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CCTA/WHO AFRICAN CONFERENCE ON ANCYLOSTOMIASIS

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CCTA/WHO AFRICAN CONFERENCE ON ANCYLOSTOMIASIS

Report

The African Conference on Ancylostomiasis,¹ jointly organized by the Commission for Technical Co-operation in Africa South of the Sahara (CCTA) and the World Health Organization (WHO) and held at Brazzaville from 22 to 29 August 1961, was opened by the Minister of Public Health, His Excellency Dr R. Mahouata, on behalf of the President of the Republic of the Congo (Brazzaville). He thanked the organizations which had arranged the meeting, and welcomed the participants.

Dr F. J. C. Cambournac, Director of the WHO Regional Office for Africa, and Dr E. T. Verdier, Scientific Secretary of the CCTA Scientific Council for Africa South of the Sahara, thanked the Congolese authorities for their hospitality and the Minister of Public Health for his words of welcome.

Dr M. Vaucel was elected Chairman of the Conference and Dr J. Fraga de Azevedo, Vice-Chairman; Dr R. Elsdon-Dew and Dr J. Gillet acted as Rapporteurs.

1. INTRODUCTION

It is estimated that one-fourth of the world's population is infected with *Ancylostoma*, particularly in warm humid regions. The importance of this intestinal verminosis as a cause of morbidity varies according to ecological conditions.²

This Conference was convened: (1) to assess the situation with regard to ancylostomiasis in Africa South of the Sahara (its prevalence and gravity); (2) to discuss survey methods and study the factors governing the distribution of the disease; (3) to study the pathogenesis of the disorders it causes; (4) to discuss the methods to be applied for its treatment and prophylaxis.

¹ The Conference adopted the spelling "ancylostomiasis" in English and "ankylostomiasis" in French.

² The term "ecological" is used here in its widest sense.

2. GEOGRAPHICAL DISTRIBUTION

Ancylostomiasis is very widespread in Africa south of the Sahara. Its prevalence is not uniform and for many regions no accurate data are available.

The information available is drawn either from hospital statistics, which indicate the part this disease plays in general morbidity, or—less frequently—from epidemiological surveys. The Tables in Annex 1 (page 20) give some of the results obtained from surveys made preparatory to this Conference. It would be desirable to carry out uniform surveys in the various regions of Africa so as to obtain comparable results and define the respective distributions of the two parasites in question—*Ancylostoma duodenale* and *Necator americanus*.

2.1 Prevalence and gravity of the disease

2.1.1 *Epidemiological survey methods*

The choice of survey methods will depend upon the aim in view (basic research, applied epidemiology, morbidity and treatment). It should be made clear whether the survey relates to ancylostomiasis alone or to intestinal verminoses as a whole, since this may modify the techniques and procedures to be employed. The importance of integrating such surveys with other investigations in a given region must be remembered since by this means the inconvenience to inhabitants can be minimized, particularly when no treatment which will justify the operations in their eyes is to be applied.

Representative population groups (by age, sex, occupation, etc.) must be very carefully selected so as to satisfy the requirements of up-to-date epidemiological and statistical methods.

Comparison with results obtained in non-infected or only very slightly infected communities or individuals is of assistance in estimating ancylostomiasis morbidity. It should be remembered that the prophylactic or therapeutic measures decided upon by the health authorities will be based on the results of these surveys, which should include clinical, haematological and coprological examinations. The most suitable survey time is about six weeks after the end of the rainy season when *Ancylostoma* infestation is at its highest.

2.1.2 *Parasitological techniques*

The choice of technique for stool examination will depend upon the aims of the survey. Techniques will differ according to whether it is intended

to search simultaneously for other intestinal parasites or to determine only the prevalence and intensity of infection with the two *Ancylostoma* species, with a view to the adoption of selective treatment or of effective treatment against more than one parasite.

Both practical and technical problems arise, such as the distance between the central laboratory and the population examined, and the availability of laboratory equipment and technicians. The professional abilities and the efficiency of the staff must also be taken into account.

There are no simple, quick and cheap methods which are sufficiently sensitive for detecting all intestinal parasites and are at the same time suitable for quantitative use. Qualitative methods, such as direct stool examination, possibly supplemented by the concentration technique, enable helminth eggs and protozoa to be identified, and provide data on the prevalence of intestinal parasitoses.

In view of the simplicity of direct microscopic stool examination, some authors have attempted to standardize this method so as to make it quantitative. Nevertheless, the standard method described would call for accurate photometric measurement of the density of the suspension, and this renders it impracticable under mass survey conditions, particularly in the hands of inexperienced technicians. If the survey covers a very wide area, and if the examination of fresh stools in a relatively short time is not feasible, it is preferable to preserve the specimens collected in merthiolate-iodine-formol medium or in formalized solution.

For diagnosis of slight infection, confirmation of cure, or laboratory experiments, more sensitive enrichment or concentration techniques can be used such as flotation and centrifugation (Clayton-Lane) or simply flotation (zinc sulfate or sodium chloride) etc.

In ancylostomiasis surveys it is very important to know the intensity of infection and to be able to apply quantitative techniques which give numerical results. The Stoll & Hausheer standard dilution technique is the best known and most used. The original description of this method is given in Annex 2, page 21.

The test-tube filter-paper culture method has been used recently in Japan and Taiwan. This method has the advantage of permitting ready differentiation between infections due to *A. duodenale* and to *N. americanus* at the infective larval stage. It is also suitable for detecting *Strongyloides stercoralis*. The details of this method are given in Annex 3, page 27.

2.1.3 Haematological techniques in relation to clinical symptoms

In order to facilitate ancylostomiasis surveys, it is advisable, as far as possible, to determine first of all the principal causes of anaemia in the region under consideration—nutritional factors, for example. This preliminary survey can be made either in the hospitals or in the course of a

pilot study. When preliminary information has been obtained in this way, a wider survey can be undertaken.

Since the purpose of the preliminary study is to obtain guidance for the large-scale survey, it should be carried out in some detail. In addition to measurement of the haemoglobin, at least the erythrocyte count, haematocrit reading, haemo-diffractometry and a blood film examination should be performed. When possible, it is advisable also to determine the iron and protein levels of the blood. The reticulocyte response after iron treatment provides useful indications concerning the type of anaemia and the consequent treatment. Thick-film blood examination should also be done, since malaria may be a cause of anaemia in the region under survey.

This is not, of course, a complete list of techniques; obviously other tests can be added to this basic list.

If account is taken of the information provided by the results of the preliminary study, haemoglobin determination would appear to be a satisfactory method, in the mass survey, of evaluating the degree of anaemia caused by ancylostomiasis.

The dilution technique is a simple method of evaluation adapted to field conditions, which can be applied together with the coprological examination. On the subject of the methods to be used, the Conference drew attention to the recommendations of the WHO Study Group on Iron Deficiency Anaemia.¹

Finally, in any epidemiological inquiry, the clinical symptoms of anaemia and of nutritional deficiencies must be taken into consideration.

2.2 Ecological factors

Ancylostomiasis is typical of verminoses that are dependent on well-defined physico-chemical conditions related to both the environment and the level of economic development of the population concerned.

The various stages of the life cycle of *Ancylostoma* (egg, free rhabditoid form, infective strongyloid form, and adult worm) should be studied from the ecological standpoint so as to discover the most vulnerable point in the transmission chain. The use of radioisotopes might be useful here. Attention should be paid to the soil in the region surveyed, seeing that it acts as a kind of intermediate host. Laboratory study could provide valuable information.

2.2.1 Mode of transmission

Although transmission of *A. duodenale* by the oral route is theoretically possible, in practice transmission through the skin is the only method

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1959, 182.

which need be taken into account as far as the two species of *Ancylostoma* affecting man are concerned. Unlike bilharziasis, ancylostomiasis is not contracted in water. The larvae live in a damp environment, and after coming into contact with the skin they penetrate it when it dries.

2.2.2 *Climatology and type of soil*

Ancylostomiasis is a helminthic disease of hot humid regions; in temperate climates the existence of this disease depends upon the degree of heat and humidity. In tropical regions where there is a clear-cut rainy season, it is generally considered that the most favourable transmission periods are those at the beginning and end of the rains. The torrential rain washes out the soil and carries the eggs and larvae far from their original site. However, the ground may remain moist even without rain in the presence of ground water, agricultural irrigation systems, etc. There are, in fact, certain delta regions, and particularly the Nile Delta, where the incidence of ancylostomiasis is high even though rainfall is very slight.

The physical and chemical properties of the soil must also be considered. It is well known that a soil rich in organic matter favours the larval development of the parasite whereas a sandy, quick-drying soil is less suitable.

2.2.3 *Habits of the population and their relation to the life-cycle of Ancylostoma*

Since there is no animal reservoir of the parasite in the case of human ancylostomiasis, the habits of the people constitute one of the most important factors in the epidemiology of this as of the other helminthiasis. The attitude of communities to the act of defecation is based on deep-rooted custom.

In towns the process of urbanization, if well organized, should put an end to habits which become more harmful as population density increases. In rural areas, on the other hand, the inhabitants usually have no latrines and are in the habit of relieving themselves as and where they can. The attention of the authorities should be drawn to the dangers inherent in indiscriminate defecation, particularly in connexion with schemes for the economic development of many African regions (barrages, irrigation works, etc.).

In the Far East, the collection of human excrement for use as fertilizer means that the infestation is concentrated at certain points so that the disease takes on something of the character of an occupational malady. In Africa, on the other hand, the utilization of human manure is limited to a few regions; the scattered populations, generally living in small villages, relieve themselves in the bush, so that the soil becomes infested over a large area around their dwellings. For economic reasons it is impossible

to forbid the use of human excrement as a fertilizer, but the necessary measures should be adopted to avoid any risk.

2.2.4 *Exposure to infested soil : children and adults*

In Africa, very high parasite rates are commonest among children. Very young children cannot use latrines: even where they exist they are not of a suitable size. In addition, at this age children do not move very far and they therefore relieve themselves in the immediate neighbourhood of the home, and even in the places where they play. This creates a highly polluted area around the family dwelling if the ground favours the development of the parasites (*Ancylostoma*, *Ascaris*, *Trichuris*).

Occupation, and particularly agricultural work, is the predominant factor in contamination. Certain types of farming (market gardens, coffee, tea and banana plantations) provide very favourable conditions for the spread of the infection.

3. PATHOGENESIS

3.1 Comparison of infections caused by *A. duodenale* and by *N. americanus*

The symptoms of ancylostomiasis due to *A. duodenale* differ according to whether the case is one of primary infection, reinfection or superinfection. A study of some 50 cases of experimental infection with several hundred larvae induced in adults to test methods of treatment revealed the following clinical picture which appears to correspond to what is seen in naturally occurring cases.

Primary infection takes the form of discrete skin lesions, catarrh, dysphagia, and nasal or auricular pruritus. The catarrh has no connexion with the lungs but is caused by irritation of the upper respiratory passages, which produces coughing, hoarseness and sore throat, beginning on the fourth day after infection and continuing for up to two weeks. Dysphagia appears on the fifth day, and disappears again very rapidly.

Duodenitis occurs at the end of the first month and continues for 1-2 months with abdominal pains, sometimes very acute, simulating gastroduodenal ulcer or cholecystitis, nausea, and disorders of taste. These signs and symptoms are accompanied by considerable diarrhoea (5-10 stools per day) which changes the general condition and causes a loss of weight amounting to several kilograms.

The severity of the functional upset in the primary infection is not related directly to the number of parasites, but to larval migration and to allergic phenomena. Blood eosinophilia reaches its maximum (60%) at the end of the third month and then begins to decrease.

Anaemia is inconstant, since it depends upon the extent of the infection: there may be none, or it may take the form of acute plastic and isochromic anaemia, differing from chronic anaemia.

Reinfection after cure produces the same symptoms as in the first attack, but in a less acute form.

Superinfection appears in the first instance in the form of allergic skin symptoms, urticaria, purpura, vesicles and pustules. Here, too, pulmonary symptoms are lacking but catarrh of the upper respiratory tract is seen. Duodenitis never recurs.

Eosinophilia rises to a transitory peak and then falls to normal. The type of anaemia depends generally on the number of worms and the cumulative effect of the superinfections: it may be slight and isochromic, medium and hypochromic, or severe and of the hypochromic, microcytic and hypoplastic type.

At the present time, it is recognized that the anaemia is due largely to blood loss, evaluation of which has been facilitated by recent studies with ^{51}Cr and ^{59}Fe . Some symptoms may also be due to toxic action.

A well-defined clinical picture is difficult to find in an endemic area. No experimental observations have been made with respect to *N. americanus*; it should be noted that it absorbs only one-seventh to one-tenth of the amount of blood taken by *Ancylostoma* and that it lives 10-15 years as against 4-5 years for *A. duodenale*.

3.2 Ancylostomiasis infection and ancylostomiasis disease

In general it may be said that, according to the number of worms he harbours, the host will either fall ill or become a carrier, maintaining a more or less precarious equilibrium with the parasite. It is difficult to determine the threshold at which anaemia appears, since its extent depends not only on the number of worms but also on age (infants and young children being particularly susceptible), sex (women usually suffer from more serious anaemia as a result of menstruation, pregnancy and lactation), nutritional status and, finally, infections and other factors affecting the reserves of iron and plasma protein. Disorders of the plasma as well as of the red blood cells occur—for example, hypoproteinaemia with oedema.

There may be racial differences in susceptibility, and research on this point would be useful.

3.3 Ancylostomiasis morbidity and malnutrition

When ancylostomiasis is present among people with an unbalanced diet (particularly in proteins) or a precariously balanced diet, its symptoms may be confusingly like those of malnutrition and at the outset it is difficult to decide whether the major symptoms are due to one cause or the other.

Apart from the iron-deficiency anaemia frequently observed in ancylostomiasis, the other symptoms, such as loss of weight, oedema, trophic disorders of the skin and its appendages, and hypoproteinemia (with hypoalbuminemia) are common to both complaints.

The association of ancylostomiasis and kwashiorkor is commonly noted even when infection is not massive: it would seem that the undernourished organism, deficient in proteins, cannot defend itself effectively against the action of the parasite, and that malnutrition aggravates ancylostomiasis.

The diet available to the African is deficient, particularly in animal protein. Though not critical, his nutritional status is certainly precarious, and most nutritionists consider that the delicate balance is liable to be rapidly upset if communicable and parasitic diseases appear.

Children are weaned between the ages of one and three years, and without any transitional period they pass from mother's milk to adult diet. The African child at this age is prone to suffer from protein and iron deficiency in the diet, and parasitic and communicable diseases.

While theoretically adequate quantities of iron are available in the diet in most African regions, the supply is no longer sufficient after the losses caused by parasites. In tropical regions, moreover, iron-deficiency anaemia unconnected with ancylostomiasis also occurs.

3.4 Role of associated parasites

African populations are known to suffer frequently from several intestinal infections. In considering the problem of ancylostomiasis, the extent and the gravity of ascaridiasis cannot be ignored. Trichocephaliasis and strongyloidosis are also widespread, particularly in certain areas. The pathogenic enterobacteria are also frequent, especially *Shigella*. It is reasonable to suppose that the pathological effect of ancylostomiasis is aggravated by at least some of these associated infections.

Finally, the question of the transmission of the enteroviruses by *Ancylostoma* must be considered.

3.5 Natural and acquired immunity

It would be logical to assume that the various degrees of resistance to reinfection or superinfection induced by other groups of pathogenic agents would also be induced in the case of ancylostomiasis. With other nematodes it has in fact been possible to produce immunity experimentally, or to observe its existence under natural conditions, after a first infection. This applies in particular to *Haemonchus contortus* in the sheep and *Trichostrongylus calcaratus* in the rabbit. Resistance to reinfection has

been definitely established experimentally in the case of *Ancylostoma caninum* and *Uncinaria stenocephala*.

There are, however, as yet no data showing the existence of this phenomenon in human ancylostomiasis, although it is a curious fact that the distribution and prevalence of this disease in a community may retain the same pattern for years in the various age-groups.

The symptoms are generally more acute in children and gradually weaken with age—which would support the idea of the development of a certain degree of resistance.

Observations in Argentina and Nigeria, and others made more recently in West Africa, seem to indicate that resistance in a given community increases with age and that the degree of resistance varies according to race.

The role of nutrition in the development of resistance must also be borne in mind.

4. TREATMENT

Treatment of ancylostomiasis has two aspects: elimination of the parasite (treatment of the cause) and restoration of the depleted stores of iron (treatment of the anaemia). In serious cases with severe anaemia this combined treatment sometimes needs to be preceded by one or more blood transfusions in order to improve the patient's general condition. This is, of course, an emergency measure.

4.1 Anthelmintic treatment

The ideal drug should be non-toxic and without side effects, completely effective after a single treatment, cheap, easy to administer, and agreeable in taste. Epidemiologically speaking, the treatment should achieve total elimination of all pathogenic parasites, but with the therapeutic weapons available at the present time it may be considered satisfactory if the number of worms is reduced to a point where the clinical symptoms disappear and the transmission of the disease is reduced.

At the present time there are two products of choice for the treatment of ancylostomiasis: tetrachlorethylene and bethovenium hydroxynaphthoate. The older drugs, such as carbon tetrachloride, oil of chenopodium, thymol and hexylresorcinol, are considered to be more toxic or less effective.

4.1.1 *Tetrachlorethylene*

Tetrachlorethylene is widely used in both human and veterinary medicine.

The total active dose for a one-day treatment is 5-6 ml for adults and 0.10-0.12 ml per kg body-weight for children. The drug must be administered on an empty stomach, in single or divided dose. The use of a wetting agent (Tween 80) appears to facilitate its administration and perhaps increase its efficacy. Since the product is laxative, no purgative need be given.

Its toxicity is one-tenth that of carbon tetrachloride; according to the authors, minor symptoms, either digestive (vomiting, diarrhoea) or neurological (headache, somnolence, vertigo, a drunken feeling) are observed in 10%-50% of cases. The drug should not be administered to persons whose general condition is poor or who have liver trouble.

Some authors advocate repetition of the treatment after 8-10 days in heavily infected cases.

4.1.2 *Bephenium hydroxynaphthoate*

The first tests with bephenium hydroxynaphthoate were made in 1958. The LD₅₀ for the mouse is 8 g/kg.

This drug is usually administered in the morning on an empty stomach in a single dose of 2.5 g of bephenium base (5 g of bephenium salt) for adults, and half this amount for children under two years of age. Purging is not necessary.

The drug is well tolerated, although certain side effects have been mentioned, varying in intensity, according to the different authors (emetic effect, gastric pain, diarrhoea). *A. duodenale* is more susceptible to this drug than is *N. americanus* and it has more effect on adult than on immature worms. The percentage of cures obtained with a single dose appears to be satisfactory.

In heavily infected cases some authors advocate repetition of the treatment, either on three consecutive days or at intervals of 7-10 days. Since the drug is well tolerated, such repetition does not involve any serious disadvantages.

At the present time, tetrachlorethylene and bephenium are considered as promising drugs for the treatment of ancylostomiasis. Nevertheless, there is a need for wider experiments in Africa in order to compare their efficacy with that of other anthelmintics.

4.1.3 *Combined treatment : tetrachlorethylene and bephenium*

In view of the fact that bephenium appears to have a more selective action on *A. duodenale*, while tetrachlorethylene seems to be effective against *N. americanus*, some authors employ the following combined treatment : 2.5 g of bephenium base together with 5 ml of tetrachlorethylene

for adults. Very favourable results have been obtained, and the side effects are not very marked.

4.1.4 *1-Bromonaphthol*

1-Bromonaphthol is used in Japan with satisfactory results. It could be usefully tried out in Africa.

4.2 Treatment of anaemia

Iron can be administered by the oral route. Parenteral administration acts more rapidly, but is not necessary. Iron in the form of suppositories has no effect. (Associated erythropoietic deficiencies, if any, may be corrected at the same time.)

4.3 Choice of drugs in the presence of associated helminthiases

From the prophylactic point of view an anthelmintic effective against various helminthiases would be of value. Both bephenium and tetrachlorethylene are effective against *Ascaris*, and bephenium appears also to be active against *Trichostrongylus*.

Dithiazanine, which acts on *Strongyloides*, *Trichuris* and *Enterobius* is unfortunately not very effective against *Ancylostoma* or *Ascaris*. Where there is massive *Ascaris* infection it must be remembered that treatment may give rise to complications, including even intestinal obstruction.

For confirmation of clinical cure, stools should be examined three weeks after the end of treatment if the patient has been living in a non-endemic area for at least two months. In endemic regions, if the patient has been exposed to the risk of reinfection, stool examination is of no value unless it is negative after several tests at sufficiently widely spaced intervals.

5. PROPHYLAXIS

As with any parasitic disease, attempts can be made to interrupt the life cycle at more than one point. In theory, it is possible to reduce or eliminate transmission by ridding all infested persons of parasites, by preventing contamination of the soil for a period equal to the maximum life-span of the adult worm, by rendering the soil unsuitable for the development of larvae, or by preventing contact between the skin and infested soil for a sufficiently long period.

In practice, however, the incidence of ancylostomiasis reflects the standard of living of the population, and its reduction is directly dependent on improvement of economic and social conditions which, given the

present situation in African countries, cannot be expected to be rapid. Victory over the parasite is not for tomorrow, but nevertheless a start should be made and the control methods available should be put to the best use.

In order to reduce the intensity of the infection and decrease transmission, environmental sanitation measures should be applied and health education as well as treatment should be initiated.

5.1 Environmental sanitation

Environmental sanitation measures aim at preventing soil pollution by the faeces of persons infested with parasites. The ideal measure would be the installation of sewerage systems, but these are expensive and could be provided only in certain large urban centres with a main water supply. In rural areas, schemes for installing latrines should be promoted, after careful preliminary study. Care must be taken that the number of latrines is sufficient, that they are actually used, and that they are kept in reasonably good condition. The improvement of housing and of the area immediately around dwellings (by asphaltting, for example) would greatly help to reduce transmission. The World Health Organization has published two monographs describing the different methods of treating wastes and the various types of latrines.^{1, 2} Environmental sanitation measures should not, however, be undertaken in isolation but in association with a health education campaign. A considerable time will elapse before the effect of such a programme on the prevalence of ancylostomiasis becomes apparent.

In some densely populated areas of Africa, it has been pointed out, human excreta are used as fertilizer. For economic reasons it is impossible to oppose this practice, which may in any case render large quantities of eggs and larvae harmless if certain precautions are taken during the collection and composting of the manure. Heat treatment is not easily applied, but storing over a sufficiently long period under sealed conditions results in the destruction, by fermentation, not only of *Ancylostoma* eggs and larvae, but also of other intestinal parasites. The addition of chemicals such as calcium cyanamide might destroy the eggs and larvae while at the same time improving the quality of the fertilizer.

In some parts of Asia this aspect is already under study; in Africa, the local conditions under which faecal matter is used should be examined.

Biological control might also be envisaged, in particular along the lines of using fungi to combat certain nematode plant parasites.

¹ *Design and operation of septic tanks : Third European Seminar for Sanitary Engineers*, 1953, Geneva (*World Health Organization : Monograph Series*, No. 18).

² Wagner, E. G. & Lanoix, J. N. (1960) *Excreta disposal in rural areas and small communities*, Geneva (*World Health Organization : Monograph Series*, No. 39).

5.2 Health education

The Conference stressed the paramount importance of health education, which is one of the fundamental public health measures necessary to attain the objectives of such programmes.

Health education aims at obtaining the collaboration of all classes of society. It is frequently hampered by the difficulty of changing, and even uprooting, long-established customs apparently cherished by the populations concerned, so that progress can only be slow. Nevertheless, health education must be begun.

Particular emphasis should be laid upon the necessity for health education of schoolchildren, especially in those regions where the rapid development of schools cannot fail to exert an influence on social behaviour. It is also essential to make a direct approach to women, since they have a great and lasting influence on family life and the household.

With reference to the methods and means to be employed for the development of health education programmes and the training of health educators in tropical areas, the Conference referred to that part of the report on the African Seminar on Environmental Sanitation dealing with health education of the public.¹

Finally, the responsible authorities should be informed with regard to the fundamental importance of soil sanitation as an effective means of combating not only *ancylostomiasis*, but also other intestinal diseases (both infectious and parasitic) which undermine the health of the people. The general use of footwear is not a practical proposition for the greater part of the African population.

5.3 Mass treatment

Ideally, of course, the parasite should be eradicated, but this is beyond present possibilities. Administration of anthelmintics cannot, by itself, eradicate the infection, but it can reduce it to a tolerable level. For this reason, it would be wise to concentrate on controlling the disease, since it causes the greatest damage to public health and hinders the economic development of the region affected.

Suitable products are now available: they are easily administered in a single dose, well tolerated, and effective.

The following points should be borne in mind in mass treatment programmes:

(a) The most favourable period for the campaign is immediately after the period of maximum infection. In tropical Africa, this is generally about two months after the end of the rainy season.

¹ World Health Organization, Regional Office for Africa (1956) *Seminar on Environmental Sanitation in Africa, Ibadan, Nigeria, 1955: Report*, Brazzaville (Unpublished mimeographed document WHO MH/AS/117.56).

(b) A preliminary survey should be made to determine which age-group is the most seriously affected, and mass treatment should be first applied to that group.

(c) Anthelmintic treatment should be accompanied by iron treatment.

(d) The possibility of adding iron to the diet should be considered.

(e) The drug to be used, its dosage, and the intervals between treatments should be very carefully determined.

The success of any mass campaign for the control of ancylostomiasis depends upon the attention paid to the above-mentioned points. The Conference therefore expresses the hope that pilot units to study these questions will be organized in several African countries.

6. SUGGESTIONS FOR RESEARCH: IMMUNOPROPHYLAXIS

In view of the very relative efficacy of the means at our disposal for ancylostomiasis control, there is no reason to neglect any longer the promising prospects offered by immunization methods, already successfully applied in the prevention of bacterial and viral diseases. Any idea of immunization against Metazoa appears to be illusory. However, recent successes against zoonhelminths in veterinary medicine justify the hope that similar results may be obtained in human medicine. An example of a success in this field is the effective protection against acute parasitic bronchitis in bovines which can be produced by inoculation with irradiated larvae of *Dictyocaulus viviparus*. The first experimental tests on monkeys using human parasites (avirulent strains of *Schistosoma japonicum*) have shown that it is possible to produce active immunity in these animals.

The Conference expressed the hope that present efforts to develop a vaccine against *A. duodenale* would be successful; the results already obtained with *Uncinaria stenocephala* and *A. caninum* justify such a hope.

Our knowledge of the host/parasite relationship and of tissue and defence mechanisms is far from adequate. Basic research to elucidate this immunological problem is of the highest importance as a guide to the discovery of an effective vaccine.

Study of the metabolism of the worm in its various free and parasitic phases (which calls for *in vitro* culture in an axenic medium) might provide useful indications with regard to the nutritional requirements of the parasite in competition with its host, and might lead to the discovery of more effective therapeutic products.

Special attention should be paid to the role of diet in increasing resistance to the parasite.

Research on somatic antigens, on metabolites and on the antibody-antigen reaction might lead to the development of a method of serodiagnosis, or might open up new prospects in the field of immunization.

7. RECOMMENDATIONS

The Conference recommends :

- (1) that methods and techniques for epidemiological surveys be standardized as far as possible, since such standardization is necessary for the operation of the pilot projects proposed in this report ;
- (2) that carefully planned and strictly controlled comparative tests of anthelmintic drugs be made in accordance with up-to-date drug-testing procedures, a preliminary study having, of course, been made of the criteria of cure ;
- (3) that the gap in the bibliographical record between the publication in 1922 of the *Bibliography of hookworm disease*¹ and the coverage of the years 1950-60 provided by a *List of references on ancylostomiasis*² prepared by WHO be filled ;
- (4) that, in view of the importance of ancylostomiasis and other helminthiases in Africa, the responsible authorities should undertake adequate prophylactic measures, and that campaigns to this end should, as far as possible, be combined with other public health programmes such as those in the field of nutrition ;
- (5) that research be undertaken on the biology and physiology of *Ancylostoma* for the purpose of improving present methods of therapy and prophylaxis ;
- (6) that meetings be held at fairly frequent intervals for the exchange of ideas and information.

ACKNOWLEDGEMENTS

The participants in the Conference wish to express to the Government of the Republic of the Congo (Brazzaville) their gratitude for the hospitality and facilities offered them during their stay in Brazzaville, and to thank the Minister of Public Health, who was kind enough to open the meeting.

¹ Rockefeller Foundation, International Health Board (1922) *Bibliography of hookworm disease*, New York (Rockefeller Foundation Publication, no. 11).

² World Health Organization (1961) *List of references on ancylostomiasis*, Geneva (Unpublished mimeographed document WHO AFR/ANCYL/CONF/4).

Annex 1

RESULTS OF PREPARATORY RESEARCH STUDIES

TABLE 1. PREVALENCE OF ANCYLOSTOMIASIS IN AN URBAN ENVIRONMENT (BRAZZAVILLE, 1961) ACCORDING TO COPROLOGICAL ANALYSIS

Age (years)	0-2	3-5	6-10	11-20	21-30	31 +
<i>A. duodenale</i> and <i>N. americanus</i>	12.7%	38.8%	68.3%	71.4%	55.1%	51.2%

TABLE 2. DISTRIBUTION OF EGG-LOAD PER GRAM OF STOOL BY AGE AND SEX IN 563 SUBJECTS *

	Number examined	Percentage negative ^b	Number of eggs per gram of stool ^a			
			1000	2000-4000	5000-9000	10 000 +
Children (0-14 years)	218	12	28%	33%	12%	15% (A)
Males (15 years and over)	177	8	29%	21%	17%	25% (B)
Females (15 years and over)	168	5	41%	33%	14%	7% (C)

* From villages close to Harbel, Liberia (193), to Ibadan, Nigeria (127), and to Brazzaville, Congo (243: rural inhabitants).

^a The egg-counts indicated are for "F" (formed) stools and the counts for children up to the age of seven years have been adjusted so as to make them comparable with the counts for other age-groups. The average counts for all groups, except the last, emerge clearly from the table. For the 10 000 + group, the averages have been calculated on the basis of "F" stools, and (in number of eggs per gram of stool) are:

(A) Children 0-14 years	17 000
(B) Adult males	17 000
(C) Adult females	16 000

The eggs/mg/F figures can be obtained by moving the decimal point three places to the left.

^b Infestations not apparent under examination.

TABLE 3. PREVALENCE OF *N. AMERICANUS* AND *A. DUODENALE* ACCORDING TO THE TEST-TUBE FILTER-PAPER TECHNIQUE IN THE AREAS COVERED BY TABLE 1*

	Percentage of carriers of <i>N. americanus</i>	Percentage of carriers of <i>A. duodenale</i>
Children (0-14 years)	80	38
Males (15 + years)	93	41
Females (15 + years)	91	34

* Afuko excluded owing to absence of *A. duodenale* in that area.

Annex 2

DILUTION EGG-COUNTING TECHNIQUE FOR HOOKWORM, *ASCARIS*, *TRICHURIS* AND OTHER PARASITES *

The dilution egg-counting technique, originally devised for use in the study of culture conditions affecting the free-living stages of hookworm, was first employed as an aid in epidemiologic investigations in Puerto Rico in 1922, as a substitute for earlier attempts at the counting of hookworm eggs in aqueous faecal smears.¹ When the method was initially described,² the desirability was noted for the establishment of counts from a fairly large portion of the original stool, good comminution of the faecal material in the diluent, prompt withdrawal of the dilution drop from the population of eggs in suspension, and accurate count of the ova removed. The latter requires the aid of a microscope equipped with a mechanical stage for examination of the measured drop that has been mounted under a cover-slip of appropriate size.

Preferred practice for many years has been to transfer the faecal sample, without weighing, directly into a flask with an elongated neck, so that 4 ml of the excrementitious material displaces the meniscus of decinormal sodium hydroxide from the level of the 56-ml mark to the level of the 60-ml mark.³

This one-fifteenth dilution permits drops of 0.075 ml to be withdrawn for counting, and these represent 1/200 or 5 mg of the original specimen. (Drops of 0.15 ml may instead be counted, thus 1/100 or 10 mg of the

* Prepared by N. R. Stoll, Sc.D., The Rockefeller Institute, New York, USA.

original specimen; 5-10 mg represents the amount of faecal material available for reasonable scrutiny in the usual smear.) Lytic effects on the bacteria and saponifying effects on constituents in the faecal matrix are produced by the action of the sodium hydroxide. The result is an odourless slide-preparation of high visibility in which the eggs are free and are not obscured by debris. Ascaris eggs gain a distinctive appearance through the "blooming" or loss of their outer (albuminous?) coating.

The author's preferences concerning details of the method follow.

1. Start with a faecal specimen of generous size (as large as a hen's egg). Classify, for consistency, as F (formed), SF (soft-formed), M (mushy), MD (mushy-diarrhoeic).

2. Add decinormal (0.4%) NaOH to a dilution egg-counting flask calibrated at the 56-ml and 60-ml levels. (See specifications for dilution egg-counting flasks, page 26). Alternatively the faecal sample may be weighed, and uncalibrated flasks employed.

3. With a swab-stick (or bamboo splinter), carefully add faeces to the flask until the prepared meniscus of 56 ml has risen to the 60-ml mark.

4. Add several glass beads of 5- to 6-mm diameter, rubber-stopper the flask, and shake, taking care to shake "against" the rubber stopper. In routine practice, the preparation is then set aside for several hours or overnight; some slight additional shaking then completes the comminution.

5. When ready to do the egg-counting, again gently shake the flask in order to resuspend the eggs, and promptly withdraw a drop of 0.075 ml. The pipette of choice for this sampling has a bore and length of such size as to allow the 0.075-ml level to be visible above the top of the flask. (See specifications for dilution egg-counting pipette graduated at 0.075 ml, page 26.) With a pipette on which the rubber bulb is properly seated, a full squeeze withdraws the egg-containing diluent to the mark. Overdrawing and gradually expelling excess fluid to obtain correct volume in the pipette results in loss of eggs, as does delay in sampling after shaking the flask to suspend eggs in the diluent.

6. Expel the measured 0.075- (or 0.15-)ml drop on to a microscope slide by holding the pipette vertically, and apply a cover-slip, keeping the edges of the latter parallel to the edges of the slide. Desirable size of cover-slip depends both on the size of the microscope slide in use, and the degree of pigmentation in faecal specimens. The object is to count all the eggs in the preparation. There are advantages in using a slide of dimensions 37 mm × 75 mm (1½ in. × 3 in.) if the mechanical stage of the microscope

permits. Use may be made of a smaller cover than 25 mm² when the preparation is unusually clear (as from M and MD specimens)—perhaps of 22 mm² or even of 18 mm². Thinner preparations with larger covers (22 mm × 30 mm, 24 mm × 32 mm, etc.) speed up counting by virtue of the resultant increased visibility, especially if the faecal specimens turn out to be excessively pigmented, as was found in two of the three African areas studied.

Oculars of 5× or 6× and objectives of 10× (16 mm) are usually employed, but under proper conditions a lower-power objective may be useful.

7. Start at the upper right-hand or upper left-hand corner of the cover-slip (due to reversal of image, this appears to the observer as the lower corner), and traverse a zone the width of the microscopic field all the way across the width of the cover-slip; examine the entire preparation in successive zones. (A Chinese parasitologist may wish to count in zones traversed vertically.) The rule is followed that if the microscopist is interrupted during counting he goes to the "end of the row" before stopping.

8. A hand tally-counter (Veeder) is of invaluable assistance. It is the author's practice to count all the egg species present: hookworm, roundworm, whipworm, etc. The Veeder counter is habitually used to carry the hookworm totals, and a mental tally kept of the other eggs (occasionally transferred as a partial count to a sheet of paper).

9. One slide count of a 0.075-ml drop multiplied by 200 gives the number of eggs per gram (EPG) of the original specimen (there is insufficient difference between one ml and one gram of faeces to warrant a special label for results from a counting flask prepared by displacement versus weighing of the faeces).

10. After the counts on a specimen, or a series of specimens, have been performed, they are listed with a notation of the consistency of the stools from which they were derived (F, SF, M, MD). The counts are then brought to "Basis F". This is done by multiplying EPG counts from SF specimens by 1½, from M specimens by 2, and from MD specimens by 3.

The reason for "Basis F" is simple. If the egg output of infected individuals reflects the size of the infections they carry (i.e., number of worms), egg counts per gram measure such egg outputs only insofar as the individuals concerned have comparable *faecal* outputs. It has been found that in general the amount of F faeces passed per day per person is roughly comparable within a given population unit; also that soft, pulpaceous M faeces on a daily basis bulk to about twice the volume of F,

and therefore the eggs are present in half the F number per gram, and their number must be doubled to compare on "Basis F". The added SF and M ratios were likewise found of value. In a given region most of the specimens will be apt to fall into the classification for one of the consistencies, usually F, so that usually only a minority of the counts need the adjustment to "Basis F".

11. A further requirement applies to counts from small children. In general it appears that children to the age of 2 years pass about one quarter, and children of 3 and 4 years about one-half, the amount of faeces of adults in the same area.⁴ (Strangely enough, children aged 5 years and over appear, within the probable error, to have faecal outputs comparable to adults.) For comparison with egg-counts from adults in a given community survey, the EPG for children aged 0-2 years is discounted by 3/4; for children aged 3-4 years is discounted by 1/2. Thus, a 10 000 EPG count obtained respectively from a child of 2, a child of 4, and an adult, would be listed as 2500 EPG, 5000 EPG, and 10 000 EPG for comparison of size of infections.

It should be noted that the qualifications mentioned under items (10) and (11) apply with equal force to the results secured by any method of faecal examination for the presence of ova. The prodigious fecundity of intestinal helminths obscures the force of these qualifications when mere presence or absence of eggs is sought; with enumeration of the eggs they need consideration if comparability of the information is a desideratum.

12. Not even the above considerations encompass all the variables involved in the flow of eggs to the outer world in the intestinal contents. Greater reliability of the status of the egg output for a given individual is obtained by securing counts on additional faecal specimens from that individual. Every additional specimen egg counted improves the stability of the measure for the individual infection. Pre- and post-treatment egg-counts based on less than three specimens counted per individual have an unnecessarily large probable error; five counts are preferable for investigative purposes. In community surveys the individual fluctuations are absorbed in the averaging of counts from several individuals within given egg-count intensities. Caution needs to be exercised, however, in determining, both for successive samples from one individual, and for those from many individuals in community surveys, whether specimens are actually contributed by the donors to whom they are ascribed.

13. While EPG numbers have widely appeared in parasitological literature over the years, there is considerable convenience in working with the counts as EPMg. This requires no more than placing a decimal point three figures to the left, and using the resultant smaller EPMg figure.

In practice this means that from an F specimen an 0.075-ml slide-count of five hookworm eggs represents a suggested minimal positive grouping; and a slide-count of 50 or more places it in the very heavy worm-burden classification.

Supplementary Suggestions

A. Faecal specimens are preferably refrigerated before preparing the dilution counting flasks, and the latter may also to advantage be refrigerated when the laboratory is at tropical temperatures. Sealed containers for the specimens will reduce desiccation, but if only slight drying has occurred they may be cautiously rehydrated before tubing.

B. Classification of consistency is simple. "F" and "SF" have retained the bowel-cast; "M" (mushy) specimens have slumped; if mushy specimens tend to flow slightly on tilting they are classified "MD". Examination of "D" (liquid) specimens produces unreliable counts, and these are used only for diagnostic purposes.

C. A stock bottle, conspicuously labelled, of 4% (i.e., N/1) NaOH, is a convenience in the laboratory, portions to be reduced to the N/10 value as needed.

D. In filling displacement flasks, the routine is first to fill them approximately to the 56-ml level, and then "trim" them, with the aid of a Pasteur pipette. They should be trimmed accurately, so that the entire encircling 56-ml mark on the neck of the flask shows *under* the meniscus of the clear diluent, when the mark is held at eye-level.

E. Occasionally it is desirable to rescue for counting a specimen that contains less than 4 g. One way is to add a provisional mark to the neck of the tube at the 58-ml level, and use a half-size sample, care being taken to record that the dilution is twice the usual one.

Test-tubes, with lip, of 20-mm × 200-mm size (taking a No. 2 solid rubber stopper) are also useful for small specimens, or in "emergencies". If calibrated at the 28-ml and 30-ml levels, they permit a 2/30 dilution. For successive use of such tubes the 28-ml and 30-ml levels may be carefully etched on the glass with a diamond pencil. Any provisional marks then made, as with a red China ("wax") marking pencil for convenience during tubing, may be restored by referring to the etchings after later routine washing of the tubes.

F. With practice, the addition of the proper amount of faeces into the neck of the flask (or the test-tube), can be performed without smearing the glass.

G. "Best practice" involves having the faecal specimens and "tubing" done in a room that is either well-ventilated or has a suction fan.

Routine work with faeces deserves somewhat the same consideration as chemical work with hydrogen sulfide.

The dilution counting itself is preferably done in another room of the laboratory, as the treatment with NaOH N/10 produces practically odourless preparations.

Specifications

Dilution egg-counting flasks

Pyrex, or equivalent; constructed by fusing a neck of 20-mm-diameter glass tubing to the top of a 50-ml *heavy-duty* Erlenmeyer flask. Marked with encircled lines at the 56-ml and 60-ml levels, the latter mark to be at 45-50 mm from top of tube. Permits use of a No. 4 solid rubber stopper. The flask stands without a rack.

These are sometimes listed in supply catalogues as "Stoll flasks".

Dilution egg-counting pipette graduated at 0.075 ml

To be made from heavy glass tubing, 8-mm exterior diameter, 2-mm interior diameter.

They are drawn so that there is a gradual diminution of bore to 0.7-1.0 mm at tip, and so that a volume of 0.075 ml is obtained at a distance of 8.5-10.0 cm from that tip.

Over-all length of pipette, 16.5 cm; both ends to be cautiously flame-smoothed, *without constriction* of lumen; tip also to have outer edges slightly bevelled for 5 mm.

An encircling mark to be etched (and whitened) at the 0.075-ml level, and marked 0.075.

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Annex 3

**A TEST-TUBE FILTER-PAPER METHOD
FOR THE DIAGNOSIS OF *ANCYLOSTOMA DUODENALE*,
NECATOR AMERICANUS
AND *STRONGYLOIDES STERCORALIS* ***

During the survey trip through West and Central Africa, June to August 1961, use has been made of a test-tube filter-paper cultivation technique for two purposes: first, for the determination of the presence of *Strongyloides* and/or hookworm. This aspect of the technique is simple and after short practice can be reliably performed by any skilful technician. The second opportunity presented by the use of the technique is for the differentiation of the two hookworm species, *Ancylostoma duodenale* and *Necator americanus*, in their infective larval stage. This allows the determination of the hookworms present before treatments, etc., are undertaken, but the morphological discrimination of the two species requires some degree of familiarity with larval nematode structures.

Harada et al.¹ introduced the idea of adapting the older method of cultivating hookworm larvae in faeces on a piece of filter-paper placed in a Petri-dish, by using instead the filter-paper as a strip to be inserted in a test-tube. The technique was developed by Sasa et al.² for the purpose of facilitating epidemiological surveys of hookworms and related nematodes in man and domestic animals.

Since 1958 the author has employed this technique for the study of the relative prevalence of *A. duodenale* and *N. americanus* in Taiwan. It has, in addition, been found of particular value in assessing the efficiency of anthelmintics against *Ancylostoma* and *Necator*, together with Stoll dilution egg-counts.

In due course, some modifications of the technique have been made by the author and his associates. This paper is intended to give materials and procedures of the technique as modified in the author's laboratory, and necessary precautions for its proper use.

I. Materials needed:

1. Test-tubes (18 mm × 180 mm, or 20 mm × 200 mm), and test-tube rack
2. Pasteur pipettes (20 cm long), for use with rubber bulb

* Prepared by Professor H. C. Hsieh, Director, Department of Parasitology, Kaohsiung Medical College, Kaohsiung, Taiwan, China.

3. Centrifuge tubes (15-ml), pointed; and centrifuge
4. Microscope slides and cover-glasses (18 mm × 18 mm preferred)
5. Forceps (tweezers)
6. Beakers (1000 ml, 2; 100 ml, 2)
7. Compound microscope with medium- and high-power objectives (oil-immersion unnecessary)
8. Incubator for use at 28°C
9. Water bath for use at 50°C
10. Ancyloscope

(To facilitate the examination of tubes, Sasa et al.² described a device which they termed an "ancyloscope". It is essentially a lens of low power mounted in a tube that is adjusted at a convenient angle, so that the very bottom of the inside of the culture tube can be brought into focus. The tube itself is placed upright in an adjustable holder at the focal distance, and the bottom of the tube is given lateral illumination with a beam from a shrouded electric-light bulb. Any worms present are strikingly in view in the reflected light. While the ancyloscope is obtainable from Japanese sources at a moderate cost, a substitute can be devised in most laboratories with available apparatus.)

11. Wooden applicators (about 15 cm long), or substitutes (such as bamboo splinters)
12. Strips of coarse filter-paper (15 mm × 150 mm)
13. Cellophane cut into 80-mm squares, and rubber bands to fix these squares at the top of test-tubes
14. Old newspaper (25 cm × 40 cm)
15. Distilled or heat-sterilized water
16. Lysol or other disinfectant
17. Wax pencil (china-marking pencil)

II. Procedure :

1. To each of the test-tubes in the test-tube rack add about 7 ml of distilled or heat-sterilized water.
2. Spread the sheet of old newspaper to protect the laboratory bench top.
3. Crease a filter-paper strip and lay it on the newspaper (with successive strips, move down the paper).
4. Take about half a gram of faeces with the wooden applicator and smear on to the filter-paper, leaving about 5 cm at left end of the paper unsmearcd.

(b) Body-length about 590 μ , and sheath-length about 660 μ ; sheath conspicuously striated, most clearly observed around the tail portion of body; mouth "spears" appear dark; anterior end of body (not the sheath) rounded, like the small end of a hen's egg; anterior portion of intestine as wide as oesophageal bulb; tail-end sharply pointed *Necator americanus*.

(c) Body-length about 660 μ , and sheath-length about 720 μ ; sheath less clearly striated; mouth "spears" less conspicuous; anterior end of body (not the sheath) blunt; intestine narrower in diameter than oesophageal bulb; tail-end blunted *Ancylostoma duodenale*.

3. Exceptions :

(a) Occasionally infective larvae will be found ex-sheathed and the sheath striations accordingly unavailable.

(b) It should be recalled that *Strongyloides stercoralis* has a free-living generation; male and female adults, as well as immature larvae may consequently be encountered.

IV. Precautions desirable for obtaining best results :

1. Faeces should be collected in a clean container and must not be contaminated with soil, etc. ; the specimen should remain at about 15°-30°C (59°-86°F) from time of defaecation until cultivation. Exposure of the faecal sample to strong heat or sunshine, or to refrigeration, will destroy the viability of the parasites.

2. Nearly dry faeces, or the outside layer of a small faecal sample, particularly in the dry season, should not be used.

3. Formed faeces may be softened by adding a few drops of distilled water to facilitate smearing on filter-paper.

4. A small percentage of larvae hatched on filter-paper (under the conditions specified this may be about 10%, as estimated by Hsieh & Kuo) do not come down to the water at the bottom of the test-tube. In areas where the degree of infection is small, and in post-treatment examinations of patients, more than one test-tube culture per faecal sample may therefore be desirable. Under such conditions, the author has used up to four tubes per specimen.

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