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Geachte collega,

Hierbij zend ik U 18 abstracts van wetenschappelijke bijdragen aan onze recent gehouden najaarsvergadering met het verzoek deze in TGM te willen plaatsen onder de kop

NETHERLANDS SOCIETY FOR PARASITOLOGY

Autumn meeting, LEIDEN, 30 October 1987

De volgorde van de abstracts is met potlood rechtsboven aangegeven. Uiteraard zullen wij de kosten voor extra pagina's vergoeden.

Tevens wil ik van deze gelegenheid gebruik maken U te vragen hoe het staat met de publicatie van vorige abstracts. Bij mijn weten zijn de volgende nog niet gepubliceerd: Najaar 1985 (ms 3960), voorjaar 1986 (ms 4051), najaar 1986 (ms 4147) en voorjaar 1987 (ms 4244).

Zijn er van deze al in druk verschenen? Zo ja, zoudt U zo vriendelijk willen zijn mij een kopie van de betreffende pagina's te zenden? Zo nee, hoe is de planning van de plaatsing van de abstracts?

Uiteraard hebben wij begrip voor alle problemen, zoals de heer Vogel die in het verleden regelmatig heeft genoemd, maar U kunt zich wellicht ook voorstellen dat onze leden hun abstract liefst zo snel mogelijk na indienen, geplaatst zien.

Uw reactie met belangstelling tegemoet ziend,

met vriendelijke groet,

Fred Bantman

## DNA synthesis in Plasmodium berghei

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Results of measurements on the DNA content of different life cycle stages of P. berghei were reviewed. The DNA content was determined by direct microfluorometry after Feulgen-pararosaniline staining.

Sporozoites, gametes and young blood stages are haploid. During blood-schizogony, genome duplication and nuclear division are alternating events during the last six hours of the 24-hour cycle. DNA synthesis in the male gametocyte for the production of the eight gametes takes place within 10 minutes in the midgut of the mosquito. Within 3 hours after fertilization the diploid zygote synthesizes DNA up to the tetraploid value. This DNA synthesis coincides with the time of meiosis. Some results were presented on the karyotype of the different stages and of clones of P. berghei, established by 'pulsed field gradient gel electrophoresis'. At least 12 chromosomes were distinguished. The karyotype of asexual blood stages and of gametocytes were comparable. Significant differences in the size of chromosomes were found between different clones. Moreover the karyotype of a clone changed after a long period of only asexual multiplication.

(2)

The interaction between Cooperia spp. and Ostertagia spp. in cattle.

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In this study the presence of interaction between Cooperia oncophora and Ostertagia ostertagi, nematodes which parasitize the small intestine and the abomasum of cattle, respectively, has been investigated. Interaction is of epidemiological importance when it leads to a reduced worm burden or a lowered faecal egg output. As there were some indications that interaction between C. oncophora and O. ostertagi is immunologically mediated, experiments were carried out in which calves were given some degree of immunity by inoculation with one of both species. By means of challenge infections it was examined whether this immunity affected the heterologous species or not. The effect of concurrent infections was also investigated.

During the experiments the course of infection was monitored by faecal egg counts. Other parasitological parameters, worm numbers, worm length, number of eggs in utero (indicating the fecundity) and vulval flap development of O. ostertagi females, were determined post mortem. By use of the Enzyme-Linked-ImmunoSorbent Assay (ELISA), the relative amount of serum IgG was measured to examine whether the interaction is immunologically mediated or not. Additionally, the numbers of some effector cells were counted in tissue fragments of the small intestinal and abomasal mucosa.

From the results of the experiments can be deduced that interaction between C. oncophora and O. ostertagi exists. This interaction leads to a stunted growth and development of worms, but it does not affect the worm burden or the faecal egg output.

The fact that interaction is only found in a sequential infection model implies that the host's immune response is involved by the mediation of this interaction. This thought is supported by the negative relation between parasitological parameters and heterologous antibody levels. Also the fact that



immune effector cells are more rapidly found in heterologously primed animals when compared to controls stimulates this hypothesis.

In many parasite-host models interaction between nematode species is of epidemiological importance, because its negative or positive influence on the size of the worm population or the faecal egg count. The results from the present experiments do not imply that such importance is present in the C. oncophora - O. ostertagi - calf model. However, when parameters indicating growth and development of worms are used as parameters that measure resistance it should be considered that these parameters are influenced by a previous heterologous infection.

Cerebral lesions in Plasmodium berghei infected mice:  
An immunopathological phenomenon.

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Infection of mice with P. berghei results in a biphasic mortality pattern in several mouse strains. Part of a group of mice dies early in the second week of infection whereas the remainder dies in the third week or later. Histological examinations of the brain from mice which die early reveals haemorrhages in the cerebellum and other parts of the brain. Mice which die late do not exhibit brain lesions.

Ultrastructural investigations of the brain with cerebral lesions shows an association between the vascular endothelium and monocytes. Sometimes monocytes are found even in the subendothelial area. Sticking of macrophages to the endothelium can obstruct the passage of erythrocytes. Lymphocytes can be found in association with macrophages. In addition phagocytosis by endothelial cells and degeneration of endothelial cells has been observed.

The body temperature of mice which die early decreases rapidly to values below 30°C. Mice which die late exhibit a moderate and transient decrease in body temperature. Histological examination of the brain reveals a correlation between a severely decreased body temperature and cerebral lesions.

Early death can be prevented by immunosuppressive measures such as the addition of dexamethasone to the drinking water, treatment with anti-T-cell serum, irradiation of the spleen, and splenectomy. This points in the direction of a T-cell dependent immunopathological reaction being involved in the pathogenesis of brain lesions.

Administration of serum from malaria-immune mice early during infection also can prevent development of cerebral lesions. The amount of immune serum needed can be so low as to have no effect on the development of the parasite. This suggests the masking of an immunopathological epitope by antibodies present in the serum of immune mice. The question rises whether mice can be immunized against the development of cerebral lesions.

Cerebral lesions are particularly found when mice are infected with low numbers of parasites e.g.  $10^3$  PE. When mice are infected with large amounts

of parasitized erythrocytes ( $10^7$  PE or more) no cerebral lesions occur. Probably  $10^7$  PE exhibit enough epitopes at the beginning of infection to generate immunoprotective reactions which protect against early death. When the same number of epitopes comes up in mice infected with  $10^3$  PE, the changes in immunoresponsiveness already induced before that point of time may prevent the generation of these protective reactions.

It is possible to immunize against the development of cerebral lesions in at least two more ways. Infection with  $10^7$  PE followed by chloroquine treatment two days later prevents the development of cerebral lesions during a subsequent low-dose infection. Injection of more than  $10^8$  PE, killed by repeated freezing and thawing, has the same result.

Summarizing it can be stated that an immunopathological reaction lies at the base of the development of cerebral lesion in *P. berghei* infected mice. Induction of this immunopathological process can be prevented by immunosuppressive measures or by pre-immunisation with dead or live parasites, which in itself does not protect against the proliferation of the parasite.



Trematode parasites interfere with the endocrine control of reproduction of their intermediate hosts, freshwater snails

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Most digenic trematode parasites have freshwater snails as intermediate host and a vertebrate as definite host. In our laboratory the combination *Lymnaea stagnalis*-*Trichobilharzia ocellata* is used as a model system for studying the host-parasite relationships.

Infection with digenic trematodes causes in hermaphroditic freshwater snails a strong reduction in sexual maturation and in fecundity and at the same time stimulation of body growth. The hypothesis that the effect on reproduction can be explained by the presence of a specific agent in haemolymph of infected snails interfering with the snail's endocrine system is sustained by the present data. The data clearly demonstrate the presence of such an agent, called *schistosomin*, in haemolymph of parasitized *L. stagnalis* from 6 weeks post exposure onwards. In two ultra(enzyme)cytochemical bioassays it has been demonstrated that schistosomin inhibits the effects of the gonadotropic hormones Calfluxin (CaFl), a neuropeptide produced by the neurosecretory caudodorsal cells (CDC), and of the dorsal body hormone (DBH), secreted by the endocrine DB, upon their target organs at the level of the receptor complexes. It is supposed that the already in an early stage of infection observed decrease of hormone receptors in the reproductive tract is also directly or indirectly caused by schistosomin.

The presence of schistosomin in haemolymph of infected *L. stagnalis* appeared to be a general phenomenon in snail species infected with digenic trematodes. Also haemolymph of species

that are intermediate hosts for parasites that cause Schistosomiasis in man and in domestic animals, appeared to contain schistosomin.

The question whether schistosomin is species-specific was investigated in the CaFl-bioassay. Schistosomin of *L. stagnalis* seems rather species-specific. Schistosomin of *Biomphalaria glabrata* infected with *Schistosoma mansoni*, on the other hand, had some effect on target tissue (albumen glands) of *L. stagnalis*, but not on that of *Bulinus truncatus*. This indicates that there might be some structural similarity among schistosomins of different species.

Studies to isolate and characterize schistosomin(s) of infected *L. stagnalis* are in progress. These attempts are favoured by the recent development of a rapid binding-assay. In this assay fluorescent CaFl is added to a crude receptor preparation of target tissue. The amount of unbound CaFl is measured and appeared to increase in the presence of schistosomin. This supports the supposition that schistosomin acts as an antagonist of CaFl.

This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.



Characterization of the epitopes on the 45/48 kD and 25 kD proteins on the gametes of Plasmodium falciparum

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It has been reported that Plasmodium falciparum sexual stages contain at least two target antigens of transmission blocking antibodies: one expressed on the surface of newly emerged gametes (Mr: 45,000/48,000) and the other one on the surface of zygotes/ookinetes (Mr: 25,000). Characterization of these antigens showed that both antigens are glycoproteins and that the 25 kD protein is also acylated.

In order to discover the contribution of the carbohydrate groups to the epitopes on the 45/48 kD and 25 kD proteins, the immunological reactivity of these antigens was tested after (1) modification (by treatment with periodate) and (2) cleavage (by trifluoromethanesulfonic acid) of the carbohydrate groups. Additionally the 25 kD protein was also deacylated (with KOH) and tested in order to ascertain the contribution of the acyl group for the functionality of the epitopes.

The results of these experiments demonstrated that the carbohydrate groups on the 45/48 kD proteins were likely to be an integral part of the epitopes or contribute for a great part to the stability and maintenance of the epitopes that are recognized by monoclonal antibodies. The carbohydrate group of the 25 kD protein does not structurally contribute to the epitopes on this protein; but the acyl group on this protein is likely to be a part of the epitopes or contributes to the stability and maintenance of epitopes recognized by specific anti-25 kD monoclonal antibodies.

This information might be of great relevance for the development of a transmission blocking vaccine based on these antigens.

## Human Oesophagostomum infections in Northern Togo and Ghana

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Oesophagostomiasis in man is considered to be a very rare infection.

The pathology is caused by the formation of small tumors in the intestinal wall. These tumors contain larvae of *Oesophagostomum spec.*

The taxonomic status of the species involved remains obscure which is partly due to the fact that adult, lumen inhabiting worms have rarely been found. Only Leiper, in 1911, has recovered a few adults from stools of an asymptomatic patient in Nigeria.

It was supposed that the adult stage is not normally reached in man.

Clinical and pathological studies initiated by Gigase et al (1987) indicated a high incidence of symptomatic cases in Northern Togo. The present study on the prevalence and distribution of Oesophagostomum infections in man was based on a systematic charcoal-culture of stool samples from northern Togo and Ghana. Young children (less than 5 years of age) were less infected than older children and adults. The prevalence ranged considerably, in those over 5 years of age: in some villages almost no infections were found, in others 30-40 % were infected. Females are consistently more infected than males. Adult worms could be recovered by treatment with pyranthel pamoate. Further studies are being undertaken.

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Discharge of *Eimeria*-oocysts by lambs at pasture.

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During the period march-august on nine sheep-farms faecal samples were taken to examine the discharge of coccidian oocysts. The adult ewes were sampled once before lambing. After birth the lambs were sampled 16 times with regular intervals. Of the adult ewes 96,5% showed discharge of oocysts. The average level of oocyst discharge was low and varied between the farms from 145 to 1330 oocysts per gram of faeces (OPG). On four of five farms where young ewes ( $\pm 1$  year) and older ewes were kept separately, the young ewes showed a significantly higher OPG than the older ewes.

Soon after turning out the lambs showed a rise in oocyst discharge. On most farms a maximum was reached between 2 and 5 weeks after turning out. This average maximum OPG on the farms ranged from 49.000 to 186.000. During the following 2 months the oocyst discharge decreased, just like the individual variation. From 19 weeks after the average birthdate the mean OPG was lower than 10.000. In both the adult ewes and the lambs always polyinfections with at least four *Eimeria* species were observed. *E.ovinoidalis*, *E.parva* and *E.ovina* were the dominating species. *E.granulosa* and *E.intricata* were only sporadically observed.



CIRCULATING SURFACE-ANTIGENS IN DIPETALONEMA VITEAE INFECTION

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We characterized an in-vitro-released-(IVR)-fraction of adult Dipetalonema viteae.

Using mouse-sera raised against this IVR-fraction (three proteinmolecules of 67, 58 and 56 kD) we localized the IVR-fraction in the surface (hypodermal structures) of adult D. viteae, performing IFAT on cryostat-sections. The IVR-fraction could be detected circulating in serum of D. viteae-infected hamsters using immunoblotting. Another non-hamster protein of 30 kD was detected also. Mouse-sera raised against IVR-fraction show no cross-reaction with other anti-helminth-sera in ELISA.

This IVR-fraction, species-specific, parasite-derived and circulating in host serum, can be used to investigate the importance of in vivo circulating products for protective immunity, immunosuppression, better immunodiagnosis and general pathology in filariasis.

Intervet

## Intravenous vaccination of calves against *Dictyocaulus viviparus*

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Five groups of av. 5 Friesian calves were reared wormfree. At the age of 8 and 12 weeks groups A and C were vaccinated orally with gamma-irradiated larvae. Group B received the same larvae, exsheathed, intravenously (v. jugularis). Groups D and E remained unvaccinated. At the age of 14 weeks the 5 groups were challenged with 10,000 infective larvae : groups A, B and D via the oral route, and groups C and E i.v. After challenge all calves were turned out to uncontaminated pasture, and observed until slaughter, 6 weeks later. In i.v. challenge control group E all animals showed severe symptoms of husk and 3 out of 5 died within 3-4 weeks p.i. (av. no. of worms in lungs was 1525). Oral vaccination reduced the number of adult worms in group C for 99%. In the oral challenge control group D symptoms were also severe but no one died, and av. no. worms at slaughter was 746. Reduction of adult worms was 98% in group A and 94% in group B. Comparison of control groups D and E already demonstrated the fatal consequences of by-passing the intestine. Parasitological protection was satisfactory in all 3 vaccinated groups. The i.v. vaccinates (B), however, showed severe lung lesions, least weight gain and highest antibody titres. Histomorphological alterations indicate a predominant gut mucosal immunity in the orally vaccinated animals, whereas in the i.v. vaccinates the contrary was found. This leads to the conclusion that the stimulation of mucosal immunity at the port d'entree of the parasite is an advantage for the host's health.

(10)

IDENTIFICATION OF LEISHMANIA PARASITES BY DNA RECOMBINANT PROBES.

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DNA hybridization offers three methods for the detection and /or characterization of Leishmania parasites.: Southern blotting, dot blot and in situ hybridization. Dot blot and in situ hybridization might be performed with total as well as recombinant DNA probes whereas for Southern blotting recombinant probes are necessary.

Previously we demonstrated the potential of DNA hybridization techniques for the identification of Leishmania parasites by dot blot and in situ hybridization with total DNA probes. A good hybridization signal was obtained, but the specificity was low. To increase the specificity we started a hybridization study with recombinant DNA probes. Recombinant DNA probes of genomic libraries from nuclear and kinetoplast DNA and cDNA libraries were used. Recombinant DNA probes were shown to hybridize in all three hybridization systems. Kinetoplast derived probes showed no interaction with nuclear DNA whereas cDNA probes did not hybridize with the kinetoplast DNA. This has been nicely demonstrated in in situ hybridization assays. Of the limited number of clones that have been screened extensively none of them has been found to be strain specific. Kinetoplast clones show a high degree of isolate specificity, whereas cDNA clones display considerable cross-hybridization between related Leishmania strains. Southern blotting might become an additional technique for the classification of Leishmania strains since several recombinant clones are able to differentiate groups of isolates on the basis of hybridization signal and pattern. Dot blot and in situ hybridization look promising for clinical application but have to await the advent of new more strain specific probes.



(11)

DETECTION OF SPECIFIC ANTIBODIES  
IN KALA-AZAR AND CHAGAS' DISEASE  
BY MEANS OF A SIMPLE  
MICRO-AGGLUTINATION TEST

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## ABSTRACT

The introduced immuno-diagnostic techniques for the diagnosis of visceral leishmaniasis (kala-azar) and American trypanosomiasis (Chagas' disease), are of limited value in poorly equipped laboratories and under conditions prevailing in the field.

An easily applicable direct agglutination test (DAT) for the diagnosis of visceral leishmaniasis and Chagas' disease has been developed. Following trypsin treatment, promastigotes of L.d.donovani and epimastigotes of T.cruzi were stained with Coomassie brilliant blue. The formalin preserved antigen suspensions were used in V-well microtitre plates and the test was read visually.

Active and recently treated kala-azar cases had titres of 1:51200 or higher. Only 24 out of 1497 samples collected from healthy controls and patients with other diseases, including African and American trypanosomiasis (Chagas' disease), reacted at 1:6400 serum dilution or higher. The DAT proved to be of value for the differential diagnosis of kala-azar and of Chagas' disease in Brazil.

In the DAT for Chagas' a titre of 1:1600 could be considered the cut-off point; the sensitivity and specificity were 93.0% and 92.4% respectively. Treatment of chagasic sera with 2-mercaptoethanol (2-ME) had no influence on antibody titre levels. In contrast, titres in sera from patients with visceral leishmaniasis, African trypanosomiasis and auto-immune disorders, subjected to similar treatment, showed remarkable decline.

The performance of the DAT for the diagnosis of visceral leishmaniasis was evaluated in comparison with IFAT and ELISA. The sensitivity (96.9%) and specificity (97.6%) of the DAT were comparable to those obtained in IFAT. Lower sensitivity (62.5%-96.9%) and specificity (70.6%-92.0%) levels were found in ELISA.

Being simple and economical to apply, the DAT is recommended for sero-diagnosis and sero-epidemiological studies of visceral leishmaniasis and Chagas' disease.



## GIARDIASIS, ASPECTS OF HUMORAL IMMUNE RESPONSE.

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Forty sera from children and adults (1-54 years of age) with symptomatic Giardia lamblia infections were investigated for the presence of anti-G. lamblia IgG and IgA antibodies. ELISA with peroxidase-conjugated antihuman IgG and IgA (Inst. Pasteur) has been applied in polystyrene microtiterplates to the patients' sera and to 17 sera from non-infected healthy adults. The antigen used consisted of washed, intact parasites, who were grown to late log-phase in modified BI-S-33 medium (Diamond et al. 1978). The humoral immune response was found to be age dependent. In sera from patients below the age of 8 years, no significant IgG and IgA antibodies were found. Other patient and control sera showed a considerable variation in serum reactivity. When the mean extinction + 2 standard deviations of the readings from 17 healthy controls is taken as the lower limit of positivity, we were able to detect IgG antibody in 42% of the sera from adults, and IgA antibody in 48%. Humoral IgG and IgA responses occurred independently. By combining IgG and IgA detection, 68% of sera from adult patients could be discriminated from control sera.

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Trans R Soc Trop Med Hyg 1978; 72: 431-432.

SEX-SPECIFIC-SURFACE-ANTIGENS IN DIPETALONEMA VITEAE

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Evidence was obtained that a Dipetalonema viteae antigen of 30 kD, circulating in the blood of the infected host, the hamster, originated from the surface (hypodermis) of the adult worm (C.M.Adema et al).

Thus far mixed populations have been used. Culturing adult male and female worms separately resulted in a difference of colour of the RPMI medium; this suggested that different products were released, that could be sex-specific-surface-antigens.

To gain knowledge about a possible antigenic difference between the surface of male and female worms, according to the method of Betschart (et al) an extract was prepared of the surface in which the muscle layer, the hypodermis, the basal and a part of the median zone of the cuticle were dissolved.

SDS-PAGE and immunoblotting with serum directed against D.viteae circulating antigen showed the presence of sex-specific-antigens in the extracts: in the female extract a 94 kD band and in the male extract 2 bands with molecular weights higher than 94 kD and a 30 kD band of which the molecular weight corresponds with the circulating antigen that previously was proven to be hypodermal (C.M.Adema et al).

The thin extracted outer layers of the cuticle were used for immunizing mice. Sera were tested using IFAT on frozen sections of adult male and female worms and gave specific epicuticle and cuticle reactions, with sex cross-reactivity. This suggests that there are common surface-specific-antigens in male and female worms. Using this method the possible presence of sex-specific-antigens in the extracted cuticle could not be demonstrated, but the higher intensity of the reaction of the sera directed against female cuticle points at the presence of such antigens in the extracted cuticle. The use of monoclonal antibodies to detect them would help.

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Adema CM, Vetter JCM. Circulating surface-antigens in Dipetalonema viteae infection. Unpublished report.



Has the incidence of human toxoplasmosis decreased in  
The Netherlands?

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The policy of the serological laboratory of the Academic Hospital of the Free University (Department of Clinical Microbiology and Hospital Hygiene; head: Prof.Dr. D.M. Maclaren) concerning the serodiagnosis of toxoplasmosis was evaluated for the years 1984-1986. Over this period, circa 400 requests for serodiagnosis per year were met. In a large proportion, clinical indications for these requests were ill-defined or were rather suggestive of other diseases.

Initially, serodiagnosis was largely based on the results of an IFAT-IgG. Only limited opportunities to send sera to a reference laboratory for further testing existed. The introduction of a capture ELISA-IgM medio 1985 greatly improved diagnostic facilities, resulting in generally well-interpretable results.

Surprisingly few cases of congenital toxoplasmosis were encountered. The incidence of acquired toxoplasmosis with clinical manifestations was low as well. In at least 60% of the cases with the clinical diagnosis chorioidoretinitis suggesting toxoplasmosis indications for an active infection were found.

The results from the testing of sera send in for screening purposes (transplantation patients, gravaidae, etc.) and from patients in which the symptomatology was not characteristic for toxoplasmosis were joined together and used to estimate prevalence and incidence rates of toxoplasmosis in the general population in the region. The rates thus obtained were compared with those calculated by van der Veen and Polak (J. Hyg. Camb. 85: 165-74 (1980)) for the area around Tilburg (The Netherlands) in 1978. It seemed justified to conclude that the incidence of human toxoplasmosis has decreased considerably in The Netherlands over the last ten years. Possibly a reduction of over 50% occurred.



Giardia lamblia - infections at the University Hospital Rotterdam  
Dijkzigt/Sophia Children's Hospital

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From 1981 - 1986 routine microscopical examination of the stools of 9245 outpatients and inpatients seen at the University Hospital Rotterdam was carried out according to the method of Ridley and Hawgood (1). Most stool samples were from outpatients with abdominal complaints with or without diarrhoea. Giardia cysts were detected in 5.1% of the stools. On average 2.7 samples per patient were examined a few days apart. From the 469 patients with positive stools for cysts of Giardia, 129 (27.6%) were immigrants and had a history of recently being abroad, nearly half of them from the Mediterranean region. The other 340 patients were living in or near Rotterdam. 76.5% of these patients were, according to their name, of Dutch origin. Infections occur in all age groups. The highest frequency (13%) of the number of patients with Giardia - positive stools were found in the age group of 2-4 year. It appears that endemic Giardia-infections are predominant.

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1. Ridley D S, Hawgood B C. J. Clin. Pathol. 1956; 9: 74-76

*Lymnaea stagnalis*: functional characteristics and lectin binding  
of a monoclonal antibody recognized hemocyte subpopulation

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The monoclonal antibody LS1 recognizes a membrane epitope of a hemocyte subpopulation in *Lymnaea stagnalis*. The percentage of LS+ hemocytes is higher in juvenile pond snails than it is in adults. In both groups, LS1+ hemocytes have the morphology of immature cells. Functional characteristics of LS1+ hemocytes and lectin binding to these cells were studied. In both age groups, LS1+ hemocytes are phagocytically less active and have a lower lysosomal enzyme (peroxidase) contents as compared to LS1- cells. The proliferative activity, as measured by the incorporation of bromodeoxyuridine, is much higher for LS1+ hemocytes than it is for hemocytes lacking the epitope. The lectins RCA and STA recognize surface carbohydrate moieties present on subpopulations of hemocytes only. The LS1+ hemocyte population virtually always has the surface carbohydrate recognized by STA, the LS1- population never shows binding of RCA. Our results support the findings that the LS1 epitope is a membrane marker of less differentiated hemocytes in both juvenile and adult *L. stagnalis*. Furthermore, they suggest a relation between the presence of the LS1 epitope and the binding of STA. The absence of the LS1 marker may correlate with the absence of a sugar recognized by RCA.

## ANTIGENIC COMPOSITION OF THE CUTICLE OF ONCHOCERCA VOLVULUS.

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In helminth infection the surface of parasites plays an important role in host-parasite interactions.

We obtained the cuticle of Onchocerca volvulus by means of microdissection. The composition of the cuticle was analysed using SDS-PAGE, ELISA, IFAT and monoclonal antibody technology.

Cuticle-fragments obtained by microdissection were extracted by sonication in NaDOC and BME, ultracentrifugation and dialyzation. This extract was analyzed by SDS-PAGE and stained for protein (coomassie) and for glycoproteins (silver staining).

A difference in proteincomposition of the cuticle and the internal organs was found. Glycoproteins form a mayor part of the extract. We produced MOAB's against cuticle-fragments, which in ELISA ... reacted with cuticle-extracts only, and not with the extracts of internal organs. IFAT using cryostate-sections of O.volvulus supports these findings.

We conclude that microdissection offers the possibility of isolating the organs of O.volvulus separately. Furthermore IFAT and ELISA indicate differences in the antigenic composition of the isolated organs.



Prevalence of Trypanosoma brucei rhodesiense infections in the population of South Luangwa Valley, Zambia as indicated by an immunofluorescence assay.

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Between April and June 1987 a survey on human trypanosomiasis was carried out in South Luangwa Valley, Eastern Province, Zambia. Three areas were studied: Masumba, Kakumbi and Chibembe, in the reach area of Kamoto Hospital. 1649 people above 12 years of age were seen: 507 in Masumba, 597 in Kakumbi and 545 in Chibembe. From each person thick and thin blood films were made on overhead transparencies according to the method of Beljaev (WHO, Delhi, India) and stained by Field's stain. Blood was stored on Whatman no. 4 filter paper. Testing for specific antibodies against T.b. rhodesiense was carried out with an indirect immunofluorescent assay, developed at the Institute of Tropical Medicine at Antwerp, after elution of the sera from the filter paper. A mixture of two different antigenic variants of T.b. rhodesiense from Uganda was used as antigen in the test. Preliminary results show that 76 (4.6%) of 1648 sera gave a weak reaction. Ninety sera were found positive, of which 78 (4.7%) did react with only half of the antigen in the mixture and 12 sera (0.7%) reacted strongly with both antigens.

Reaction	Masumba	Kakumbi	Chibembe
weak	19 (3.7%)	23 (3.8%)	34 (6.2%)
positive, ½ of antigen	16 (3.2%)	33 (5.5%)	29 (5.3%)
strongly positive	3 (0.6%)	2 (0.3%)	7 (1.3%)

In only 1 thick blood film (from Kakumbi area) of the positive sera trypanosomes were found. Malaria parasites (Plasmodium falciparum and a single P. ovale) were seen in 904 (55.8%) of 1620 thick blood films: Masumba 235(= 47.4%), Kakumbi 352(= 59.4%), Chibembe 317(=59.7%)