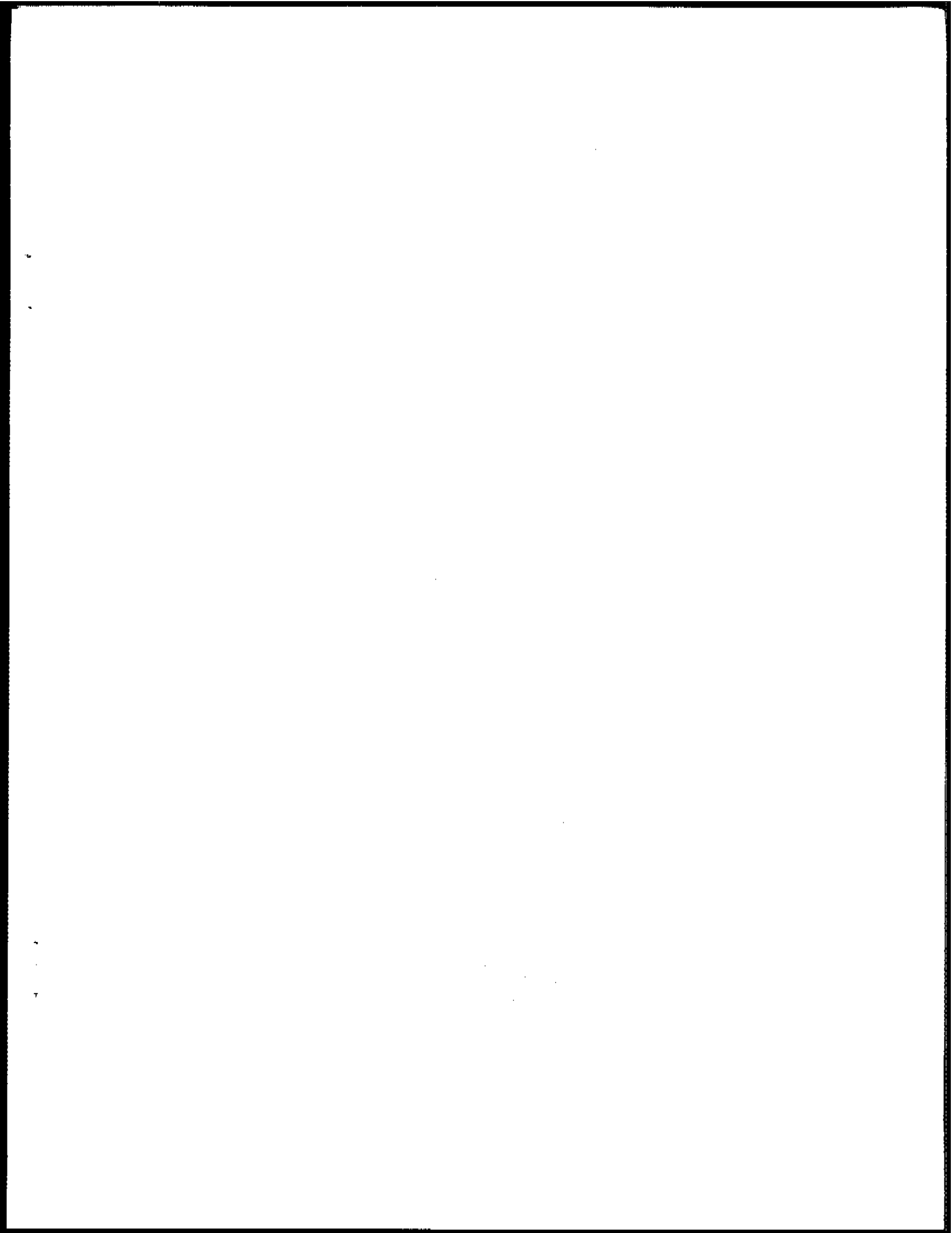
34.5 per cent. of parasitologically or clinically positive subjects and 38.2 per cent. of negative subjects gave a positive complement fixation test. However, it should be mentioned that the results here vary considerably according to the filarial species involved (10.4 per cent. positive tests among carriers of W. bancrofti alone and 60.0 per cent. among carriers of O. volvulus alone). This test gave mediocre results with filariasis caused by sanguicolous microfilariae (W. bancrofti and D. perstans), but acceptable ones with onchocerciasis, a form of filariasis with dermal microfilariae. Subject to a few improvements, this test might be as useful as the intradermal test, at least as concerns onchocerciasis.

The results do not differ in relation to sex for the two tests used. On the other hand, they vary according to the locality in relation to the filarial species or the type of filarial association predominant there.

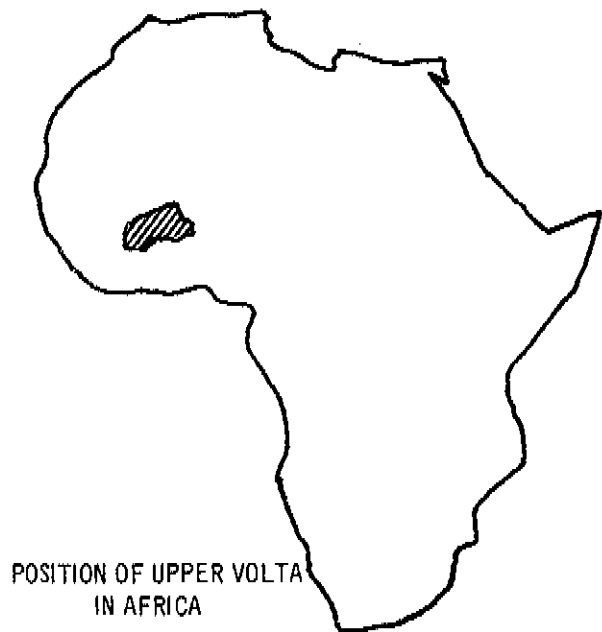
Finally, in regard to the influence of age, no pronounced variation was observed for the complement fixation test, among either positive or negative subjects. The same applies to the intradermal test among positive subjects. On the contrary, in the case of the latter test, a large increase in positivity with age among negative subjects was noted.

ACKNOWLEDGEMENTS

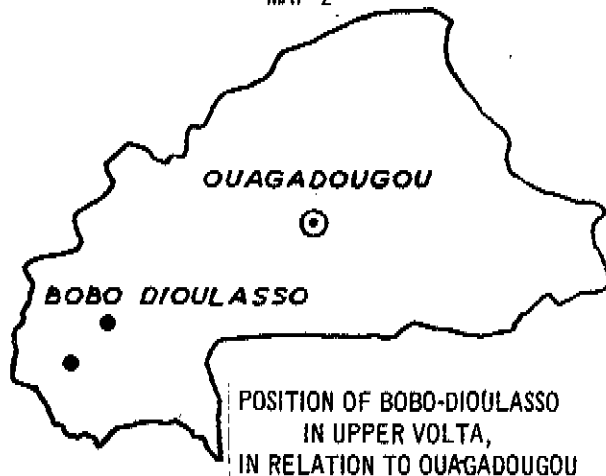
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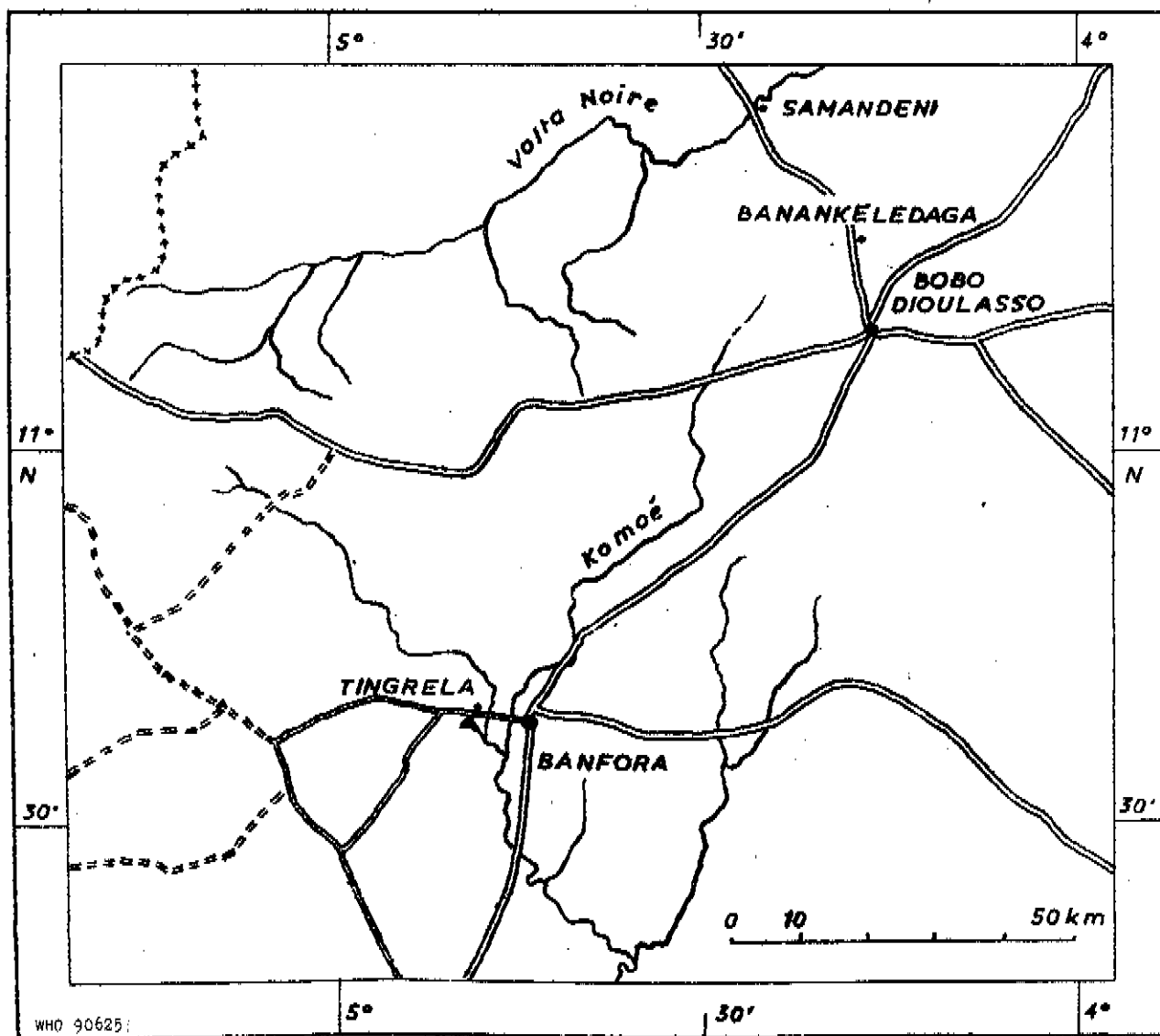
MAP 1



MAP 2



MAP 3



POSITION OF THE THREE VILLAGES SURVEYED IN RELATION TO BOBO-DIOULASSO AND BANFORA